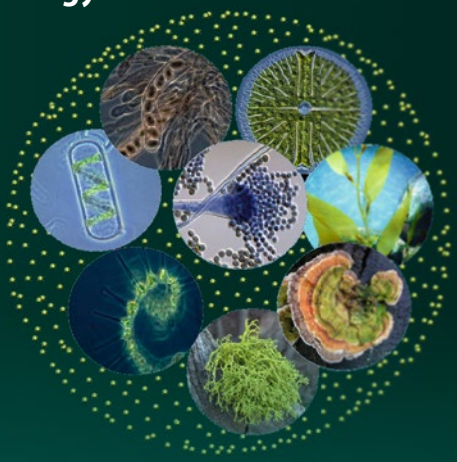
Advances in Environmental Microbiology 6



Christon J. Hurst *Editor*

Understanding Terrestrial Microbial Communities

Springer

Advances in Environmental Microbiology

Volume 6

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When I was born into this life during 1954 there was an older brother named Preston waiting for me! I am not certain about what my brother thought of the changes which my presence brought to his existence, but there I was and we became fairly inseparable. Not even his red haired Raggedy Ann, which had been my brother's 1952 Christmas present, came between my brother and I. Although, Raggedy Ann may have been a more patient listener.

Raggedy Ann, Preston and Christon in December 1954

My favorite childhood memory with my brother was when we played train ride in the basement of our house. Our parents had found some cardboard boxes and arranged the boxes as part of a circle on the basement floor. During many evenings, my brother and I would then each sit down inside of a box and pretend that we were taking an imagined journey.
A few years later, a sister named Embeth was born into our lives. Raggedy Ann had by then disappeared but Embeth came into this world with red hair, and we three Hurst children began a true life journey together.

Preston, Christon and Embeth in 1960

My little sister was a good listener, and my favorite childhood memory with my sister was when I used to read books to her and show to her the illustrations in the books as I turned the pages. Embeths favorite books for our reading time were the Mrs. Piggle-Wiggle series by Betty MacDonald.
The three of us siblings have grown older together and I happily remember journeys with them. Eventually, my brother and I took train rides on the Algoma Central Railway in Ontario, Canada, for canoeing trips with our Boy Scout troop. One time, Preston and I along

with his wife and son took a journey to Nashville, Indiana, not by train but by automobile and that was a particularly good day!

Preston and Christon Hurst in Nashville Indiana on October 2nd 2002

And many years later, I eventually took a trip to Germany with my sister during which I signed the contract to begin this book series.

Embeth at Cafe Winuwuk near Bad Harzburg Germany on July 25th 2013

I have proven to be more durable than was the Raggedy Ann doll, although perhaps I still am not as patient as a listener. And, my sister finds delight each time I show to her a new book that I have published. When this volume is printed I will read the dedication to my sister and show to her the pictures as I turn these front pages. Embeth will then smile and say to me, "Chrissy, you know that I now could read that for myself". My brothers comment will be "Chrissy, we need to find some new cardboard boxes and set up a train on the floor". And so, I lovingly dedicate my work on this book to Preston and Embeth who are my two dearest friends.

Series Preface

The light of natural philosophy illuminates many subject areas including an understanding that microorganisms represent the foundation stone of our biosphere by having been the origin of life on Earth. Microbes therefore comprise the basis of our biological legacy. Comprehending the role of microbes in this world which together all species must share, studying not only the survival of microorganisms but as well their involvement in environmental processes, and defining their role in the ecology of other species, does represent for many of us the Mount Everest of science. Research in this area of biology dates to the original discovery of microorganisms by Antonie van Leeuwenhoek, when in 1675 and 1676 he used a microscope of his own creation to view what he termed "animalcula," or the "little animals" which lived and replicated in environmental samples of rainwater, well water, seawater, and water from snow melt. van Leeuwenhoek maintained those environmental samples in his house and observed that the types and relative concentrations of organisms present in his samples changed and fluctuated with respect to time. During the intervening centuries we have expanded our collective knowledge of these subjects which we now term to be environmental microbiology, but easily still recognize that many of the individual topics we have come to better understand and characterize initially were described by van Leeuwenhoek. van Leeuwenhoek was a draper by profession and fortunately for us his academic interests as a hobbyist went far beyond his professional challenges.

It is the goal of this series to present a broadly encompassing perspective regarding the principles of environmental microbiology and general microbial ecology. I am not sure whether Antonie van Leeuwenhoek could have foreseen where his discoveries have led, to the diversity of environmental microbiology subjects that we now study and the wealth of knowledge that we have accumulated. However, just as I always have enjoyed reading his account of environmental microorganisms, I feel that he would enjoy our efforts through this series to summarize what we have learned. I wonder, too, what the microbiologists of still future centuries would think of our efforts in comparison with those now unimaginable discoveries which they will have achieved. While we study the many wonders of microbiology, we also

further our recognition that the microbes are our biological critics, and in the end they undoubtedly will have the final word regarding life on this planet.

Christon J. Hurst in Heidelberg

Indebted with gratitude, I wish to thank the numerous scientists whose collaborative efforts will be creating this series and those giants in microbiology upon whose shoulders we have stood, for we could not accomplish this goal without the advantage that those giants have afforded us. The confidence and very positive encouragement of the editorial staff at Springer DE has been appreciated tremendously and it is through their help that my colleagues and I are able to present this book series to you, our audience.

Cincinnati, OH Christon J. Hurst

Volume Preface

If the world suddenly were to be without its microbes, then none of the plants and animals which we perceive as comprising higher levels of life in our ecosystem could survive. This book presents a summary of knowledge regarding the natural terrestrial microbial processes which represent a key component of maintaining healthy life on our planet. The authors begin by explaining how microorganisms sustain the soil ecosystem through a recycling of carbon and nitrogen. That basic knowledge is followed by chapters which describe integration of soil microbiology processes into ecosystem science, usage of natural processes to achieve successful bioremediation including the accomplishment of safe and effective landfill operation, and design of composting processes which can help us to reduce the amount of wastes that we place into landfills. This book also presents an understanding of how human land usage patterns, including restoration efforts, affect soil microbial communities and how wetland microbial communities respond to anthropogenic pollutants. The book concludes with an understanding that many of the fungi which function by environmentally recycling the carbon and nitrogen of organic materials do sometimes begin their degradative action too soon, with the result being infectious diseases that are destructive of plants and can injure or even kill vertebrate species.

I am tremendously grateful to Andrea Schlitzberger, Markus Spaeth, and Isabel Ullmann at Springer DE, for their help and constant encouragement which has enabled myself and the other authors to achieve publication of this collaborative project.

Cincinnati, OH — Christon J. Hurst

Contents

Chapter 1
Carbon Cycle Implications of Soil Microbial Interactions

Kelly I. Ramin and Steven D. Allison

Abstract The soil environment contains the largest pool of organic carbon on the Earth's surface, with soil carbon residency and flux controlled by microbial metabolism. Despite the fact that microbial interactions have metabolic implications, these interactions are often overlooked in conceptual models of the soil carbon cycle. Here, we hypothesize that microbial interactions are intrinsically coupled to carbon cycling through eco-evolutionary principles. Interactions drive phenotypic responses that result in allocation pattern shifts and changes in carbon use efficiency. These changes promote alterations in resource availability and community structure, thereby creating selective pressures that contribute to diffuse evolutionary mechanisms. The outcomes then feed back into microbial metabolic operations with consequences for carbon turnover, continuing a feedback loop of microbial interactions, evolutionary processes, and the carbon cycle.

1.1 Introduction

Soil holds the largest store of carbon on Earth, estimated to be >2300 Pg C (Jobbágy and Jackson 2000). Flux rates of carbon from the soil exceed anthropogenic emissions by up to ten times yearly (Chapin et al. 2002). Owing to the scale of soil carbon inputs into the atmosphere, and major concerns over human disruption of the global carbon cycle, it is important to understand the drivers of the soil carbon flux. Because microbes are responsible for the degradation and transformation of organic matter, soil carbon cycling is dependent upon microbial metabolism (Falkowski et al. 2008). Yet microbial processes that govern the turnover of carbon in the soil are not fully understood (Prosser 2012).

Microbial processes have been difficult to study owing to the microscale at which they take place, the spatial and temporal fluctuation of conditions in the soil, and the incredible diversity of interacting organisms and abiotic parameters. With advancements in molecular tools, the diversity of the soil biota and its associated carbon

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cycling potential have become more resolved. Many stressors in the soil environment have been explored for their impact on carbon cycling. Yet less attention has focused on how microbial interactions influence the evolution and phenotypic expression of microbial traits that affect carbon cycling in the soil environment. This chapter will therefore discuss the impact of microbial interactions on traits involved with carbon cycling.

For the purposes of this chapter, interactions will be defined as processes driven by one microbe that have either positive or negative effects on survival or reproduction of one or more other microbes. We will focus on interactions that influence phenotypic expression and genotypic capacity of traits with consequences for carbon cycling. We propose that microbial interactions act as pressures that result in changing the cellular allocation of resources underlying these processes. These pressures alter fitness cost/benefit ratios and ultimately impact carbon cycling.

This chapter also aims to address how microbial interactions influence community structure. Community structure may be important to carbon cycling if organisms show inter-taxa variation in their capacity for carbon cycling and if the breakdown of carbon is limited by cellular processes (Schimel and Schaeffer 2012). There is extensive evidence that changes in microbial community structure have impacts on carbon turnover (Balser and Firestone 2005; Matulich and Martiny 2014). More broadly, changes in diversity are often linked to altered functioning (Tilman et al. 2001; Bell et al. 2005). Interactions that alter diversity at the microsite, such as niche partitioning, or prevention of competitive exclusion, such as non-transitive interaction networks and negative frequency-dependent selection, therefore, will likely have effects on community carbon cycling (Cordero and Datta 2016).

Microbial interaction networks therefore cannot be decoupled from the soil carbon cycle. The purpose of this chapter is to explore the implications of microbial interactions in soil carbon cycling (Fig. 1.1). We hypothesize that changes in allocation patterns resulting from interactions will lead to both ecological and evolutionary consequences for carbon cycling. Furthermore, we hypothesize that microbial interactions have important ramifications for community structure that feed into associated community functioning. While these metabolic constraints on carbon transformation and shifts in allocation that change the fate of carbon may take place at the microsite, evidence suggests that microbial metabolic processes collectively scale up and contribute to carbon cycling at the ecosystem level (Brown et al. 2004; Elser 2006; Sinsabaugh et al. 2015). Therefore, the effect of microbial interactions on soil carbon flux potentially has relevance across multiple spatial and temporal scales, including the global scale over decades to centuries.

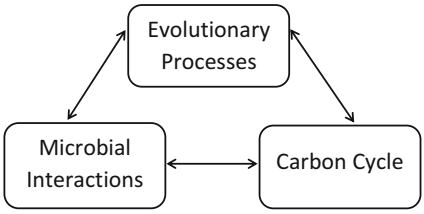

Fig. 1.1 A conceptual diagram of the feedback between microbial interactions, evolutionary processes, and the carbon cycle

1.2 Allocation Patterns

Microbial growth has been shown to drive soil organic matter (SOM) decomposition, indicating that metabolic mechanisms that impact growth rate have a large influence on soil carbon dynamics (Neill and Gignoux 2006). While growth rate is partly determined by rRNA copy number, or codon usage bias (Vieira-Silva and Rocha 2010; Stevenson and Schmidt 2004; Goldfarb et al. 2011), carbon use efficiency (CUE) is phenotypically variable and depends upon maintenance costs. As a metric, CUE defines the amount of growth achieved per unit of acquired carbon and may be an important control on carbon sequestration in soil (Allison et al. 2010; Bradford and Crowther 2013). Maximum possible microbial CUE has been estimated at approximately 60% of acquired carbon being assimilated into biomass or ATP but declines with growing maintenance costs (Schmidt and Konopka 2009). Maintenance costs vary with conditions and may increase with temperature, nutrient limitation, starvation, physiological stress, allocation to storage, extracellular products, and transporters (Lipson 2015; Matsumoto et al. 2013).

Microbes often face competition for limited resources in the soil environment. The investment in acquiring resources, part of cellular maintenance costs, generally lowers the overall metabolic efficiency of the cell (Teixeira De Mattos and Neijssel 1997). The phenotypic response of microbes living in resource-limited conditions includes synthesis of enzymes that acquire limiting resources to maximize uptake rates, synthesis of enzymes targeting alternative forms of the limiting resources, a decrease in anabolism to match the uptake of the limiting resources, and use of storage polymers to compensate resource deficiencies (Harder and Dijkhuizen 1983; Schmidt and Konopka 2009).

Metabolic theory posits that thermodynamics define absolute constraints on the uptake, transformation, and secretion of energy and matter, as well as the rates of these processes (Brown et al. 2004). These controls over energy and matter fluxes also dictate ecological interactions among organisms by defining a bacterium's ability to grow, produce molecules that impact surrounding bacteria, respond to declining resources, or counter chemical attacks. The cellular response to interactions may lead to a shift in allocation of resources that impacts the rate of carbon turnover and its ultimate fate in the soil environment. These metabolic interactions influence what percentage of acquired carbon is transformed and immediately released into the atmosphere, converted to biomass or extracellular products, or stored as recalcitrant compounds in the soil.

Many of the effector molecules associated with maintenance costs are proteins. Protein production requires the greatest amount of energy and resources of all microbial processes (Koch 1985). Even under optimal conditions, maximum growth rate is limited by macromolecular synthesis, energy production, and transport of molecules, all processes driven by proteins. Therefore, allocation of resources toward nongrowth protein synthesis represents a decrease in fitness (Chubukov et al. 2014). This burden creates a strong selective pressure for microbes to reduce nonessential protein production.

In addition to the increase in resource-acquiring mechanisms, microbes in the soil alter their growth rates and production levels of other potentially costly molecules in response to interactions. Toxins attack predators and competitors for nutrients. Defense systems respond to interspecific assaults. Biofilm polymeric substances protect microbes against desiccation and antibiotics while slowing diffusion of nutrients away from the producing cells. Siderophores chelate iron to make it bioavailable. Production of these may also represent a decrease in fitness for a microbe.

1.2.1 Interaction-Mediated Phenotypic Plasticity

Phenotypic plasticity is beneficial in highly heterogeneous environments, allowing microbes to adjust their response to a range of conditions. This has the potential to ameliorate the severity of circumstances causing negative fitness effects for the microbe on a short-term scale. Phenotypic plasticity arguably carries costs with its maintenance, though. Evolutionary biologists have analyzed the costs and limits on phenotypic plasticity (DeWitt et al. 1998), as well as constraints on the evolution of plasticity. A loss of plasticity may be due to accumulation of mutations or loss of genes if their products are unused or being produced by other community members (Murren et al. 2015). Multiple studies have found loss of core metabolic genes in obligate symbiotic, parasitic, or commensal microbes. In contrast, some free-living microbes have streamlined their genomes by maintaining core functional genes while reducing the relative amount of intergenic spacer DNA and number of paralogous genes (Giovannoni et al. 2014; Solden et al. 2016). Microbes must balance their capacity for plasticity with the burden of DNA replication, immediate ecological and environmental pressures, and availability of genetic material through horizontal gene transfer (HGT).

1.2.1.1 Interaction Agents in the Soil Environment

Interaction-induced phenotypic alterations are often initiated via direct contact, metabolic by-products, or diffusible autoinducer molecules that interact with regulatory pathways, such as quorum signals, volatile organic compounds (VOCs), or even toxins (Effmert et al. 2012; Decho et al. 2011; Davies et al. 2006; Straight and Kolter 2009). Multiple studies have shown coordinated phenotypic responses to environmental or competitive stressors within and between populations (Challis and Hopwood 2003; Rigali et al. 2008). When this occurs, autoinducers are considered signals. In some cases, however, phenotypic responses are induced that are not part of an effort to enact a cooperative, coordinated response. For example, it is possible that some autoinducer producers may force metabolic changes in other microbes for their own benefit, which is termed coercion. Some microbes appear to have evolved the capacity for “cross talk” or the ability to eavesdrop on heterospecific

autoinducers in the surrounding environment. These autoinducers are known as cues (Traxler and Kolter 2015; Netzker et al. 2015; Federle and Bassler 2003; Diggle et al. 2007a, b).

Microbial interactions may act to alter the expression of various traits that have implications in carbon cycling, such as growth rate and production of extracellular products. The production of many exoproducts is temporally and spatially modulated through intercellular signals within and between populations (Diggle et al. 2007a, b; Huang et al. 2013; Strickland et al. 2013), as may be differentiation and predatory behavior (Straight et al. 2006; Müller et al. 2014; Schuster et al. 2003). Autoinducers are also involved in efficiency sensing: detection of diffusion rates to optimize production amounts of extracellular products (Hense et al. 2007). The impact of autoinducers on fitness for an individual microbe in relation to its community, through both competition and cooperation, confers a level of importance that is reflected in the capacity for a wide diversity of genes for signals found in many microbes (Challis and Hopwood 2003; Krug et al. 2008; Schuster et al. 2003). Furthermore, as mediators of interactions that result in altered expression of functional traits, autoinducers are fundamental to ecosystem function (Seneviratne 2015; Zhuang et al. 2013).

Autoinducer efficacy and persistence in the soil environment are affected by the size and adsorption properties of the autoinducer molecules and may be altered by pH and the ratio of clay to organic material (Traxler and Kolter 2015; Subbiah et al. 2011; Lv et al. 2013). Mineral soil is comprised of approximately 50% air- and water-filled pores, which are temporally and spatially dynamic (O'Donnell et al. 2007). This creates a high surface area within the soil matrix, on which many soil microbes form biofilms. Biofilms alter the autoinducer potential of a community through changes in diffusion rates, redox gradients, and pH (Stewart 2003; Decho et al. 2011). Additionally, some microbes produce degrading enzymes, agonists, and antagonists of autoinducer molecules (Wang and Leadbetter 2005; Xavier and Bassler 2005). Not only do these compounds serve to manipulate microbial interactions, but some of the degraded products may form new carbon and nutrient sources and act as antimicrobial compounds or iron chelators (Leadbetter and Greenberg 2000; Kalia 2013).

Another direct mechanism that may force interspecific changes in microbial phenotype, and hence shifts in resource allocation, is contact-dependent inhibition (CDI) (Ruhe et al. 2013; Blanchard et al. 2014). This not only causes shifts in resource allocation and a decrease in growth for the CDI-producing cell but also decreases in growth or death of the recipient. This mechanism requires close proximity for action, conditions that arise in soil microbial biofilms.

Finally, microbes may cause changes to neighboring cells' phenotypes through indirect agents. Metabolic by-products can change the local abiotic conditions, such as pH, creating stressful conditions and altering metabolic efficiency of neighbors. Likewise, metabolic by-products can alter efficiency as newly available resources that benefit neighbors through cross-feeding.

1.2.1.2 Soil Biofilms

Biofilm formation is important to many soil microbes for survival. It offers protection against several soil environment stressors such as predation, desiccation, and toxin exposure (Matz and Kjelleberg 2005; Mah and O'Toole 2001; Roberson and Firestone 1992; Jefferson 2004). The prevalence of biofilm formation among bacteria, estimated to be at 99% of taxa, supplies evolutionary evidence of life in biofilm as an important adaptation. Though fungi, algae, protozoa, and yeast also grow in biofilms alongside bacteria, the primary focus in research of biofilms has been on bacteria (Jass et al. 2002; Vu et al. 2009). Regardless of taxonomic identity, biofilms establish conditions that alter contact between microbes by immobilizing the biofilm cells next to each other, forming barriers to inhibit interactions, or altering diffusion rates of extracellular molecules.

The exact composition of biofilms varies widely but contains polysaccharides, proteins, lipids, nucleic acids, and other biopolymers such as humic substances, along with the resident microbes. While some of the matrix can be easily degraded as a nutrient source, humic substances are resistant to degradation, contributing to long-term soil carbon stocks (Flemming and Wingender 2010). The combined, three-dimensional matrix of molecules is broadly termed "extracellular polymeric substances," or EPS. Each species of bacteria produces a distinct set of polysaccharides and proteins for their respective EPS, which is integrated into multispecies biofilms (Vu et al. 2009). Biofilm matrix architecture varies widely based on EPS molecular structure and environmental conditions, with the different architectures impacting important physical parameters of microbial existence, such as diffusion gradients (Flemming and Wingender 2010). The dramatic change in phenotype that accompanies the transition to a sedentary lifestyle within a biofilm makes it difficult to isolate the changes in cellular efficiency or changes in allocation of resources due to production of EPS. However, initial colonization is marked by high production of metabolically expensive carbon compounds and proteins, so an immediate reduction in growth might be expected. In fact, a decline in growth has been observed in some cases (Burmolle et al. 2014; Mah and O'Toole 2001).

The transition from a planktonic lifestyle to a biofilm is accomplished through multiple changes in gene expression. Many of the differentially expressed genes associated with the transition from planktonic to biofilm life code for metabolic function and starvation responses (Stewart 2003; Jefferson 2004; Donlan 2002; Booth et al. 2011; Sauer and Camper 2001; Prigent-combaret et al. 1999). These changes in gene expression can be initiated by environmental cues but have also been observed to be engendered through intercellular autoinducers (Parsek and Greenberg 2005; Jefferson 2004). For example, Lopez et al. (2009) found that a diverse set of natural molecules that cause potassium leakage by temporarily creating membrane pores in *Bacillus subtilis* were responsible for inducing biofilm formation. These molecules are produced by other strains as well as *B. subtilis* itself. They proposed that a membrane receptor was likely able to detect lowered intracellular

concentrations of potassium and initiate a transcriptional response leading to biofilm production.

Though the specific interacting molecules were not always determined, several other studies have shown either induction or an increase of biofilm formation in strains of bacteria grown together versus when grown in monocultures (Burmolle et al. 2007; Bleich et al. 2015; Shank et al. 2011), whereas other studies have found inhibition of biofilm production (Powers et al. 2015). Monoculture biofilm formation may be a cooperative mechanism (West et al. 2007); however, induction of biofilm production by heterospecific strains could also mean that biofilm formation is a defensive or coercive strategy.

Through the progressive stages of development of a biofilm, colonizers transform their created biofilm environment through cell autoinducers, waste products, and degradation of soil organic matter (SOM) (Stewart 2003). This transformation creates microenvironments that magnify spatial and temporal heterogeneity within the biofilm due to restricted diffusion, leading to changes in microbial phenotype relative to available resources and interacting organisms (Stewart and Franklin 2008). Some microbial processes also have bistable switches that respond to intercellular autoinducers that may affect the phenotypic heterogeneity displayed within a mature biofilm (Chai et al. 2008; Dubnau and Losick 2006). These mechanisms that increase heterogeneity may lead to an increase in community- or population-level efficiency through specialization in tasks and reduction of the unicellular burden of enzyme production, or a reduction in the waste of resources through cross-feeding, and may act to alter soil carbon turnover rates (Folse and Allison 2012; Jefferson 2004; Bernstein et al. 2012; Ackermann 2015; Huang et al. 2013).

The physical structure of EPS in the soil affects microbial processes and interactions by affecting diffusion rates. As the amount of EPS accumulates, diffusion rates of oxygen, nutrients, and waste products decrease, creating conditions that might decrease growth rates through nutrient limitation, triggering of a stress response, and transition of metabolism to inherently less efficient anaerobic respiration or fermentation (Stewart 2003; Mah and O'Toole 2001; Prigent-combaret et al. 1999). Thus, it is possible that conditions generated through biofilm structural and chemical differentiation created by indirect microbial interactions lead to lower metabolic efficiency. Likewise, the stress response that has been noted in biofilms represents a shift toward allocation of resources to maintenance (Schimel et al. 2007).

Alternatively, decreased diffusion associated with the EPS matrix may benefit microbes. Extracellular products that are available to and benefit all members of a community—or public goods—such as enzymes, quorum molecules, and siderophores, remain closer to the producing cell, increasing its return on investment (Burmolle et al. 2014; Flemming and Wingender 2010). Because restricted diffusion effectively lowers the productive need of these molecules, it may allow the producing cells to devote more of their resources toward growth, improving metabolic efficiency and biomass accumulation. One study showed that 63% of four-species biofilm-producing consortia synergistically increased biofilm production relative to strains grown independently in the lab (Ren et al. 2014). The highest-producing four-

species consortia contained a dominant biofilm producer, *Xanthomonas retroflexus*; however, all of the interacting species in that group increased in both biofilm production and relative cell number compared to monoculture biofilms. Only 2 of the 35 combinations of 4-species consortia showed decreased biofilm production relative to monocultures.

1.2.1.3 Growth and Dormancy

Interactions among microbes, whether positive or negative and direct or indirect, have the potential to affect growth and soil carbon cycling. Exploitation competition between microbes is indirect and involves depletion of a common limiting resource, with the winner having a higher capacity for resource acquisition. Higher resource acquisition increases growth rate, effectively starving the loser of resources. An evolutionary focus on this strategy may only be successful when resources are available (Stevenson and Schmidt 2004; Goldfarb et al. 2011; Moorhead and Sinsabaugh 2006). Given the highly variable availability of resources, it is unsurprising that the soil environment hosts a wide diversity of microbial growth strategies, beyond the simple dichotomy of copiotrophs and oligotrophs (Ernebjerg and Kishony 2012; Vieira-Silva and Rocha 2010). Yet the ability of microbes to maintain relatively high growth rates down to nanomolar or micromolar concentrations of substrate due to the maximization of uptake suggests a strong selective advantage for exploitative competition (Schmidt and Konopka 2009).

Indeed, some bacteria may have evolved measures to manipulate their growth rate as a competitive measure. By switching to a high-growth rate low-yield strategy, bacteria disproportionately acquire available resources even though their metabolic efficiency declines (Pfeiffer et al. 2001; Lipson 2015). While this low-yield strategy might not immediately improve fitness, it functions to decrease fitness of competitors by reducing resources available for their growth. This strategy has the effect of increasing carbon turnover and flux but is only beneficial under conditions with high rates of resource diffusion (Lipson 2015). Therefore, this mechanism would likely only occur at the surface of biofilms where high diffusion rates of oxygen and resources take place.

Additionally, interference competition, in which competitors directly and aggressively fight over resources, often supports exploitative efforts. Some microbes may respond to nutrient stress, which is associated with exploitative competition, by slowing growth and producing growth inhibitory antibiotics (Rigali et al. 2008; Cornforth and Foster 2013; Garbeva and de Boer 2009). This slowed growth may accompany an allocation toward cellular maintenance costs of antibiotic production, but it has also been proposed that the slowed growth is a preemptive protective measure against antibiotic attacks (Mah and O'Toole 2001). The reason why slowed growth imparts protection is unclear. However, because resistance to antibiotics may also carry a fitness cost, the slowed growth could be associated with this shift in allocation away from growth and toward resistance (Andersson and Levin 1999; Andersson and Hughes 2010; Dykes and Hastings 1998). Garbeva et al. (2011)

found differential regulation of ribosomal protein and stress response genes along with induction of antibiotic production, suggesting that slowed growth is partly due to a cellular stress response. Slowed growth may also be caused by production of coercive molecules to suppress antibiotic production in a neighboring cell or to trigger antibiotic production in a third cell that is forced into the role of bodyguard (Tyc et al. 2014; Abrudan et al. 2015; Galet et al. 2014). Given the fitness cost of production of some growth inhibitory molecules, it is surprising that one study found 33% of soil bacteria constitutively produce antibiotics, lending credence to the hypothesis that antibiotics may also serve as autoinducers (Tyc et al. 2014).

Dormancy or a reduced metabolic state will have indirect fitness consequences for a population by freeing up for their kin the resources that microbes otherwise would have consumed for themselves (Ratcliff et al. 2013). These microbes may be the persister cells noted in biofilms that are more inclined to switch into a dormant or reduced metabolic state (Stewart and Franklin 2008). In the soil environment, approximately 80% of all bacteria are in a dormant state (Lennon and Jones 2011). Though the reduced metabolic state is energetically prudent, the cost of going into this state is not zero. Multiple metabolic processes must first prepare for cellular shutdown, including production of machinery to go into and out of dormancy, as well as resting structures (Lennon and Jones 2011). Ultimately, microbial interactions affect the rate at which neighboring microbes transition into a dormant state, either through exploitation or kin selection, thus altering soil carbon turnover rates.

1.3 Evolution of Traits with Carbon Cycling Consequences

Studies of social evolution are often performed using microbes due to their relative simplicity. Even though laboratory experiments often cannot specifically prove that the evolutionary response to selective pressures in the experiment is solely due to the interaction and therefore social behaviors, these experiments inform about potential mechanisms that may occur through interactions and, as such, are important to begin understanding how evolution impacts carbon cycling (Rainey et al. 2014). Social behaviors have fitness effects for both the actor and the recipient. Cooperative behaviors can be mutually beneficial, in which both the actor and recipient receive positive fitness results, or altruistic, in which the actor does not. Likewise, competitive behaviors are broken down into selfish, with the actor receiving a fitness benefit while the recipient is harmed, or spiteful, with both being harmed (Hamilton 1964). Natural selection acts on genetic variation, often a single, specific locus in microbes (Mitri and Foster 2013). For many social evolutionary mechanisms, relatedness is determined at one specific gene, such as for a public good or toxin (Table 1.1).

Pressures that shift the cellular balance away from reproduction, such as those that occur through microbial interactions, act as selective forces that may have implications for carbon cycling. The higher the incurred cost to fitness and the longer it occurs, the more likely a change in allocation will lead to evolutionary changes. Presumably, costly traits, such as production of extracellular goods, will be

Table 1.1 Potential effects of microbial interactions on soil carbon cycling

Interaction type	Effect upon soil carbon storage	Potential mechanisms
Exploitation	−	Rate of SOM degradation increases with increasing growth of exploiting population
Decrease in CUE	−	Reduction in biomass accumulation and increasing amount of carbon released as CO_2
Toxin production	±	Metabolic production costs may decrease carbon storage but growth inhibition might increase it Reduction in niche overlap may contribute to increased SOM degradation
Signal degradation	+	The targeted population will be unable to function cohesively in SOM degradation
Coercion	±	Effects are dependent upon what action is being coerced
Dormancy	+	Reduces total SOM degradation if dormancy caused by stressors other than nutrient limitation
Cross-feeding	−	Rate of SOM degradation increases, but yield may decrease
Syntrophy	−	Streamlines metabolic processes and facilitates SOM degradation in anoxic environments
Siderophore cheating	±	May increase or decrease SOM degradation depending on the relative metabolic costs of siderophore production and growth rates of the cheater and producer
Enzyme cheating and Black Queens	+	Reduction of degradation of SOM by lowering total enzyme production
Biofilm cheating	±	Increased carbon allocation to EPS and humic substances increase storage though associated production costs may cause greater CO_2 flux
Soil pore formation	−	Facilitates access to SOM, oxygen, and water resulting in increased degradation

maintained if the benefit outweighs the fitness cost. Benefits to a producing cell may be direct, as is the case with enzymes that scavenge high-energy resources, or indirect, such as a reduction in competition for resources.

Conversely, costly traits may be maintained if the cost of loss increases, as occurs with enforcement tactics carried on mobile genetic elements (MGEs).

1.3.1 Horizontal Gene Transfer

Many of the genes responsible for microbial interactions and carbon cycling are part of the accessory genome, which constitutes upwards of 90% of a bacterial taxon's pan-genome (Touchon et al. 2009; Haq et al. 2014; Rankin et al. 2011). The accessory genome—those genes contained within a microbe that are shared through

HGT via mobile genetic elements (MGEs) such as transposons, bacteriophages, and plasmids—predominantly codes for secreted proteins but can also encode metabolic traits and pathways (Falkowski et al. 2008; Ochman et al. 2000; Nogueira et al. 2012). The more complex pathways may be difficult to transfer, however, because of their multigene nature and incongruity with preexisting pathways (Schimel and Schaeffer 2012). This has likely led to the deeply conserved nature of these large metabolic units (Martiny et al. 2015).

Transmission of MGEs increases at higher cellular densities (Sorensen et al. 2005; McGinty et al. 2013). Biofilms promote HGT by creating a matrix for microbes to interact closely for conjugation, maintaining the naked DNA of lysed cells in proximity to the biofilm's residents for transformation, and even potentially facilitating viral infection for transduction (Donlan 2002; Flemming and Wingender 2010; Hausner and Wuertz 1999; Burmolle et al. 2014; Sorensen et al. 2005; Molin and Tolker-Nielsen 2003). Because of this, it is likely that plasmids and bacteriophages have incorporated genes that facilitate biofilm formation to ensure their own propagation (Jefferson 2004; Madsen et al. 2012). Therefore, the biofilm acts as a reservoir of genetic information, allowing rapid adaptation to fluctuating conditions, and redefinition of an ecological niche (Haq et al. 2014; Norman et al. 2009).

Because many of the genes carried on MGEs code for public goods that are secreted from the cell, the potential loss of public goods by diffusion implicitly increases the cost of production to the cell and likelihood of gene ejection. As is the case with whole organisms, MGE success depends upon propagation. To resolve a potential conflict of survival between the host and the MGE, an evolutionary compromise has been observed in which the biosynthetic cost of secreted and outer membrane proteins is often lower than those for purposes elsewhere in the cell, improving the likelihood of the MGE maintenance within the cell (Nogueira et al. 2009; Smith and Chapman 2010).

It is important to consider that MGEs have also evolved mechanisms of forced maintenance. These mechanisms impact interactions between microbes as well as metabolic efficiency through shifts in allocation of resources toward fabrication of MGE products. For example, addiction complexes contain a toxin-antitoxin complex, with the antitoxin degrading more rapidly than the toxin (Zhang et al. 2012). Because the toxin remains in effect for a longer period than the antitoxin, the cell loses immunity upon loss of the MGE, and fitness lowers to zero.

Through MGEs a picture emerges of how function and interactions feed into one another. Microbes create biofilms that favor HGT, and MGEs contain traits that impact neighboring cells. The toxin-antitoxin complexes force production of their products while killing local cells that have not acquired the same complex. Depending on what other genes might be carried with these complexes, this may also have a large impact on the production of public goods that are involved in carbon cycling or sequestration. Even without a toxin-antitoxin complex, the associated increase of relatedness involved with HGT creates a dynamic of kin selection that promotes production of public goods encoded on MGEs (McGinty et al. 2013). Despite this immediate and localized increase in relatedness, HGT is thought to

contribute to the larger process of speciation (Boto 2010). In fact, genes associated with secreted proteins have been found to evolve at a relatively high rate (Nogueira et al. 2012), which may have more downstream effects on carbon cycling as discussed in the continuing sections.

1.3.2 Cheaters

Cheating is an evolutionary strategy that either eliminates the cost of production of a public good for the cheater while using the goods produced by others or disproportionately increases access to a limiting resource for the cheating microbe. The success of any cheating strategy is density dependent, as a competitive strategy only has benefits insomuch that it is distinct among its competitors (West et al. 2007; Ross-Gillespie et al. 2007). It also depends upon diffusion rates, spatial structure, and available resources. Despite the population-level benefit of cooperative public good production, cheating is a strategy that commonly arises (Allison et al. 2014; Darch et al. 2012; Kim et al. 2014). Multiple mechanisms exist to buffer populations against cheaters, including those associated with MGE maintenance, but the rapid generation time of microbes combined with the relatively high evolvability of genes for secreted products suggests that cheating mutations may occur often (Travisano and Velicer 2004; Diggle et al. 2007a, b; Popat et al. 2015).

Cheating with EPS production in biofilms alters allocation of resources, metabolic efficiency, and growth through an increase in production of EPS. Cells at the surface of a biofilm experience higher resource and oxygen levels. Cheaters have arisen with an increased ability to produce biofilm compounds, effectively pushing themselves to the surface of the biofilm to acquire more of these resources while suffocating the wild-type strain (Xavier and Foster 2007; Kim et al. 2014). This allocation to biofilm polymers, however, comes at the expense of reproduction as indicated by lower density of cheater cells compared to wild-type cells. Genetic analysis confirms that increased competitive ability was not achieved through faster growth but through increased biofilm polymer production (Kim et al. 2014).

Because microbial growth is positively correlated with SOM degradation, this competitive interaction, resulting in a decreased growth rate, may represent slowed carbon turnover and lower relative biomass. Depending upon the molecular composition of the produced EPS, more resistant forms of soil carbon may be formed. However, the increased allocation of resources toward production of EPS may be associated with decreased metabolic efficiency and consequently a greater proportion of acquired carbon being respired.

When members of a population are producing the same public goods, it is evolutionarily expedient for an individual microbe to evolve a loss of production. Because the cheater is still being provided with communal public goods, the loss of function represents an increase in fitness and has a positive competitive effect against surrounding producers. An example of public goods commonly involved in cheating is siderophores. Because soil is often an aerobic environment, bioavailability of iron

is limited. Siderophores chelate iron, an important element involved in many metabolic pathways. Many species of bacteria and fungi produce multiple types of siderophores and their receptors, some that are more metabolically costly than others (Dumas et al. 2013). It is common for cheaters to arise that pirate xenosiderophores, which are siderophores produced by other species. Cheating is done by expressing xenosiderophore receptors that are likely acquired through HGT (Cornelis and Bodilis 2009). There is often a concomitant reduction in the production of endogenous siderophores and a subsequent increase in fitness (Galet et al. 2015; Miethke et al. 2013). Traxler et al. (2012) found that siderophore piracy can be used to reduce the growth of competitors, as well. In their experiment, *Amycolatopsis* sp. AA4 arrested development of *Streptomyces coelicolor* through manipulation of iron availability via siderophore production and then through piracy of *S. coelicolor* siderophores. The shifts in allocation of resources through siderophore cheating lead to changes in metabolic efficiency and growth rate, thereby altering the fate of carbon in the soil.

Extracellular enzymes are another public good that are subject to cheating. Enzyme cheating potentially has a high impact on soil carbon turnover because enzymes are the proximate agents by which microbes access SOM and begin degradation and subsequent mineralization of carbon. Some extracellular enzymes can also contribute to production of recalcitrant SOM in the soil (Burns et al. 2013), affecting the amount of carbon that is sequestered. As with siderophores and EPS, their production costs are relatively high, potentially resulting in reduced fitness through allocation of carbon and energy toward enzymes and a lowered metabolic efficiency. This cost is a trade-off with the increase in fitness resulting from higher resource acquisition due to enzyme activity (Allison et al. 2011).

Enzyme cheaters have been shown to benefit from high diffusion rates, especially as the cost of production rises (Allison 2005; Allison et al. 2014; Folse and Allison 2012). In biofilms, where a majority of microbes grow in the soil, loss of enzymes through diffusion will be partially mitigated by the EPS matrix. This decreases overall cheater success because enzymes remain localized to the producers and because producers form patches that exclude cheaters (Allison 2005; Allison et al. 2014). Likewise, low nutrient concentrations allow producers to form insulated patches against cheaters (Mitri et al. 2011; Nadell et al. 2010, 2016).

1.3.3 Black Queen, Cross-Feeding, and Syntrophy

With higher percentages of cheaters, a public good supply is reduced, causing a decline in both producers and cheaters. However, in nutrient-limited environments that impose slowed growth rates, such as mature biofilms, the relative number of cheaters in communities might be more likely to stabilize (Morris et al. 2012). The Black Queen Hypothesis stipulates that the stabilization of cheaters in a population of public good producers occurs when producers are forced to be helpers, individuals that collectively make a minimum required amount of a vital public good that can sustain both populations, thereby ensuring their own survival (Morris et al. 2012).

This requires close proximity of the producers and cheaters. Furthermore, Morris (2015) suggests that, over time, cheating may arise in helpers for other public good traits that the first cheater still produces, creating an auxotrophy.

Stabilization of a mutual auxotrophy was shown in an experiment in which *Escherichia coli* strains were grown together that had null mutations for different amino acid production pathways (Pande et al. 2014). The cross-feeding mechanism created a division-of-labor fitness advantage over the ancestral strain that was stabilized through negative frequency-dependent selection for the pair of metabolic dependents. The benefit received by auxotrophic microbes is reflected in a recent analysis that shows the widespread nature of this strategy (Solden et al. 2016).

In the case of enzymes, decreasing production, either through cheating or as mutual auxotrophy, may slow carbon turnover in the soil by decreasing the relative amount of active enzymes to break down carbon substrates relative to the number of individuals in the community (Folse and Allison 2012; Oliveira et al. 2014). Alternatively, cheating may facilitate a transition to a more efficient community metabolism that frees cellular resources to be allocated toward growth. This may increase total carbon consumption in the community and the rate of carbon turnover (Pande et al. 2014).

Cross-feeding interactions have arisen through optimization of metabolic pathway length (Pfeiffer and Bonhoeffer 2004; Costa et al. 2006). Cross-feeding is pervasive in microbial communities and is often associated with metabolic traits whose expression can be altered dependent upon the surrounding circumstances (Ponomarova and Patil 2015). Production of ATP in metabolic pathways involves a stepwise series of multiple enzymes. These enzymes are energetically expensive and resource intensive. By eliminating some of the ATP-generating steps, an overflow metabolism occurs, in which the resource is only partially oxidized and then secreted from the cell (Teixeira De Mattos and Neijssel 1997). This increases the ATP production rate, though at the expense of yield (Pfeiffer and Bonhoeffer 2004). In microbes adapted to resource limitation, overflow metabolism occurs with excess carbon supplies. The partially oxidized compound is then used by another microbial community member.

More complex cross-feeding patterns have been found in nature. These syntrophies, or "obligate mutualistic metabolisms," are broadly described as a service mutualism, in which one species provides a chemical resource in exchange for a benefit from its interacting partner, such as removal of a secreted waste product (Harcombe 2010; Bull and Harcombe 2009). Syntrophies are largely anaerobic processes that are beneficial to the participants because they shift the metabolic reactions toward thermodynamic favorability for the producing cell while providing resources for the recipient (Morris et al. 2013; McInerney et al. 2008). Biofilms can function to keep interacting partners in close proximity (Little et al. 2008), ultimately increasing carbon turnover in mature biofilms with anoxic regions.

1.4 Community Structure

Diversity-function relationships generally show a positive, asymptotically saturating relationship between species richness and ecosystem function (Tilman et al. 2001; Bell et al. 2005; Tiunov and Scheu 2005; Langenheder et al. 2010). The saturation point is likely related to redundancy of functional traits that are held within a community (Allison and Martiny 2008). Conversely, there are contrasting results of a "negative complementarity effect." This decreasing function with increasing diversity has been hypothesized to be caused by competitive interactions (Becker et al. 2012; Jousset et al. 2011; Van der Wal et al. 2013; Szczepaniak et al. 2015). These conflicting patterns with increasing species diversity indicate that a community's composition may have an impact on its overall function.

Mouillot et al. (2011) showed that functional diversity, rather than taxonomic diversity, was more predictive of ecosystem multifunctionality, with a few specialist species contributing disproportionately to primary production and degradation. However, complex, multigene traits are more deeply conserved than simple traits like the ability to utilize a simple carbon substrate (Martiny et al. 2013, 2015; Zimmerman et al. 2013; Berlemont and Martiny 2013). Despite the rampant nature of HGT, it appears as though even simple traits are not distributed completely randomly on a phylogenetic tree as would be expected if these traits were inherited horizontally.

Microbial community composition has been shown to affect ecosystem function in multiple studies (Tiunov and Scheu 2005; Bell et al. 2005; Langenheder et al. 2010; Reed and Martiny 2007). A recent literature synthesis that investigated the relationship between altered community composition and related function found that 75% of the papers that explicitly tested for a link between community structure and processes found a statistically significant link (Bier et al. 2015). When examining the link between community and function, the available techniques used to evaluate who is present in the community often do not take into account the metabolic states of the individual members of the community, which may be altered by microbial interactions (Baldrian et al. 2012; Lennon and Jones 2011; Schimel and Schaeffer 2012).

Community composition only indirectly controls the turnover of soil carbon by altering the genetic potential of the community and the context within which microbes operate. Ultimately, it is microbial physiology that directly controls carbon turnover (Allison 2012). Schimel and Schaeffer proposed that in order for the community composition to impact soil carbon turnover rates, (1) the organisms must vary in the functional traits that they possess and (2) that the biological reactions they facilitate must be either the rate-limiting or the fate-controlling step in carbon breakdown. The authors argue that the rate-limiting step is more likely due to abiotic soil constraints, and therefore, it is the fate-controlling step that likely shows a relationship between microbial community composition and function.

1.4.1 Community Composition Is Determined by Microbial Interactions

Microbial interactions, along with available resources and conditions, determine the composition of microbial communities. The resource competition theory posits that the species with lower resource requirements will outcompete other species with higher requirements when they are both limited by the same resource. However, species can coexist if they are either limited by different resources or if they have nearly identical resource requirements (Tilman 1981). Soils are spatially and temporally heterogeneous, though. Microsite variation alters the outcome of many competitive interactions beyond the resource competition theory, as do the additional competitive mechanisms such as toxin production (Hibbing et al. 2010; Cordero and Datta 2016). A strategy that works in one location may not be as effective in the neighboring location. For example, in environments with low nutrient levels or low diffusion rates, the competitive ability conferred by a rapid growth rate is diminished (Dechesne et al. 2008). Rapid depletion of resources upon initial colonization of a substrate surface and increase of EPS will likely create that scenario, increasing coexistence.

1.4.1.1 Spatially Defined Interactions

The proximity of microbes to each other is relevant to interactions and microbial processes. Importantly, HGT shapes communities and their function and increases with microbial density and activity (van Elsas and Bailey 2002). However, in a soil simulation parameterized using photos taken at the microscale in soil, Raynaud and Nunan (2014) determined that the average distance between microbes in the soil is 12 μm, with distances decreasing and aggregation increasing closer to the surface of the soil. In lower-density bulk soil, the average number of interacting species was 11 ± 4 within 20 μm, whereas in the higher-density rhizosphere, it was closer to 284 ± 30 species (Raynaud and Nunan 2014).

Results from modeled two-species interactions show that spatial separation may result from microbial interactions, with antagonism leading to self-segregation and mutualism to homogenization (Blanchard and Lu 2015). Separation allows microbes to coexist that might normally compete (Ettema and Wardle 2002; Dechesne et al. 2008). So, while competitive exclusion may occur on a very small scale, diversity is maintained through the larger soil ecosystem, with stability of some communities dependent upon spatial structure (Kim et al. 2008). Furthermore, as was previously discussed with biofilms, each microsite is changed through the interactions it hosts, contributing to temporal heterogeneity.

Non-transitive interaction networks have been studied to determine how diversity can be maintained despite antagonistic interactions using rock-paper-scissor dynamics. Spatial structure allows sensitive strains to survive close to toxin-producing strains through shielding by strains resistant to the toxin (Kerr et al. 2002; Narisawa

et al. 2008). This dynamic functions in communities with one toxin-producing strain or in communities with diverse toxin producers, with diverse toxin production leading to increased ecological stability (Biernaskie et al. 2013; Prasad et al. 2011; Kelsic et al. 2015). These models, however, do not account for the effects of antagonism strength on microbial interactions. For example, synthesis of communities with varying strengths of bacteriocin action suggests that potent bacteriocins led the producers to extinction by stimulating heightened attack responses from their opponents, whereas weak bacteriocins supported coexistence through mild responses (Majeed et al. 2013).

1.4.1.2 Inhibition and Reduction of Niche Overlap

Similar to the effects of physical separation with the previous examples, non-transitive interaction networks are applicable to modulation of antagonism through multispecies interactions. Neighboring cells can decrease antibiotic production of a focal species' antagonist, eliminating negative fitness impacts on the focal species and allowing all three to coexist (Tyc et al. 2015). In this respect, the identity of interacting species plays a strong role in ecological processes. Abrudan et al. (2015) demonstrated that inhibitory interactions were reduced by induction of antibiotic production combined with suppression of antibiotic production in competing species, which allowed maintenance of diversity. Moreover, they found that interactions were environmentally mediated.

Species with high niche overlap are predicted to be more competitive with each other (Freilich et al. 2011). Often, this means that phylogenetically related species engage in stronger competition than do more phylogenetically distant species (Jousset et al. 2011). Sympatric *Streptomycete* species showed higher degrees of antibiotic inhibition and reciprocated production than with allopatric *Streptomycete* species, with niche overlap being positively correlated with antibiotic inhibition (Kinkel et al. 2014; Vetsigian et al. 2011). This result supports the hypothesis that antibiotics function to mediate community interactions in attempts to reduce niche overlap. Indeed, sublethal levels of antibiotics altered independent growth rates of several *Streptomyces* strains on distinct substrates, as well as their range of substrate use (Jauri et al. 2013). Niche overlap declined in 56% of the isolate-isolate-antibiotic combinations, suggesting that sublethal antibiotics acted as an "escape from competition" mechanism. Consequently, antibiotic production may be an instrument to initiate niche differentiation, leading to speciation. Even monoculture biofilms undergo adaptive diversification to eliminate intraspecific competition and form synergistic communities through spatial partitioning and cross-feeding, which leads to higher productivity (Poltak and Cooper 2011).

1.4.1.3 Fungal Interactions

Fungi act as ecosystem engineers, creating pores that form new habitats and mining new resources for other microbes. Soil pore structure has been observed to be nonrandom in nature, with a highly structured bacterial distribution (Young and Crawford 2004). Using this evidence, in an experimental manipulation, Crawford et al. (2012) found that at scales below 53 μm, fungal hyphae were highly correlated with soil pore organization. Additionally, increasing the fungal/bacterial ratio increased soil aggregate formation, indicating that soil community structure plays a role in aggregate stabilization and pore formation in soil. The pores are speculated to improve local conditions for the engineering species by opening up channels for oxygen exchange and increasing water flow potential. These effects increase nutrient exchange and bacterial colonization through increased connectivity and potentially increased carbon turnover. When varying hydration conditions were modeled as a function of the pore matrix potential, microbial dispersal increased dramatically (Kim and Or 2015). In addition, fungi facilitate bacterial movement in conditions with low water potential by providing a highway for bacterial biofilm formation and motility (Pion et al. 2013). Highly mobile bacteria species stimulated migration by less-mobile species along fungal hyphae, with no obvious fitness decline (Warmink et al. 2011; Warmink and Van Elsas 2009).

Fungi have been noted to dominate the litter horizon in a forest ecosystem, with the fungal/bacterial ratio evening out in the organic horizon (Baldrian et al. 2012). Bacteria often benefit from fungi due to the fungal release of extracellular enzymes that create nutrient "hotspots" or metabolic intermediates from degrading recalcitrant carbon sources (Van der Wal et al. 2013; Tolonen et al. 2014). Increases in bacterial biomass are correlated with increasing fungal biomass in soil microcosms (Šnajdr et al. 2011). Bacteria have lower yield than do fungi, though, so changes in the fungal/bacterial ratio have implications for ecosystem CO_2 flux (Lipson et al. 2009). Additionally, the area around fungal hyphae has been postulated to have concentrated horizontal gene transfer (HGT), including HGT occurrences between bacteria and fungi (Zhang et al. 2014), which has the potential to increase carbon turnover through transfer of carbon-degrading traits.

1.4.2 Evolutionary Feedbacks on Carbon Cycling

Though multiple pairwise evolution experiments have been performed in the laboratory, the relevance of these experiments for communities of interacting microbes is unclear (Turcotte et al. 2012; Johnson and Stinchcombe 2007). Various pressures imposed by interactions with multiple species simultaneously may result in microevolution of a population that cannot be accounted for in simple two-species experiments (Johnson and Stinchcombe 2007). Even though it is possible to

extrapolate the fundamental niche of an organism, ecological interactions alter the niche, resulting in an altered range of conditions that permit survival.

Diffuse evolution refers to evolution that is caused by one species' effect on the evolving species but depends upon multiple other species within the environment. Diffuse coevolution occurs when the selection is reciprocal (Strauss et al. 2005). Research on the interplay between ecological interactions and evolutionary mechanisms is still in its early stages (Johnson and Stinchcombe 2007). By impacting evolutionary rate and direction, diffuse coevolution alters interactions between members of a community of microbes, which then feeds back on function, further affecting community interactions (Lawrence et al. 2012; Fussmann et al. 2007; Schoener 2011).

The community context of diffuse evolution may indicate that whole communities evolve together through direct or indirect mechanisms (Little et al. 2008; Barraclough 2015). Generally, it has been seen that over time, antagonistic communities evolve to be less competitive, with this effect increasing with increasing diversity (Fiegna et al. 2015a). Competition was observed to cause a decrease in resource use diversity, associated with a decrease in relative growth rate and yield compared to the ancestral strain, though this effect saturated at higher species richness (Fiegna et al. 2015b). The decrease in growth rate and yield mirrors microbial adaptation to resource-limited conditions, with increased uptake machinery and enzyme production for resource acquisition (Schmidt and Konopka 2009).

Further eco-evolutionary dynamics were highlighted in an experiment performed by Lawrence et al. (2012) whereby evolution in a community of four strains of bacteria was observed over several generations. The researchers found that the strains grown in a community evolved faster than did those same strains in monoculture. Additionally, the interacting species evolved resource use divergence, and cross-feeding on metabolic waste products, indicative of character displacement and positive interactions. Three of the four community-evolved species did more poorly than their ancestors when grown in monoculture, revealing some degree of coadaptation. When compared to the community of ancestors, the evolved community had smaller population sizes, but higher CO_2 flux rate, suggesting a decrease in community CUE. This experiment demonstrated that community interactions can increase evolutionary rates above selection caused by abiotic pressures alone. It also reveals that adaptations caused by community interactions may function to transform environmental conditions, strengthening selective pressures and altering carbon cycling through evolution-induced metabolic shifts.

1.5 Conclusion

Through effects on physiology, public goods, and evolution, microbial interactions play a large role in soil carbon cycling. The rate of microbial metabolism controls uptake, transformation, and allocation of carbon (Brown et al. 2004). Because microbial interactions change phenotypic allocation of carbon and drive selection

that alters metabolic traits, these interactions are tied to the carbon cycle. Furthermore, the dynamics of populations and communities are largely determined by the rate of metabolism and the metabolic products of the member organisms. Waste products, biofilm formation, and growth rate affect a microbe's neighboring cells. Finally, ecosystem processes of energy flux and biomass production are also determined by metabolism. Sinsabaugh et al. (2015) showed an allometric relationship between extracellular enzyme-substrate and production-biomass reactions, indicating that these are linked metabolic processes with relevance at the ecosystem scale.

Though some techniques have been developed to study microbes in the soil environment (O'Donnell et al. 2007), the ability to determine ecological and evolutionary processes at the microscale is still limited. The determinants of soil community structure and all of its associated network interactions are yet uncertain, as is the ability to deduce its functional capacity relative to spatial and temporal conditions (Prosser 2012). Multi-omics only offer a snapshot of community structure and function and contain multiple biases and computational bottlenecks with increasing sample sets (Hahn et al. 2016; Widder et al. 2016; Nesme et al. 2016).

Models provide an alternative approach to extrapolate microbial metabolic processes, interactions with the abiotic and biotic environment, and effects on soil carbon cycling and storage (Kim and Or 2015; Allison 2012; Liang et al. 2011; Allison et al. 2010; Widder et al. 2016). Metabolic reconstructions are able to predict cellular and community processes such as biomass yields, formed consortia, evolution, stress adaptations, and the impact of specific phylotypes (Oberhardt et al. 2009; Khandelwal et al. 2013; Carlson and Taffs 2010; Harcombe et al. 2014). Furthermore, metabolic models can be used to explore ecosystem-level processes (Klitgord and Segre 2011). Combining models with new microscale experiments could rapidly advance a predictive understanding of microbial interactions in soil. Such efforts are critical given the myriad mechanisms by which microbial interactions potentially influence the carbon cycle.

Compliance with Ethical Standards

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Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Chapter 2
Qualitative and Quantitative Aspects of the Modern Nitrogen Cycle

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Abstract The biogeochemical cycling of nitrogen was recognized and largely defined in the nineteenth century. Although great detail about both the processes and the organisms that carry them out have been reported during the 100+ years since the definition of the cycle was completed, only two new processes were added to the cycle, anaerobic ammonia oxidation and industrial fixation of N_2 by the Haber-Bosch process. The latter process has injected large amounts of reactive nitrogen into the global environment such that many locations have excess nitrogen present that accelerates eutrophication, emission of greenhouse gasses, destruction of ozone, and some direct health effects. Nevertheless, nitrogen is still the primary limitation on food production, and as the world population continues to increase, the demand for nitrogen fertilizer will continue to drive fixation of N_2 into reactive nitrogen faster than denitrification and anammox can return it to the atmosphere as the nonreactive N_2.

2.1 Introduction

The modern nitrogen cycle is a story of dichotomy. On one hand, nitrogen availability for plant culture has always been, and still is, the largest limitation of total food production for the entire planet. Yet, at the same time, excesses of reactive nitrogen (Nr, which includes all forms of N except for N_2) threaten clean waters with eutrophication, the atmosphere with smog, ozone depletion, greenhouse gasses, and acidification of condensation and precipitation which, in turn, can accelerate the acidification of soils and surface water. Much of the development of agriculture has revolved around the question of how best to provide adequate nutrients to plants, when the plants are harvested and the nutrient carried away from the field with each harvest. Over a period of time, the length of which would depend on the native soil fertility and the crops being grown and harvested, the soil will be depleted in the nutrients that support the crop growth. Although all nutrients are derived from the

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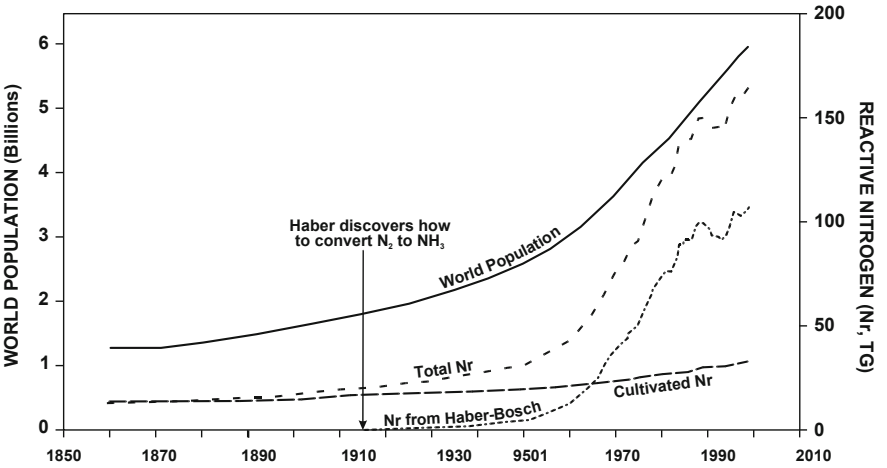

Fig. 2.1 Association of rapid global population growth with large-scale production of reactive nitrogen (N_r) by the Haber-Bosch process. Total N_r represents all sources of reactive N (including fossil fuel combustion, lightning, nonagricultural biological N fixation, biomass burning, etc.). Cultivated N_r represents that derived from cultivation of legumes, rice, and sugarcane. Figure redrawn from Galloway et al. (2003)

soil, it is nitrogen that most often and most rapidly becomes limiting for plant growth (Food and Agriculture Organization 1985; Robertson and Vitousek 2009; Lamer 1957). Manipulation of the nitrogen cycle by human intervention has allowed food production to increase such that the global population has increased from just under one billion in 1750 to over seven billion today (Smil 2004; Galloway and Cowling 2002) (Fig. 2.1), and it is estimated that in the absence of fertilizer nitrogen, the current population could be only about 60% of its current state (Smil 1997, 2004).

Although Rutherford's discovery of nitrogen as an element did not occur until 1772, the recognition that enhanced plant growth from addition of organic materials to soil used for growing plants had been known for centuries. The belief that Native Americans originated the practice of adding a fish to planted corn is actually a myth. Although Squanto did teach the Pilgrims the technique (Ceci 1975), he is thought to have learned the practice during visits to Europe as a slave. Nevertheless, it is documented that the Pilgrims did, indeed, fertilize their corn, barley, and "pease" with fish, and the practice helped them produce enough crops to turn the colony into a permanent settlement (Heath 1963). Even though neither Squanto, his European captors, nor the Plymouth colonists knew of nitrogen and its importance, it was clear to them that some material contained in the dead fish was highly beneficial to the growth of their crops. Indeed, in sixteenth-century France, a craftsman by the name of Palissy recognized that adding to the soil certain materials, all organic matter at that point in time, benefited the growth of local crops (Galloway et al. 2013). Of course, Palissy had no more knowledge of nitrogen as an essential element than did Squanto, but he did understand that grasses and other plants grew well around

animal droppings and that by collecting those droppings and adding them to gardens or other croplands, growth was enhanced.

Rutherford's 1772 report was the first that definitively identified nitrogen (not named as such until Chaptal's 1790 use of the term "*nitrogène*"), but several others, notably Cavendish, Scheele, and Priestly, were conducting experiments similar to those of Rutherford with burned or "dephlogisticated" air, which was nearly all nitrogen (Smil 2004). The role of the new element in biology, including plant growth and nutrition, was not formally recognized until 1836, when Boussingault pointed out the need by plants for nitrogen as a nutrient. He was studying legumes at the time and in 1838, while he did not understand the mechanism, reported that legumes could, somehow, extract nitrogen from the atmosphere (Smil 2004). Very shortly thereafter, von Liebig recommended the addition of nutrients, including nitrogen compounds, to the soil (Von Leibig 1840). Thus, scientists began to understand why "Squanto's fishes" worked as they did.

Recognizing the uptake of nitrogen by organisms and the release of nitrogen back to the environment upon their decay, coupled with Boussingault's observations about legume incorporation of atmospheric nitrogen, the cyclical nature of nitrogen transformations in the environment was first established by Reiset (1856), who observed the release of nitrogen back to the atmosphere. Indeed, as Payne (1986) suggested, the nitrogen cycle could easily, and appropriately, be named the Reiset cycle.

The environmental transformations of nitrogen other than uptake and release (assimilation and mineralization) followed closely on the heels of Reiset's paper in 1856. Although Pasteur predicted a role for bacteria in converting NH_4^+ to NO_3^- in 1862 (Prosser 2011), it was Schloesing and Muntz (Schloesing 1873; Schloesing and Muntz 1877a, b, 1879) who clearly showed the dependence of nitrification on microbes by using antiseptic treatment and heating to eliminate microbial activity, thus stopping nitrification. Shortly thereafter, Winogradsky (1890) isolated organisms from soil that catalyzed the nitrification process and, along with his earlier paper on sulfur-oxidizing bacteria (Winogradsky 1889), established chemoautotrophy as an important bacterial way of life.

A very few years earlier, Gayon and Dupetit (1883, 1886) observed the removal of nitrate during anaerobic decay of organics. They were able to isolate the first denitrifying organisms, thus explaining Reiset's observations on the return of gaseous N to the atmosphere, establishing denitrification as an important biological process in the nitrogen cycle. Indeed, denitrification has long been considered to be the balancing agent between the pools of nonreactive atmospheric nitrogen (N_2) and N_r (all nitrogen except N_2), that is, the counter to biological nitrogen fixation.

The latter process, biological nitrogen fixation, was discovered around the same time as nitrification and denitrification by Hellriegel and Wilfarth (1889) who built on the earlier observations of Boussingault. Hellriegel and Wilfarth determined that the nodules (which were once thought to be insect galls) on the roots of legumes were the source of the nitrogen available to the plants and that the nodules contained bacteria. However, Martinus Beijerinck was the first to isolate nitrogen-fixing

bacteria from nodules (the N-fixing symbionts were isolated substantially before free-living prokaryotes) after noticing that the nodules did not form when the plants were grown in sterilized sand and that the plants were only successful in that case when nitrogen was added to the sterile sand (Beijerinck 1888). He was mystified, however, that the isolates would not make NH_4^+ when grown outside the root nodules. Shortly thereafter, Winogradsky was able to isolate an anaerobic nitrogen-fixing organism he named *Clostridium pasteurianum* (Chung and Case 2001), although Beijerinck's later isolation of the free-living nitrogen-fixing bacterium *Azotobacter* (Beijerinck 1901) was a much more meaningful discovery.

Thus, at the beginning of the twentieth century, the natural, biological nitrogen cycle was understood, at least at the general phenomenological level. Only two more processes have been added to the cycle in 100+ years to follow, although detail, nuance, and capable organisms have been added to each of the processes that were first identified in the nineteenth century. One of the newly discovered processes, anaerobic ammonium oxidation, or anammox, was discovered very recently, being first described in 1995 by Mulder et al. (1995) after being observed in wastewater sludge. The other major process to be discovered can hardly be considered natural, but the industrial fixation of atmospheric N (nonreactive N) to ammonia by the Haber-Bosch process has altered the quantitative operation of the global nitrogen cycle drastically. It is the reactive nitrogen (N_r) generated by Haber-Bosch that created the dichotomy of having too much N_r cycling through the planet while N_r still limits food production.

2.2 Processes Old and New

Nitrogen cycles are depicted in many ways, depending on the ideas that the creator wishes to emphasize. All the depictions tend to include, however, assimilation of N from the environment by plants and microbes, consumption of plant N by animals, and mineralization of NH_4^+ from animal excretions and decaying organic matter, along with the oxidations and reductions of inorganic N species (Fig. 2.2). The redox reactions include nitrification, denitrification, dissimilatory nitrate reduction, anammox, and nitrogen fixation. Note that the biological nitrogen cycle is largely a series of oxidation-reduction reactions. The nitrogen molecule donates or accepts electrons rather easily, and there are well-known and environmentally important species of nitrogen at nearly every oxidation state from −3 to +5 (Fig. 2.3). Oxidation reactions yield energy, and so a number of organisms have evolved to use reduced species, especially ammonium (NH_4^+) as a source of energy while fixing CO_2 as the carbon source. Oxidized species, e.g., nitrate (NO_3^-), can serve to accept the electrons from the oxidation of organic matter under anaerobic conditions. The cycling of inorganic species of N through series of oxidation and reduction reactions separates the N cycle (along with the biological cycles of sulfur, iron, and a few

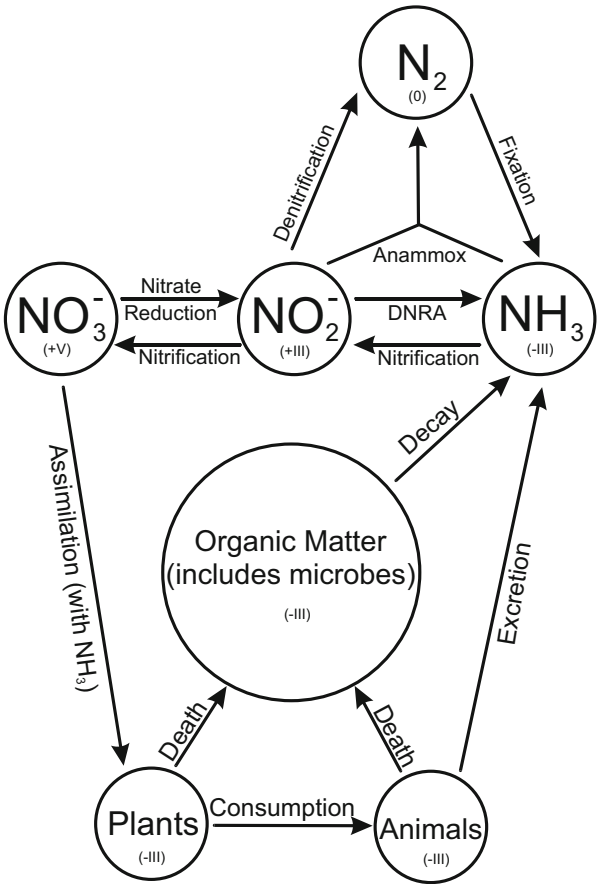

Fig. 2.2 A simplified depiction of the currently understood nitrogen cycle. The numbers at the top represent the oxidation states of the relevant inorganic forms (organics are all −3). Processes recognized after the discoveries of the nineteenth century are represented by dashed lines. Anammox is the anaerobic oxidation of ammonia, and DNRA represents dissimilatory nitrate reduction to ammonia

other, primarily metallic, species) from those, such as phosphorus, which have only assimilation and mineralization as component processes.

2.2.1 Immobilization and Mobilization (Uptake and Mineralization)

The incorporation of nutrients into biomass and the subsequent release into the environment, either by waste elimination or by decay upon death, are known by several synonymous names. Agronomists use *immobilization* to represent the uptake of nutrients by organisms (usually organisms other than crop plants) that renders the nutrient unavailable for crop production; *mobilization*, then, refers to the release of the nutrient (usually upon death and decay of organic residues) to an available inorganic form suitable for uptake by crop plants. *Assimilation* is a more general

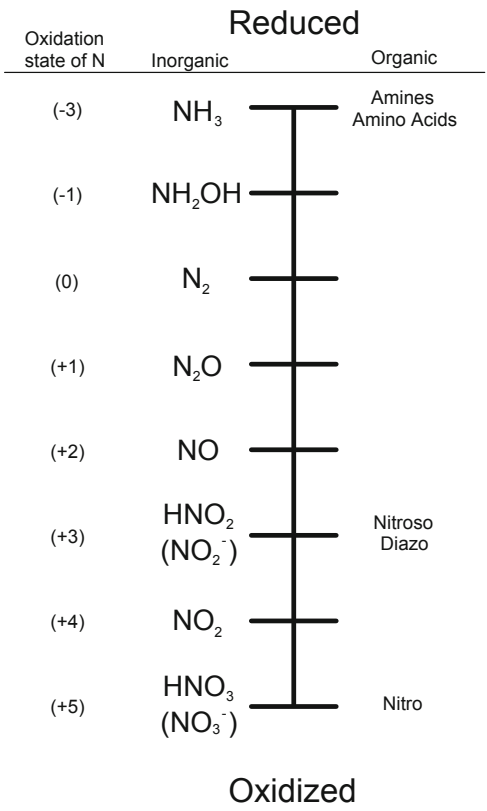

Fig. 2.3 Various oxidation states of nitrogen illustrating environmentally or biologically important compounds for each of the states

term that refers to the incorporation of any nutrient (including organic molecules) into biomass. In this case, assimilation can refer to crop or non-crop species at any taxonomic level. *Mineralization* is the term most often used for the release of inorganic materials from decaying (organic) materials. *Uptake* is simply the incorporation of an element or compound into a cell or an organism. No change of form is implied or necessary (although it could occur), and assimilation into biomass is not congruent with uptake. For example, many plants, including a number of leafy green vegetables, take up excess NO_3^- and store it internally as NO_3^- rather than reducing it to NH_4^+ and assimilating it into amino acids. Uptake could be synonymous with immobilization, therefore, but not necessarily assimilation.

A key process in assimilation of nitrogen taken up as an oxidized form (usually NO_3^-) is reduction of the N to the -III state (assimilatory nitrate reduction) and incorporation as ammonium into amines and amino acids. For plants and microbes that can tolerate and take up ammonium, this process does not occur when NH_4^+ is the N source.

The relationship of the nitrogen cycle to organic carbon is an intimate one, as it is for a variety of elements, including sulfur, iron, phosphorus, oxygen, etc. (Fig. 2.4). Note that several of those elements also have redox reactions that serve similar

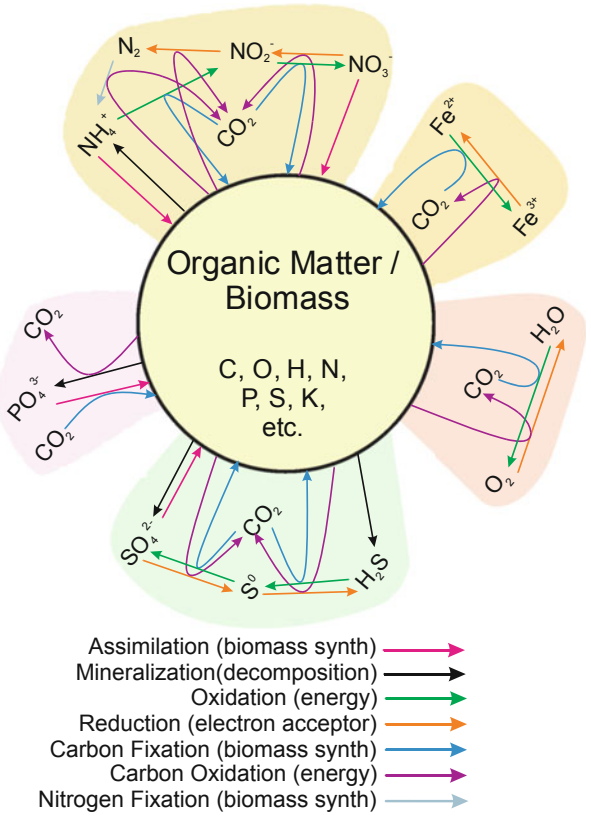

Fig. 2.4 Examples of several elemental cycles including ones in which redox processes are important components and ones in which redox reactions do not play a role. Note that all of the cycles interact and intersect with the cycling of carbon. Figure reprinted from Blum and Mills (2012) with permission of the publisher

functions as seen for nitrogen species. Typically, the amount of nitrogen assimilated (whether through nitrate reduction or incorporation of ammonium) is controlled by the mass of the organism produced. Nitrogen assimilation is related to that mass by the C:N ratio of the growing organism. The C:N ratio of microbes ranges from around 5:1 to around 15:1, but 10:1 is often used as the "typical" value due to its similarity to well-decomposed, humified organic matter. Plants can take on a wide range of C:N values depending on the nature of the plant material being analyzed. Most plants are composed largely of carbohydrates (cellulose, hemicellulose, sugars, etc.), so we generally think of them as 40% carbon, because the mass of a carbohydrate taken as CH_2O is 40% carbon by mass. Thus, the C:N ratio of the plant material is controlled by the nitrogen content and has little to do with the carbon content. The C:N ratio in plants varies widely from species to species, and within a plant species, the age of the plant plays an additional and critical role (Table 2.1). Young, green plant tissue that is actively synthesizing protein for growth can have very narrow C:N ratios, but as plant material ages and becomes senescent, the C:N ratio often widens substantially.

When the plant dies, the starting C:N ratio that the plant deals with is the C:N ratio of the plant material at the time of plant death. For organic material with wide C:N

Table 2.1 C:N ratios of selected organic materials

Material	C:N	References
Straws and stover		
Rye straw	82:1	USDA (2011)
Wheat straw	80:1	USDA (2011)
Corn stover	57:1	USDA (2011)
Pea straw	29:1	USDA (2011)
Hays		
Mature alfalfa	25:1	USDA (2011)
Legume hay	17:1	USDA (2011)
Young alfalfa	13:1	USDA (2011)
Cover crops		
Rye (mature)	37:1	USDA (2011)
Rye (vegetatively growing)	26:1	USDA (2011)
Hairy vetch	11:1	USDA (2011)
Manures		
Rotted barnyard manure	20:1	USDA (2011)
Beef manure	17:1	USDA (2011)
Other		
Marine phytoplankton	5.6:1	Redfield (1934)

Here residues are emphasized because they are the primary source of materials for microbial activities rather than healthy vegetative plants

ratios (say 80:1), the amount of nitrogen in the material is inadequate for complete decay of the organics. Thus, the nitrogen is recycled internally within the decomposing organic matter—microbe system (perhaps with immobilization of nitrogen from the soil) such that carbon is excreted as CO_2, while N is retained. The result is a narrowing of the C:N ratio within the organic matter; the humifying organics effectively become enriched with N (Fig. 2.5). At some point (usually at C:N ratios around 35:1 to 30:1), the amount of N is sufficient such that carbon actually becomes limiting. At this point, N will be released from the decaying material to the environment (i.e., mobilization). The process continues until the organic matter approaches the C:N ratio of the decomposing microbes which is usually about 10:1.

2.2.2 Nitrification

Nitrification is the oxidation of nitrogen from the −3 oxidation state (NH_4^+) to the +5 state (NO_3^-). The reaction occurs in two steps, carried out by different bacteria, in a process resembling a "bucket brigade." First, NH_4^+ is oxidized to NO_2^- (+3) with an energy yield to the ammonia-oxidizing bacteria (AOB) of about 58 kcal (243 kJ) per mole of N oxidized. Subsequently, the NO_2^- is oxidized by nitrite-oxidizing bacteria (NOB) to NO_3^-, a reaction that yields only 18 kcal per mole (75 kJ) to the organisms. The differential energy yield partially explains why NO_2^- rarely accumulates in the

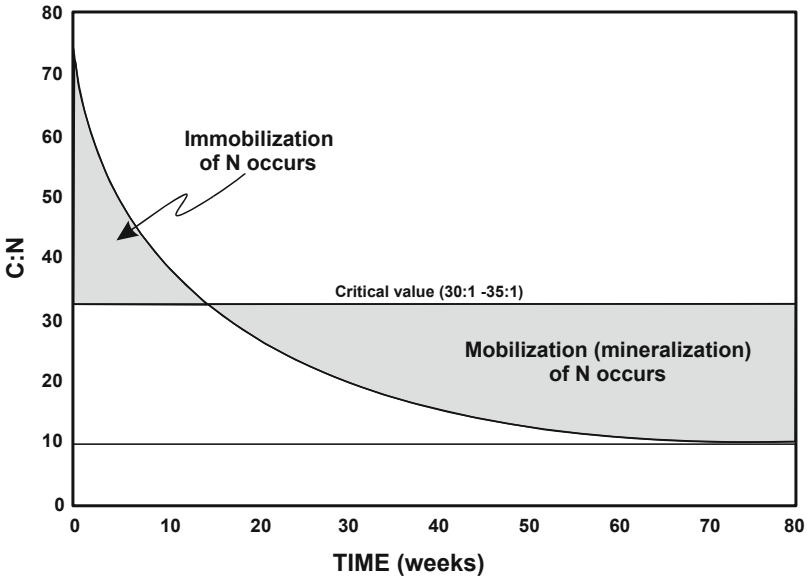

Fig. 2.5 Change in C:N ratio of decomposing organic matter (including attendant microbes). As the material ages, carbon is released more rapidly than nitrogen which is retained or even taken up from the surrounding environment (immobilization). Once the critical point is reached, however, nitrogen is actually in excess of what is needed for microbial growth given the carbon supply, and from there on, nitrogen is mineralized and released to the environment

environment; each mole of N oxidized by the NOB yields only 31% of the energy gained by the AOB in the oxidation of ammonia such that the NOB are (relatively) near starvation and quickly take advantage of any NO_2^- molecule released by the AOB. Nitrification is important in nearly all aerobic environments and, in some, is an important component of the carbon cycle as well. For example, CO_2 fixation by nitrifying bacteria accounts for up to 12–32% of the primary production in the global marine environment (Yool et al. 2007)

Although a number of genera have been identified, the guild of nitrifiers is not a diverse group in terms of numbers of taxa. Two main genera of nitrifying autotrophs were identified early: *Nitrosomonas* represented the AOB (at least in soil), and the NOB were *Nitrobacter*. A number of other nitrifying bacteria have been identified, however, including several genera of AOB and several NOB (Table 2.2). Additionally, the relatively small guild of organisms is spread rather widely across the *Proteobacteria*, and its members are included in two monophyletic lines. The *Gammaproteobacteria* houses *Nitrosococcus oceani*, while *Nitrosomonas* (including *Nitrosococcus mobilis*) is included in the *Betaproteobacteria* along with *Nitrosovibrio*, *Nitrosospira*, and *Nitrosolobus* (Purkhold et al. 2000). These organisms are closely related, and some have suggested they be reclassified into a single genus (*Nitrosospira*) (Head et al. 1993; Purkhold et al. 2000). That opinion is not universally held, however, and evidence for maintaining these organisms as separate

Table 2.2 Distribution of nitrifiers (AOB and NOB) among bacterial phyla

	Class within proteobacteria			
Guild	α	β	γ	δ
AOB	–	*Nitrosomonas* *Nitrosovibrio*[a] *Nitrosospira*[a] *Nitrosolobus*[a]	*Nitrosococcus*	–
NOB	*Nitrobacter*	–	*Nitrococcus*	*Nitrospina* *Nitrospira*[b]

[a]Some authorities have made a case for lumping these genera into *Nitrosomonas* (e.g., Head et al. 1993; Spieck and Bock 2005); however, others have offered evidence to keep the genera as closely related but separate from *Nitrosomonas* and from each other (Teske et al. 1994)
[b]Some sources list *Nitrospira* in the *Deltaproteobacteria* (Teske et al. 1994), although at present, the prevailing sentiment is to place it in a phylum of its own: *Nitrospirae* (Ehrich et al. 1995; Lücker et al. 2010; Spieck and Bock 2005)

genera has been offered (Teske et al. 1994). Isolation of a chemolithotrophic, ammonia-oxidizing archaeon was reported by Könneke et al. (2005), such that the acquisition of energy from the oxidation of ammonia is known to be present in both prokaryotic domains. While a few authors have begun to use the acronym AOM (ammonia-oxidizing microorganisms) in place of AOB (e.g., Daims et al. 2015) to indicate the presence of the Archaeal members of the guild, this chapter will continue to use AOB because of its most common usage in the current literature.

The NOB, similarly, are a small group of genera, but their phylogenetic relationships are, similar to the AOB, spread across several subgroups of the *Proteobacteria* (Spieck and Bock 2005). At this point in time, no nitrite-oxidizing Archaea have been reported. The *Alphaproteobacteria* contain several members of the genus *Nitrobacter* that are quite closely related to members of the heterotroph *Bradyrhizobium*, the denitrifier *Blastobacter*, and the facultative phototroph *Rhodopseudomonas* (Teske et al. 1994). In addition to the AOB *Nitrosococcus*, the *Gammaproteobacteria* also contains the NOB *Nitrococcus*. Two other nitrite-oxidizing genera of NOB are *Nitrospina*, which is placed in the *Deltaproteobacteria*, and *Nitrospira*, which has been classified within the *Deltaproteobacteria* by some (Teske et al. 1994) but is formally placed within its own phylum, *Nitrospirae* in Bergey's Manual of Systematic Bacteriology (Ehrich et al. 1995; Lücker et al. 2010; Spieck and Bock 2005). *Nitrospira* is quite ubiquitous, and its members have been found in terrestrial environments (Pester et al. 2014), aquatic habitats including freshwater (Hovanec et al. 1998; Daims et al. 2001), marine habitats (Watson et al. 1986), geothermal springs (Lebedeva et al. 2011), and habitats where microbes tend to form films or aggregates such as drinking-water distribution systems (Martiny et al. 2005), wastewater treatment plants (Daims et al. 2001; Schramm et al. 1998) (where they are often the dominant nitrite oxidizer), and corroded iron pipes (Ehrich et al. 1995) (Tables 2.3 and 2.4).

The two processes (ammonia oxidation and nitrite oxidation) are tightly linked, because NO_2^- rarely accumulates in the environment. As pointed out above, that linkage is, at least in a large part, due to the energetic differences between the two

Table 2.3 Denitrifying genera grouped according to distinctive physiological features[a,b]

ORGANOTROPHIC	
General aerobic	**N_2 fixing**
Pseudomonas	*Rhizobium*
Alcaligenes	*Bradyrhizobium*[j]
Flavobacterium[c]	*Azospirillum*[g]
(*Achromobacter*)[d]	*Pseudomonas*
Paracoccus	*Rhodopseudomonas*
(*Corynebacterium*)[e]	*Agrobacterium*[k]
[*Acinetobacter*]	**Animal or pathogenic**
Cytophaga	*Neisseria*
[*Gluconobacter*][f]	*Kingella*
[*Xanthomonas*]	(*Moraxella*)[l]
Oligocarbophilic	*Wolinella*[i]
Hyphomicrobium	**PHOTOTROPHIC**
Aquaspirillum[g]	*Rhodopseudomonas*
Fermentative	**LITHOTROPHIC**
Azospirillum[g]	**H_2 use**
(*Chromobacterium*)[h]	*Paracoccus*
Bacillus	*Alcaligenes*
Wolinella[i]	*Bradyrhizobium*
Halophilic	*Pseudomonas*
Halobacterium	**S use**
Paracoccus	*Thiobacillus*
Thermophilic	*Thiomicrospira*
Bacillus	*Thiosphaera*
[*Thermothrix*]	[*Thermothrix*]
Sporeformer	**NH_4^+ use**
Bacillus	*Nitrosomonas*
Magnetotactic	
Aquaspirillum	

Tiedje (1988), the original contains the appropriate literature citations

[a]Genera in parentheses are of uncertain or discontinued taxonomic status or the denitrifying species have been transferred to another genus

[b]Genera in brackets indicate that characterization as having respiratory denitrification is incomplete

[c]It has been suggested that denitrifying *Flavobacteria* are actually strains of *Pseudomonas*

[d]Most strains now considered to be *Alcaligenes*

[e]It has been suggested that the main strain studied, *C. nephridii*, is an *Alcaligenes* sp.

[f]Described as *Acetomonas* by the authors, but now designated as *Gluconobacter*

[g]*Aquaspirillum* and *Azospirillum* were derived from the genus *Spirillum*; the remaining *Spirillum* species does not denitrify

[h]*C. violaceum*, the species *C. lividum* now termed *Janthinobacterium*

[i]Originally, *Vibrio succinogenes*; cannot reduce NO_2^- to N_2O

[j]Slow-growing rhizobia (e.g., *Rhizobium japonicum*) are now designated as *Bradyrhizobium*

[k]*Agrobacterium* is not N_2 fixing but is closely related to *Rhizobium* and is usually associated with plants, due to its plant pathogenic properties

[l]All denitrifying strains of *Moraxella* are now considered *Kingella denitrificans* except for one poorly characterized soil isolate

Table 2.4 Examples of nitrogen-fixing microbes

Free-living bacteria		Associative bacteria	
Heterotrophs		**Heterotrophs**	
Aerobic	*Azotobacter*, *Beijerinckia*, *Xanthobacter*	Biocoenoses Rhizosphere Phyllosphere	 *Azospirillum*, *Bacillus* *Klebsiella*, *Beijerinckia*
Facultative aerobes	*Bacillus*, *Azospirillum*, *Klebsiella*	Symbioses Legumes	 *Rhizobium*, *Bradyrhizobium*, *Allorhizobium*
Phototrophs		Nonlegumes Termites	*Frankia*, *Nostoc* *Citrobacter*
Cyanobacteria	*Nostoc*, *Trichodesmium*, *Anabaena*	**Phototrophs**	
Purple non-sulfur bacteria	*Rhodopseudomonas*, *Rhodospirillum*	Lichens Liverworts	*Nostoc*, *Stigonema* *Nostoc*
Purple and green sulfur bacteria	*Thiocapsa*	Mosses Cycads Water ferns (*Azolla*)	*Halosiphon* *Nostoc* *Anabaena*
Archaea	*Methanococcus*, *Methanosarcina*, *Methanobacterium*		

The list is not comprehensive

reactions. Nevertheless, nitrification is not always a stable process in many engineered systems like wastewater plants (Graham et al. 2007), and the result can be a cessation of activity, an accumulation of NO_2^-, or a runaway nitrification with rapid conversion of all inorganic species to NO_3^-. Whether any of these is a problem depends largely on the desired processes operating within the plant. The instability may derive from the relatively low energy yield of the overall nitrification process and the low number of genera that possess nitrification capacity (Berman et al. 2010).

Given the close linkage between ammonia oxidation and nitrite oxidation, and the relatively low energy yield of each, it seems surprising that the two processes have remained separate and were not joined in a single organism. The combined process of conversion of NH_4^+ to NO_3^- was predicted to occur in physiological thermodynamic studies by Costa et al. (2006) who coined the term comammox (complete ammonia oxidation) to describe the hypothetical organisms. These authors argued that although there is a definite energetic advantage to a single organism possessing both parts of the nitrification process, such an organism would likely be outcompeted in many environments, by organisms which contain the two processes separately. Costa et al. (2006) argued that the separate organisms would likely have faster growth rates, whereas the combined organism would likely tend to maximize growth yield rather than growth rate. However, under conditions that could favor slow, substrate-influx-limited growth with clustering of biomass spatially in microbial aggregates in biofilms, the combined organism might then be more competitive.

Indeed, some very recent reports have documented the existence of three distinct strains of *Nitrospira* that have been cultured (Daims et al. 2015; van Kessel et al. 2015) and one uncultured strain (Pinto et al. 2015) for which sequence data are available that do oxidize NH_4^+ completely to NO_3^-. The discovery of the organisms that possess both of the nitrification reactions examined those types of habitats where Costa et al. (2006) predicted such organisms, if they exist, might live, and they and their molecular signals were successfully found there.

It is interesting to note that many of the organisms that are identified as nitrifiers can metabolize using alternate pathways. The sequences representing the uncultured organism that were reported by Pinto et al. (2015) also contain genes for metabolizing urea, presumably to make use of the ammonia for growth. The organisms cultured by Daims et al. (2015) and van Kessel et al. (2015) contain the urea transport and degradation genes as well suggesting that urea may be a universal source of ammonia for any organism carrying out the combined nitrification reactions.

In addition, however, it is becoming apparent that as a group, the nitrifiers may be more metabolically capable than we have thought previously. Some strains of *Nitrobacter* have been shown to have the capacity to oxidize acetate or pyruvate when nitrite is unavailable (Santoro 2016), and some ammonia-oxidizing marine *Thaumarchaea* can take up some organics (Li et al. 2015), and some may require organics (Qin et al. 2014). There is thought that some *Nitrospira* may degrade either cyanate or urea to feed ammonia oxidizers which, in turn, produce NO_2^- for the NOB (Palatinszky et al. 2015; Koch et al. 2015). Furthermore, there is evidence that some strains of *Nitrospira* may not oxidize nitrite, but may, in fact, oxidize H_2 to obtain energy for growth (Koch et al. 2014). Because of the way nitrification is measured, discovery of these alternate means of nitrification will likely not change the quantitative view of nitrification and its role in the overall nitrogen cycle. It is not known if the organisms that combine ammonia and nitrite oxidation generate NO or N_2O as by-products in a fashion similar to the other nitrifiers (Santoro 2016).

2.2.3 Nitrate Reduction

2.2.3.1 Assimilatory Nitrate Reduction

The reduction of nitrate requires energy, but when coupled to the oxidation of a reduced compound, organic or inorganic, the net energy yield can often support prolific microbial growth. Nitrate reduction occurs in three major pathways. For those organisms, microbes and plants, that can take up NO_3^- from the environment and incorporate it into biomass, assimilatory nitrate reduction is the means whereby the nitrate is transformed to ammonium for incorporation into amino acids through pathways whereby α-ketoglutarate is aminated to form glutamate or glutamate is subsequently aminated to form glutamine or, less common, where CO_2 is aminated through an ATP-dependent reaction to form carbamyl phosphate. Assimilatory

reduction is an important strategy for organisms that live primarily in aerobic systems where nitrification keeps ammonium concentrations low, and nitrate may be the dominant inorganic nitrogen species. Generally, assimilatory reduction is considered to be part of the assimilatory apparatus of the microbes and plants that take up nitrate to make proteins, and it is not dealt with independently. Nevertheless, its stoichiometric similarity to dissimilatory reduction makes it worthy of mention here, even if free ammonium is not a product. The reaction for both assimilatory and dissimilatory reduction of nitrate may be written as:

$$\begin{aligned} &C_6H_{12}O_6 + 3NO_3^- + 3H^+ \rightarrow \\ &6CO_2 + 3NH_3 + 3H_2O \quad (\Delta G = -1795\ \text{kJ/mole glucose}) \end{aligned} \tag{2.1}$$

Organisms that reduce nitrate for purposes of nitrogen assimilation may use the energy yielded by the reaction, but if they do, the amount is a tiny fraction of that gained through carbon (or other electron donor) oxidation exploited specifically for energy acquisition, such that assimilatory nitrate reduction effectively serves only to allow incorporation of the nitrate nitrogen into biomass. Given that the purpose of assimilatory nitrate reduction is the generation of ammonium for biomass production, the presence of ammonium in the environment precludes the need for the organism to generate the reduced nitrogenous form, and so assimilatory reduction will be inhibited.

2.2.3.2 Denitrification

Losses of fertilizer N due to denitrification are often 20–30% of the added N (Tiedje 1988) but can occasionally reach 70% (Firestone 1982). In this case, fertilizer loss means added nutrient that is not taken up by the (crop) plant and remains in the environment, usually at a location other than the site of application where, if it were retained, it could be used for subsequent crops. Losses of fertilizer differ depending on the identity of the inorganic N species present. Ammonium is an exchangeable cation, and barring conversion to nitrate by nitrification or conversion to ammonia due to high pH, it can persist in an available, exchangeable form for some time. Ammonia (NH_3) is subject to losses as vapor, so in instances of high pH (anything above 7), the nitrogen can be emitted into the atmosphere where it can fall back to earth downwind of the source.

Nitrate is lost by two mechanisms. Because NO_3^- is a mobile anion, i.e., it is not sorbed strongly in soil, it is often carried downward by percolating water. The combination of heavy fertilizer use and the mobility of nitrate make the substance the single most ubiquitous contaminant of groundwater in the United States (Canter 1997). It is so common that the US Environmental Protection Agency has placed a recommended limit of 10 mg NO_3^- N/L on water intended for drinking purposes. The limit is a recommendation because there are many parts of the country where there is no water source (especially groundwater) with a lower concentration (Canter 1997).

The final way fertilizer is lost from agricultural soil is through denitrification, the reduction of the nitrogen atom from NO_3^- (N is +V) to N_2 (N is 0). This is a process unique to prokaryotes in which NO_3^- is substituted for O_2 as the terminal acceptor of the electrons released during the oxidation of organics and several inorganic compounds. The overall stoichiometry assuming a carbohydrate electron/energy source is:

$$\begin{aligned} &C_6H_{12}O_6 + 4.8NO_3^- + 4.8H^+ \rightarrow \\ &6CO_2 + 2.4N_2 + 8.4H_2O \quad (\Delta G = -2671 \text{ kJ/mole glucose}) \end{aligned} \tag{2.2}$$

The electrons are placed on the N atom in a sequence that generates a series of compounds of sequentially lower and lower redox potential in a series as follows with the top line of numbers (+5 through 0) representing the redox state of the nitrogen atom in each molecule:

$$\overset{+5}{NO_3^-} \rightarrow \overset{+3}{NO_2^-} \rightarrow \overset{+1}{N_2O} \rightarrow \overset{0}{N_2} \tag{2.3}$$

Note that some organisms produce NO(+1) as an intermediate between NO_2^- and N_2O so that in some habitats, NO is a by-product of denitrification in a manner similar to N_2O. A true denitrifying organism is defined as one capable of carrying out the last step (i.e., having the complete reaction sequence) which requires the enzyme nitrous oxide reductase for which the *nos* gene codes.

Denitrification is generally considered to be a largely facultative, heterotrophic process with a large variety of organic molecules supplying electrons for the reduction of NO_3^- and the nitroxy intermediates. However, some inorganic compounds and elements have also been shown to be oxidized through autotrophic denitrification. For example, autotrophic strains of *Alcaligenes*, *Bradyrhizobium*, *Paracoccus*, and *Pseudomonas* can use H_2 as an energy and electron source. Additionally, several strains of autotrophic sulfur oxidizers (*Thiobacillus*, *Thiomicrospira*, and *Thiosphaera*) oxidize reduced sulfur for energy while denitrifying NO_3^- to N_2 as a terminal electron acceptor (Myrold 1998).

When the full pathway of denitrification was determined, it was clear that nitroxyanions were intermediates in the sequential placement of electrons on the nitrogen atom. NO and N_2O are both gasses and as such are prone to leak from open systems in which denitrification is occurring. When denitrification occurs in a closed system, such as a sealed laboratory microcosm, N_2 is quantitatively recovered, although NO and N_2O may be observed as ephemeral intermediates. The efflux of N_2O in open systems is strictly a physical leakage of the N_2O as it degasses from the point of production prior to final reduction to N_2. N_2O is of major environmental concern, as it is the largest contributor to stratospheric ozone depletion (Ravishankara et al. 2009), and it has a stated greenhouse gas potential (global warming potential) of about 300 compared with CO_2 whose greenhouse potential is, by definition, 1.0 (U.S. Environmental Protection Agency 2013). Denitrification is not the only source of N_2O in the atmosphere, however. Nitrification also results in N_2O flux to the atmosphere (Bremner et al. 1980). The pathways for formation of

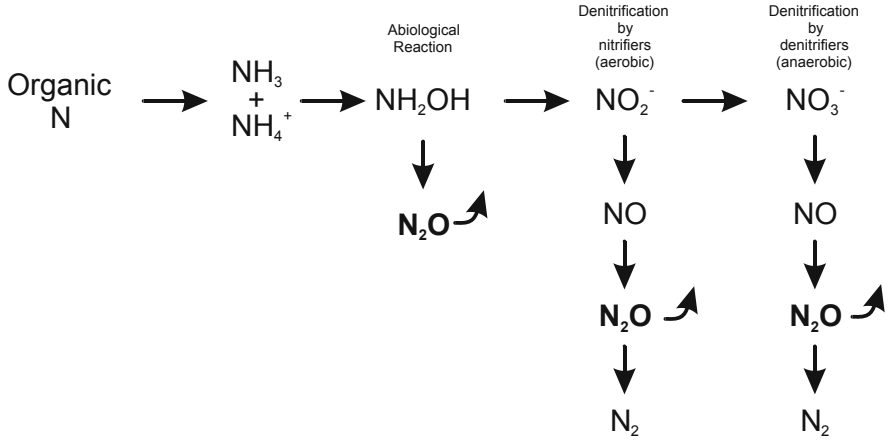

Fig. 2.6 Biological processes that produce N_2O that can flux to the atmosphere. Information for figure taken from McLain and Martens (2005) and from Baggs (2008). The upturned arrows indicate leakage to the atmosphere from the site of formation

N_2O that leaks into the atmosphere have been elucidated and reviewed (Baggs 2008; McLain and Martens 2005). In addition to denitrification, other important routes for N_2O production exist including an abiological transformation of hydroxylamine (NH_2OH) to N_2O, as well as nitrifier denitrification, an aerobic process involving nitrite reduction (Fig. 2.6).

2.2.3.3 Dissimilatory Nitrate Reduction

Dissimilatory nitrate reduction to ammonium (DNRA) serves the same purpose to the microbe affecting the process as does denitrification, except that the end product is ammonium which is often retained in the local environment rather than escaping as a gas. The stoichiometry of the reaction is exactly as seen in Eq. 2.1 for assimilatory reduction. Although DNRA (also called nitrate respiration, nitrate reduction, or even ammonification by some) has been long known, it has been recognized as an important process with major environmental and ecological significance only since the latter part of the twentieth century. Indeed, depictions of the nitrogen cycle that appeared in textbooks and articles of the 1960s and 1970s had not yet recognized the process as important and generally did not include it as an important process. As a single example, Alexander's classic text on soil microbiology (Alexander 1977) lumped assimilatory and dissimilatory reduction together and really did not consider dissimilation to ammonium as other than an interesting phenomenon that was not likely quantitatively important. The realization that substantial amounts of DNRA occurred in some habitats came about in the late 1970s and early 1980s when a number of authors began to publish works on "non-denitrification losses of nitrate" or "nitrate sinks" in (especially) coastal marine

sediments and intertidal flats (Hasan and Hall 1977; Kaspar et al. 1981; Keith et al. 1982; Kaspar 1983), and others began to point to the organisms responsible for the processes in a variety of environments (e.g., Caskey and Tiedje 1979).

Since that time, a substantial body of evidence points to the importance of DNRA in many environments, but especially coastal systems (e.g., Giblin et al. 2013; An and Gardner 2002), and in locations where there are alternating aerobic vs. anaerobic conditions such as bioreactors (Behrendt et al. 2014; Mazeas et al. 2008; Shu et al. 2016; van den Berg et al. 2015). Giblin et al. (2013) is an especially useful review of the importance of DNRA in coastal environments. Additionally, investigators have commented on the ecological differences between DNRA and denitrification. There are both advantages and disadvantages to each, depending on the system, and the advantages of the two processes operate in opposition. Denitrification represents the main way whereby reactive N (N_r) is converted to nonreactive N_2. This is obviously a global advantage as the amount of N_r continues to increase, primarily as a result of industrial fixation. For the agriculture industry, however, denitrification can represent a major loss of crop nutrient which equates with increased production cost due to the need for even more fertilizer. Furthermore, that loss only invites the fixation of even more N_r via the Haber-Bosch process. The process of DNRA, on the other hand, conserves N in the system as ammonium, NH_4^+. Thus, N_r is not removed, although that N_r does not necessarily remain at the location in which the DNRA occurred. For example, the reduced product can be lost to the atmosphere as NH_3 under the proper conditions, e.g., alkaline pH. In DNRA both nitrogen and energy are conserved within the system, as the ammonia represents the energy source for primary production by the autotrophic nitrifiers. This situation is strictly analogous to that of the sulfur cycle in coastal marine systems, where SO_4^{2-} reduction results in production of sulfide that is retained in the system as a source of energy for additional primary production by chemoautotrophic sulfur oxidizers (Howarth and Teal 1980).

The fact that DNRA competes favorably with denitrification does not follow directly from thermodynamic considerations. When respiring glucose, the free energy yield of denitrification is, theoretically, about 93% of that gained from oxygen, whereas the yield for DNRA is only about 65% (Strohm et al. 2007). These authors found that the energy yields to the cells were lower than might be anticipated from the thermodynamics and that the yield to denitrifiers was even lower than that to the organisms reducing NO_3^- to NH_4^+. This finding suggests an imbalance in ATP generation such that nitrate reducers might, indeed, be more competitive than denitrifiers under some conditions, e.g., NO_3^--limiting conditions.

Comparison of the magnitude of denitrification and DNRA on a global scale is difficult, because DNRA results in a product which continues to be recycled in the system. Some authors have made credible comparisons for the amounts of processes such as denitrification, DNRA, and assimilation for limited areas where tracer studies employing ^{15}N can help to identify the fate of the nitrate that is removed from the environment. In one such study, Marchant et al. (2014) determined the fate of nitrate in permeable intertidal sediment of the Wadden Sea. This eutrophic body

has some of the highest measured coastal denitrification rates, but only about 50% of the NO_3^- removal from the sediments can be credited to denitrification. When the $^{15}N\text{-}NO_3^-$ was added to sediments as a tracer, the dominant NO_3^- sink was denitrification (50–75%), and DNRA accounted for 10–20% of the NO_3^- consumption. These authors noted that 20–40% of the added tracer entered an intracellular pool of NO_3^- that was subsequently respired when NO_3^- became limiting. The authors further noted that a not insignificant fraction of the stored NO_3^- was actually respired by eukaryotes, a finding that has not been reported or confirmed since this recent publication but, if and when verified, could be an important part of the nitrogen story in at least some eutrophic coastal systems. Others have added credence to the importance of DNRA in coastal environments; An and Gardner (2002) noted that added NO_3^- in the form of an $^{15}NO_3^-$ amendment at about 100 μm did not stimulate denitrification, but did stimulate DNRA to levels similar to denitrification. They further speculated that high DNRA compared with low denitrification might be attributed to high sulfide concentrations, in that as opposed to nitrification and denitrification which are inhibited by high sulfide, DNRA might benefit from high sulfide due to its availability as an electron donor. Again, An and Gardner (2002) pointed out the role of DNRA in preserving nitrogen in their study site, Laguna Madre in Texas, which has only limited water exchange with other water bodies around it. Koop-Jakobsen and Giblin (2010) reported that in surface sediments of a New England salt marsh, DNRA was comparable to denitrification, but was not of major importance elsewhere (i.e., deeper in the rhizosphere, water column, etc.).

A number of studies have reported DNRA rates in various coastal environments including a variety of *Spartina* marshes (Koop-Jakobsen and Giblin 2010; Poulin et al. 2009; Smyth et al. 2013; Tobias et al. 2001a), intertidal freshwater marshes (Neubauer et al. 2005), marshes receiving nitrogen inputs from groundwater or tidal flooding (Porubsky et al. 2011; Tobias et al. 2001b; Vieillard and Fulweiler 2012), and mangroves (Fernandes et al. 2012; Molnar et al. 2013). The results indicate that the significance of DNRA varies widely and can range from less than 3% to 60–90% of the total reduction of nitrate (Giblin et al. 2013).

In soil and freshwater environments, the results are even more variable, but DNRA appears to be of less importance there relative to denitrification. In freshwater wetland sediments, Morrissey et al. (2013) demonstrated that denitrifiers were predominant in locations with rich resources, whereas those that reduce nitrate to ammonia prevailed in resource-poor locations. In a study of streams in Quebec with high NO_2^-, the NO_2^- accumulation was attributed to both denitrification and DNRA, but did not indicate a predominance of one process over the other (Corriveau et al. 2010). When examining the effect of bioturbation in sediment on the reduction of nitrate in microcosms, Nogaro and Burgin (2014) noted that denitrification was nearly always predominant. In studies of NO_3^- removal within stream sediments in several low-relief, sandy-bottomed stream through which agriculturally derived, NO_3^--rich groundwater is discharged to the stream channel, we have never observed NH_4^+ or NO_2^-, even though NO_3^- concentrations go from around 15 to 20 mg N L^{-1} at a depth of 70 cm below the sediment surface to between 1 and 4 mg N L^{-1} as the water enters the stream (Flewelling et al. 2012, 2013; Gu et al. 2007; Mills et al. 2008).

At this point in time, DNRA seems to be important in coastal marine environments under some conditions, although complete definition of those conditions has yet to be achieved. Factors that seem to influence the balance of denitrification include temperature (Ferron et al. 2009; Gardner and McCarthy 2009; Smyth et al. 2013), salinity (Gardner et al. 2006; Giblin et al. 2010), and amount and availability of both labile organic carbon and nitrate (Morrissey et al. 2013; Strohm et al. 2007). The organisms comprise both obligate and facultative anaerobes (e.g., *Clostridium*, *Selenomonas*, *Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Vibrio*) along with a few aerobes (*Bacillus*, *Neisseria*, *Pseudomonas*) and microaerophiles (*Campylobacter*) (Tiedje 1988). It is interesting to note that while denitrifiers primarily use respiratory pathways, organisms carrying out DNRA tend to be fermenters (Maier 2000).

2.2.3.4 Anaerobic Ammonium Oxidation (Anammox)

One of the two new processes in nitrogen cycling is anaerobic ammonium oxidation, or anammox. This process was first observed in bioreactors that were operated to denitrify (Mulder et al. 1995) during treatment of effluent from a methanogenic reactor. There are several newly discovered prokaryotic organisms responsible for anammox, and they are all members of the bacterial phylum *Planctomycetes*. The phylum currently contains five genera (in various stages of acceptance, hence, the names are often preceded by the term *Candidatus*, meaning the name is proposed for attribution to the organisms) (Jetten et al. 2010). Four of the genera, *Kuenenia* (Schmid et al. 2000), *Brocadia* (Kuenen and Jetten 2001; Strous et al. 1999), *Anammoxoglobus* (Kartal et al. 2007), and *Jettenia* (Quan et al. 2008), were enriched from activated sludge, while the fifth genus, *Scalindula*, was enriched from several natural habitats; the *Planctomycetes* seem to be most prevalent in aquatic zones of oxygen minima and in marine sediments (Kawagoshi et al. 2010; Kindaichi et al. 2011a, b; Lam et al. 2009; Nakajima et al. 2008; Penton et al. 2006; Schmid et al. 2007; van de Vossenberg et al. 2008; Woebken et al. 2007). Much of the available taxonomic information about the anammox-capable *Planctomycetes* comes from molecular genetic data including 16-S rRNA sequences (van Niftrik and Jetten 2012), although enrichment of the organisms, especially those in bioreactor systems, allows investigation of cell properties and physiological activity (e.g., Nakajima et al. 2008; Strous et al. 1998; Toh et al. 2002; van de Vossenberg et al. 2008; Egli et al. 2001). The anammox-capable members of the *Planctomycetes* differ significantly in terms of cell structure and cellular composition. It is beyond the scope of this writing to provide a detailed description of those differences, but an excellent review describing the organisms and their activities in detail was provided by van Niftrik and Jetten (2012), and the reader is referred to that article for that detailed description.

The anammox-capable bacteria have received much attention since the first reports in the late 1990s. Their presence is widespread in nature, and several reports have focused on their numbers in a diverse range of habitats (e.g., Dale et al. 2009;

Jetten et al. 2003; Moore et al. 2011; Penton et al. 2006; Rooks et al. 2012; Schubert et al. 2006; Woebken et al. 2007). In many cases, the enumerations are based on nucleic-acid sequence information which can yield quantitative data about the numbers of organisms, but information about their activity is not always extractable from such examinations. It is logical, therefore, to assume that the importance of the role of these interesting organisms may be either over- or underestimated when inference is made on the basis of number of copies of the biomarker gene. In many locations the organisms can be detected and even enriched, but their role in N_2 formation in the system under study may be a small one. For example, in bioreactors operated under alternating aerobic and anaerobic conditions (fill and drain) designed to reduce BOD and remove nitrogen in a liquid similar to domestic wastewater, anammox-capable organisms were detected using FISH in nearly all samples. When their numbers were compared with AOB and NOB (also enumerated with FISH counts), the nitrifiers exceeded the anammox-capable organisms by 2–4 orders of magnitude (Battistelli 2013). Clearly, one must conclude that if the anammox-capable organisms were functioning at all, their importance was much less than that of the nitrifiers. Nevertheless, the use of ^{15}N tracers can not only determine the source of N_2 emitted but can also be used to quantify the magnitude of the process (Rich et al. 2008; Song and Tobias 2011).

The existence of some organism providing a similar function as the anammox organisms was predicted several decades ago based on the observation that many parts of the anoxic deep ocean lacked the NH_4^+ expected based on the overall stoichiometry of organic inputs into those deeper zones (Broda 1977; Hamm and Thompson 1941; Richards 1965), but it was not until the discoveries of the 1990s that the riddle was solved. The amount of missing ammonia is massive, so it cannot be disputed that in many environments, anammox is, indeed, a dominant process that has global implications. Immediate benefit to wastewater processing in terms of nitrogen removal from the liquor without substantial buildup of biomass (i.e., sludge) is at least partially responsible for the high activity devoted to examining and attempting to exploit the anammox organisms in many types of bioreactors.

2.2.4 Nitrogen Fixation

2.2.4.1 Biological Nitrogen Fixation

A number of bacteria and Archaea, including heterotrophs and autotrophs, have evolved the ability to reduce atmospheric nitrogen to ammonia:

$$N_2 + 3H_2 \rightarrow 2NH_3 \tag{2.4}$$

The responsible enzyme is nitrogenase, and there is remarkable homology in the structure and function of the enzyme across all the organisms that produce and use it. There are three major guilds of bacterial nitrogen fixers: free-living autotrophs

(primarily *Cyanobacteria*), free-living heterotrophs, and symbiotic fixers. There is also nitrogen fixation within several methanogenic Archaea (Belay et al. 1984; Leigh 2000; Murray and Zinder 1984). Note that the symbioses are obligate only for the microbes to fix nitrogen. The bacterial symbionts can be found easily in soil and water, but they do not fix nitrogen outside the plant host.

Biological nitrogen fixation (BNF) is segregated into two major classes; natural BNF is that which occurs without intervention from humans. Conversely, the other type of biological nitrogen fixation is due to the active cultivation of crop plants that host symbiotic nitrogen-fixing microbes. Globally, natural BNF in terrestrial habitats adds about 58 Tg N/year which is much lower than the estimated 120 Tg N/year prior to industrialization (Fowler et al. 2013). The reason for the sharp decrease in natural BNF is considered to be the loss of suitable habitats for the process to occur. A large amount of BNF is carried out by *Cyanobacteria* in the oceans, and there is large uncertainty in the values; however, a number of investigators report values between 100 and 150 Tg N/year (Voss et al. 2013). Cultural (agricultural) biological fixation has increased from an estimated 15 Tg N/year in 1860 to around 60 Tg N/year at present (Fowler et al. 2013). Greater reliance on agricultural nitrogen fixation has improved diets in many countries where the cost of fertilizer to farmers is prohibitive. It is of interest that in areas where agriculture is practiced on the industrial scale (e.g., the United States), industrial nitrogen fertilizers are often added to legume crops such as soybean to ensure a successful yield. In these cases, the cost of fertilizer is a small portion of the total cost of crop production, and many farmers consider it valuable insurance against crop failure. Current practices for "sustainable" agriculture generally recommend reliance on legumes without additional fertilization (which inhibits BNF in both free-living and symbiotic organisms). Indeed, in many cases, inoculation with crop-specific bacteria has been recommended to enhance the nitrogen fixation process and increase yields (Pennsylvania State University Extension Service 2016). In some cases, inoculation of nonlegumes with *Azospirillum* has proven to be beneficial for nitrogen fixation and plant nutrition in the tropics (Bashan and Holguin 1997), although often inoculation of microbes into soil or water leads to rapid microbial die-off and little beneficial effect. With increasing temperatures associated with climate change, the range where augmentation of agricultural fields with microbes for purposes of enhancing nitrogen fixation may extend further into regions that are currently more temperate.

There are several factors associated with the changing climate that are anticipated to increase BNF in the near to intermediate term. For example, Fowler et al. (2015) estimate that the increase in atmospheric CO_2 (and concomitant decrease in pH) will result in an increase in BNF between 35 and 121% by the beginning of the next century. They further estimate that the expansion of the habitat for diazotrophs will result in an increase in BNF of around 27%. Several other factors listed by those authors point to additional increases in BNF that will contribute to greater concentrations of N_r in the foreseeable future, whether or not industrial fixation continues to increase.

2.2.4.2 Industrial Nitrogen Fixation by the Haber-Bosch Process

In 1909, Fritz Haber, a German chemist working with the chemical company BASF, discovered a way to react N_2 from the atmosphere with H_2 to produce NH_3. Carl Bosch, then, moved the process to an industrial scale within a year. Eventually and separately, both men received Nobel Prizes for their work in high-pressure technology. The reaction stoichiometry is exactly the same as that for biological nitrogen fixation (see Eq. 2.4). Haber did not have fertilizers in mind during his work; rather his efforts were largely directed at producing gasses to be used as antipersonnel weapons. However, because a ready source of ammonia allowed for production of many types of munitions, a large number of factories were constructed for ammonia synthesis using the Haber-Bosch process. After the end of the Second World War, the presence of these factories with no apparent use inspired their conversion to fertilizer manufacturing facilities. The drastic increase in N_r from the Haber-Bosch process seen at that time (see Fig. 2.1) is a direct result of the repurposing of those plants and the building of even more such facilities in other developed countries.

What the microbes have been able to do at environmental temperatures and pressures can only be accomplished industrially at high temperatures (400–500 °C) and pressures (15–25 MPa or 2200–3600 psi) in the presence of metal catalysts (at different times uranium and ruthenium were used, although a relatively inexpensive iron-based catalyst was developed by Bosch and is most commonly used today). The cost of industrially fixed nitrogen is tied closely to energy costs as the primary source of hydrogen is methane (natural gas), and methane is also often used to generate the high temperatures through combustion and pressures through electricity from gas-fired turbines.

At present, the global production of N_r by the Haber-Bosch process stands at about 120 Tg N/year, with 100 Tg of that going to fertilizer. The use (and, therefore, production) continues to increase, especially in developing countries. In the United States, however, nitrogen fertilizer use has leveled off at about 20 Tg N/year, as compared with 50 Tg N/year in China and about 28 Tg N/year in India (FAO 2015).

2.3 Quantitative Aspects of the Modern Nitrogen Cycle

Thus, with the addition of Haber-Bosch fixation and anammox as new processes and the elucidation of a great deal of detailed information on the organisms and processes affecting nitrogen, the nitrogen cycle appears pretty much as it did around 1900. From a quantitative viewpoint, however, the cycling of nitrogen in the environment looks very different from the days of its discovery. The injection of large amounts of N_r by human activity has resulted in a large increase in the amount of N_r residing in the soil and water of the continents, the ocean, and the atmosphere. That increase has led to eutrophication of many water bodies, especially in the coastal zone, where nutrient enrichment has resulted in severe blooms of phytoplankton, which when

they die and decay have resulted in large areas of hypoxic waters frequently referred to as "dead zones." The most notable example of such a zone is off the mouth of the Mississippi River in the Gulf of Mexico (Childs et al. 2002; Rabalais et al. 2001). The increase in N_r has also resulted in greater concentrations of nitrogen oxides in the atmosphere. These compounds have relatively long residence times and are known to contribute significantly to stratospheric ozone depletion. Indeed N_2O is now the dominant ozone-depleting substance emitted to the atmosphere (Ravishankara et al. 2009).

A comparison of the global nitrogen cycle as it existed in the preindustrial, mid-nineteenth century, around 1860 (Galloway et al. 2004), to that existing today (Fowler et al. 2013) shows many differences that can be attributed to the increase in N_r as a result of increased nitrogen fixation, both industrial and biological (Fig. 2.7). Some of the equivalent numbers in the most recent estimates (Fowler et al. 2013) were obtained by different methods than used by Galloway et al. (2004) resulting in some differences in the values obtained. While specific values may be different, they do not negate the main conclusions that nitrogen fixation, both industrial and biological, has had a profound effect on the amount of N_r cycling through the environment. For example, while Galloway et al. (2004) determined a value of biological nitrogen fixation (BNF) due to agricultural activities of 31.5 Tg N/year, Fowler et al. (2013) reported the value of 60 Tg N/year calculated by Vitousek et al. (2013) using a different approach. Similarly the value of naturally occurring BNF of 107 Tg N/year reported in the 2004 paper was in contrast to 58 Tg N/year used in the 2013 paper. Nevertheless, the total N_r input for the two reports agreed well (268 vs. 273 Tg N/year). Other differences due to methodology are also apparent, but again, the conclusions about the comparisons between the nineteenth and twenty-first centuries are not substantively affected.

Since the advent of enhanced BNF due to cultivation and the use of N_r fixed industrially through the Haber-Bosch process, emissions of NOx and NH_3 from both terrestrial and oceanic environments have increased substantially (viz., a total of around 17 Tg N/year in 1860 as compared to a total of nearly 74 Tg N/year currently). Concomitantly, therefore, deposition of both oxidized and reduced N to the contents increased from about 11 Tg N/year in 1860 to 70 Tg N/year in the first years of the twenty-first century and from 8.5 to 30 Tg N/year for the same time period over the oceans. Riverine transfers from the continents to the ocean have increased from an estimated 20 Tg N/year to as much as 80 Tg N/year. Despite the very large uncertainty in values of denitrification (Fig. 2.7), it is clear that removal of N_r has not kept pace with the increases such that the amount of N_r in the global ecosystem has increased drastically, continues to do so, and will likely continue well into the future (Galloway et al. 2004). Indeed, Fowler et al. (2015) estimate that the total production of N_r will increase to about 600 Tg N/year around the year 2100 from its current 483 Tg N/year (sum of all inputs in Fig. 2.7).

One of the most important reasons for the drastic increase in demand for N is a shift in diet in many developing countries. For example, China is increasing its per capita consumption of meat as it becomes more westernized socially. In the period from 1975 to 2012, consumption of meat in China increased from about 8 million

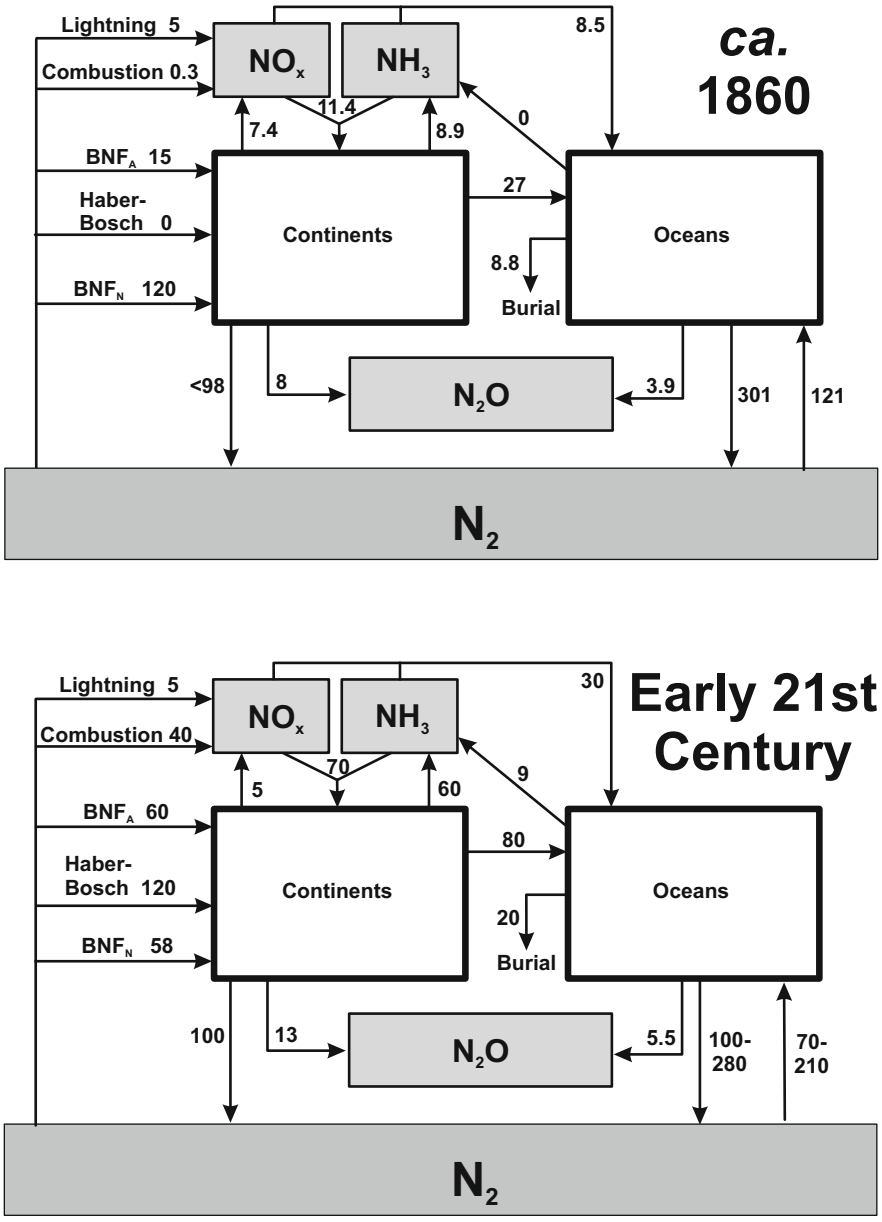

Fig. 2.7 Comparison of global N fluxes (Tg N/year) in the mid-nineteenth century and the early twenty-first century. The large inputs of N_r from industrial fixation and enhanced agricultural fixation have caused notable increases in the N_r fluxes among the pools, e.g., the riverine flux from the continents to the ocean increased from 27 to 80 Tg N/year. Storage in the pools has also increased (data not shown). Data and concept for the drawing are taken from Galloway et al. (2004) and Fowler et al. (2013)

tons to just over 70 million tons. Of that 2012 value, the largest fraction is pork, at 52 million tons (Larsen 2012). Although the per capita consumption of meat by the Chinese is substantially less than that of countries such as the United States or Australia (the two leading countries in per capita consumption), the large population there makes the total consumption much greater. The amount of nitrogen (in the form of fertilizer) needed to produce a unit weight of meat for consumption varies depending on the animal or animal product being consumed, but it is invariably well in excess of a 1:1 relationship. For example, Chatzimpiros and Barles (2013) calculated that to produce 1 unit of pork nitrogen required 7.63 units of N to be consumed as fertilizer, BNF, etc. By their calculation, milk production is less expensive in terms of N, with 6.7 units of N input to obtain a single unit of N in the milk. Beef, however, is highly N consumptive, requiring 12.65 units of N input for every unit of N in the final product. Clearly, then, conversion of diets from primarily vegetable to meat will only accelerate the demand for nitrogenous fertilizer.

2.4 Conclusion

The nitrogen cycle was nearly completely described qualitatively in the nineteenth century, i.e., nearly all the important processes had been discovered and described. Many details of the processes were added through the twentieth century, e.g., the importance of nitrification in the release of N_2O to the atmosphere. The two added processes, industrial fixation and anammox, have altered the quantitative aspects of the cycling of nitrogen drastically, perhaps irrevocably. The overall global nitrogen cycle was thought to be in balance in the nineteenth and early twentieth century, with additions to N_r from biological nitrogen fixation being balanced by the removal of N_r by denitrification. The massive input of N_r due to industrial-scale fixation by the Haber-Bosch process exceeds the rate at which the combination of denitrification and anammox can remove it and the concentration of N_r continues to increase. The resulting alteration of the environment with respect to changes such as eutrophication, additional global warming, and continued ozone depletion due to increasing concentrations of N_r remains to be seen.

Compliance with Ethical Standards

Conflict of Interest Aaron L. Mills declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Chapter 3
Integrating Soil Microbiology into Ecosystem Science

David A. Lipson and Xiaofeng Xu

Abstract There has been an increasing effort to incorporate the inner workings of soil microbial communities into conceptual and quantitative models of processes at the ecosystem or global scale. Many studies show that the characteristics of microbial species and their interactions with each other and with plants strongly influence larger-scale processes and that explicitly including microbes can improve the performance of ecosystem models. We review the current understanding of how the physiology and community structure of soil microbial communities can impact cycling of carbon (C), nutrients, and greenhouse gases and recent progress in integrating this knowledge into quantitative models of ecosystems and climate change. Microbes can be characterized by ecological strategies that influence carbon use efficiency, stress physiology, elemental ratios (stoichiometry), production of extracellular enzymes, and responses to temperature. Competitive, synergistic, and trophic interactions within soil microbial communities influence process rates and responses to climate change. Plant-microbe interactions are central in climate change responses of ecosystems and can operate by changes in nutrient cycling or through alterations in the balance of mutualists and parasites. There are trends that connect broad-scale community structure with functioning and evidence that ecological roles of microbes can be mapped to phylogeny at the genus or species level. Models that explicitly simulate microbes have included their physiological limits, growth kinetics, interactions with plants, stoichiometry, dormancy, community structure, and community interactions. Given recent advances in conceptual frameworks for microbial ecology and in techniques for describing microbial communities and computing power, further progress will depend on increased interactions between microbiologists and modelers.

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3.1 Introduction

It has long been known that microbial communities play a central role in biogeochemical cycles and potential biological feedbacks to climate change (Alexander 1964; Baes et al. 1977). Despite this recognition it has been a long-standing challenge in microbial ecology to incorporate the inner workings of microbial communities into conceptual and quantitative ecosystem models (Schimel 1995). Environmental microbial communities are notoriously diverse and dominated by species that resist attempts at cultivation (DeLong and Pace 2001; Rappe and Giovannoni 2003; Yarza et al. 2014; Youssef et al. 2015). The past two decades have seen great technical advances in describing the diversity and metabolism of uncultured microbes, and many previously uncultured bacterial phyla have recently been isolated in pure culture (George et al. 2011; Stewart 2012). Interdisciplinary studies that combine a wide range of techniques to study microbial communities and their role in the environment have become increasingly common. These studies highlight the relevance of microbial ecology and physiology for ecosystem models, but incorporating complex microbial processes and community structure into already complex models is a daunting task. Large uncertainties in coupled climate models arise from biological feedbacks; however, microbes are not yet explicitly included in models used by the Intergovernmental Panel on Climate Change (IPCC) (Hararuk et al. 2015; Wieder et al. 2015; Luo et al. 2016). Given the computational costs of adding more model components and the qualitative nature of much microbial research, it is not obvious how ecosystem models should be modified to fit the emerging understanding of how microbial communities function (Chapin et al. 2009; Lawrence et al. 2009; Todd-Brown et al. 2011).

So, when is a detailed biological understanding of soil microbial communities necessary for predicting ecosystem processes? Models assume microbial activity can be predicted from a set of environmental drivers, like temperature and soil moisture (Schimel 2001; Bloom et al. 2010). This is insufficient when:

1. Microbial activities are driven by variables that are not included in the model, such as soil texture, nutrients, trace metal availability, and specific interactions with plant species. These extraneous variables could control microbial function either directly or indirectly, through effects on the community composition.
2. The microbial community composition is unpredictable due to dispersal limitation and disturbances such as pulse dynamics or local soil properties.
3. There are community interactions such as competition, synergism, and predation that alter rates in complex ways that are hard to predict based on the standard environmental variables.

Figure 3.1 outlines the various ways that the properties of soil microbial communities and the species that comprise them can scale up to have impacts on ecosystem functions ranging from plot, regional, and global scales. In this chapter, we summarize the current understanding of how the physiology and community structure of soil microbial communities can scale up to impact cycling of C,

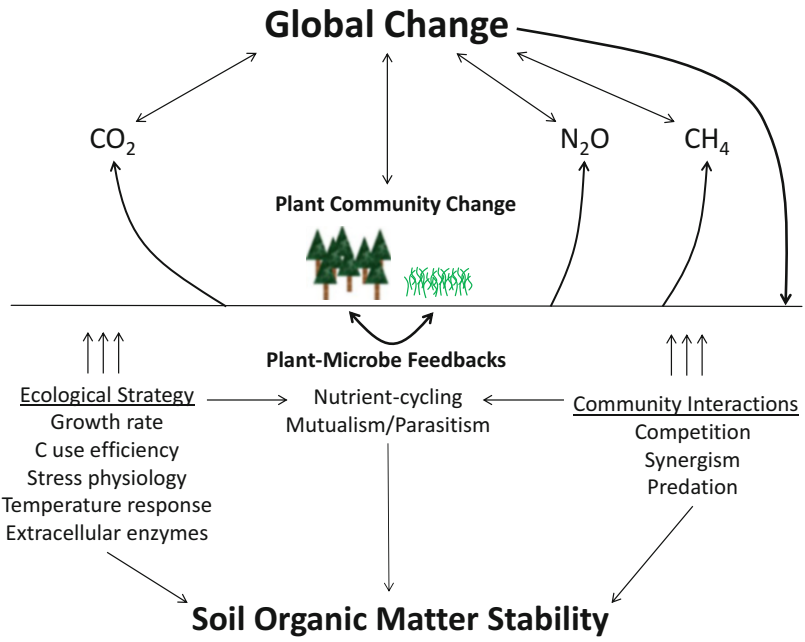

Fig. 3.1 Microbial traits and community interactions that might have significant impacts at the ecosystem and global scale. Effects are shown as arrows. Through their ecological strategies and interactions within the community and with plants, microbes impact trace gas fluxes and the C balance of ecosystems, influencing climate change, which in turn feeds back on all these processes and characteristics

nutrients, and greenhouse gases and the recent progress in integrating this knowledge into quantitative models of ecosystems and climate change. A great deal of thought has been given to this subject in recent years, and numerous useful reviews have been published on how soil microbes impact ecosystem processes (Schimel and Schaeffer 2012; Wallenstein and Hall 2012; Nemergut et al. 2014; Sinsabaugh et al. 2014; Bier et al. 2015; Ferris and Tuomisto 2015; García-Palacios et al. 2015; Nielsen et al. 2015; Zechmeister-Boltenstern et al. 2015; van der Putten et al. 2016), incorporating microbes into models (Todd-Brown et al. 2011; Treseder et al. 2012; Nazaries et al. 2013; Wieder et al. 2015; Luo et al. 2016), and the impacts of climate change and other disturbances on microbes (Bradford 2013; de Vries and Shade 2013; Griffiths and Philippot 2013; Classen et al. 2015) and their extracellular enzymes (Burns et al. 2013; Henry 2013).

3.2 Physiological Traits that Scale to Ecosystem Processes

3.2.1 Carbon Use Efficiency

Respiration by soil microbes accounts for about half of the CO_2 efflux from terrestrial ecosystems (Ciais et al. 2013). Therefore, the rate and efficiency of soil microbial respiration are key factors regulating the C balance of ecosystems. Carbon use efficiency (CUE), the fraction of C consumed by microbes that is converted to biomass as opposed to CO_2, is influenced by factors such as temperature, substrate quality, and the ecological strategies of the microbes that comprise the community. Consequently, CUE is emerging as an important characteristic of microbial communities that can strongly influence the C balance of ecosystems and their response to climate change (Lipson et al. 2009; Allison et al. 2010; Manzoni et al. 2012; Cotrufo et al. 2013; Sinsabaugh et al. 2013; Allison 2014). There is often a trade-off observed between maximum growth rate and growth yield, as thermodynamic constraints generally prevent microorganisms from being both fast-growing and efficient (Lipson 2015). This can lead to an axis representing two contrasting life history strategies: slow-growing efficient microbes that thrive under resource scarce conditions versus fast-growing, inefficient microbes that dominate under resource-rich conditions (Kreft and Bonhoeffer 2005). These trade-offs and ecological strategies that they produce have direct impacts on C cycling. A fast-growing, inefficient microbial community produces much more CO_2 per unit biomass than a slow-growing community (Lipson et al. 2009). Studies in a high-elevation forest found that seasonal changes in the soil microbial community and its predominant growth strategy had major repercussions for ecosystem respiration, in particular in the late winter, when a cold-adapted, fast-growing community developed under the thick, protective snow pack, where high concentrations of sugars accumulated due to frost damage of roots (Monson et al. 2006; Scott-Denton et al. 2006; Schmidt et al. 2009). A simple model that included microbial growth kinetics and temperature response parameters performed well in predicting ecosystem respiration (Lipson et al. 2009).

Furthermore, CUE may play a major role in microbial and ecosystem responses to climate change. A modeling study found that warming led to decreased CUE, which in turn led to reduced microbial biomass, limiting C loss from soils under warmer temperature regimes (Allison et al. 2010). A follow-up modeling study found that a trade-off between growth rate and yield would prevent adaptation of higher-yield microbes under this warming scenario, thereby maintaining this limit to C loss from soils (Allison 2014). These studies could explain earlier experimental results showing "acclimatization" of soils to warming treatments, in which the temperature sensitivity of soil respiration decreased in warmed plots (Luo et al. 2001). This so-called acclimatization has often been attributed to changes in substrate availability in warmed soils, but there are also changes in microbial community structure and function that can produce these effects (Wei et al. 2014; Osanai et al. 2015). However, it is uncertain whether global warming will result in a sustained decrease in CUE or whether microbes will adapt their CUE over time (Dijkstra et al. 2011;

Tucker et al. 2013). Reductions in CUE with warming have been reported (Steinweg et al. 2008; Manzoni et al. 2012), but there is evidence that the CUE of microbial communities may adapt in the long term (Bradford et al. 2008; Bradford 2013; Frey et al. 2013; Tucker et al. 2013). Increased temperature can also increase rates of microbial turnover in soil, which can have the opposite effect of decreased CUE, increasing the stability of soil organic matter (Hagerty et al. 2014). Interpretation of these studies is further complicated in that microbial temperature responses are sensitive to the chemistry of soil organic matter (Wagai et al. 2013), which can change in response to warming as the decomposition rates and inputs of different forms of soil organic matter shift. Similarly, mineral nutrient availability can also have a strong influence on CUE (Keiblinger et al. 2010; Manzoni et al. 2012; Sinsabaugh et al. 2016). In summary, CUE represents a strong potential link between microbial communities and the global C cycle, and so resolving the uncertainties in this area and incorporating this knowledge into coupled climate change models should be a priority.

3.2.2 Microbial Temperature Responses

Microbial responses to temperature can shape ecosystem processes in unexpected ways. The temperature sensitivity of microbial activities, such as respiration or enzyme activity, is often described as an apparent Q_{10}, the proportional increase in activity with a 10 °C increase in temperature. The Q_{10} concept was initially developed for simple chemical reactions, and so its application to complex microbial processes in the natural environment presents some difficulties. First of all, biological processes depend on enzymes, which are only functional within a certain temperature range, and so microbial growth is best described by a square root function rather than an exponential Q_{10} (Arrhenius) function. Furthermore, biological processes such as microbial respiration are the result of many different enzymes and transport processes, both within the cell and in the environment. In soils very high apparent Q_{10}s are observed around the freezing point of water, resulting from changes in the thickness of unfrozen water films in subzero soils, changes in the physiological state of microbes, and growth of microbes over time (when the Q_{10} is generated from respiration data collected in the field over a period of several weeks) (Mikan et al. 2002; Monson et al. 2006; Panikov et al. 2006; Schmidt et al. 2009). The optimal temperature ranges for microbial growth vary greatly from psychrophiles [less than 15 °C by definition, some at least as low as 5 °C (Morita 1975)] to hyperthermophiles (optimal temperatures over 80 °C), and so clearly the composition of the microbial community could affect how an environment responds to climate change in the short term. However, microbial communities appear to be well-adapted to the ambient temperatures they experience in their environment, and so changes in temperature can have a smaller impact on microbial processes than would be predicted from a model that assumes a simple Q_{10} relationship for all environments (Giardina and Ryan 2000). Maximum growth rates of bacteria are not

well correlated to their optimum temperature (Hanus and Morita 1968; Ratkowsky et al. 1982, 1983). The fastest-growing bacterium currently known, *Vibrio natriegens*, which doubles in about 9 min at 37 °C, is a mesophile (Maida et al. 2013), and one of the fastest-growing psychrophiles, *Pseudoalteromonas haloplanktis*, can give many thermophiles a run for their money (doubles in 4 h at 4 °C) (Piette et al. 2010). In the case of permafrost (permanently frozen soil layers found in polar regions), the communities in these frozen soils, despite being capable of growth at surprisingly low temperatures, are currently at suboptimal temperatures for growth (Bakermans et al. 2003; Steven et al. 2006), and melting will certainly lead to substantial losses of C (Schaefer et al. 2014; Schuur et al. 2015). However, current models do not capture the nuances of microbial activity at low temperatures. It is clear that microbial activity continues at low temperatures, for example, in permafrost and during the winter, and these activities are not yet fully incorporated into annual C cycling budgets and models (Clein and Schimel 1995; Brooks et al. 1996, 1997; Lipson et al. 1999; Monson et al. 2006; Panikov 2009; Zona et al. 2016). Despite adaptations for extreme cold, it is likely that winter microbial communities generally experience suboptimal temperatures and should be more sensitive to warming than the communities that are active in the summer. A model that incorporated seasonal microbial dynamics found large C losses in response to winter warming, when microbes were allowed to acclimate their temperature responses to changing seasons (Sistla et al. 2014). The Q_{10} values of respiration can vary by season and across altitudinal gradients, and these variations are correlated to changes in microbial community structure (Lipson 2007). These results highlight the importance of microbial characteristics and their seasonal dynamics for ecosystem processes.

3.2.3 *Stoichiometry of Microbial Cells in Relation to Soil Organic Matter*

The stoichiometric ratios of carbon, nitrogen, and phosphorus typically are expressed as C:N:P in plant litter, and soil organic matter in relation to that of microbial biomass are major determinants of C and nutrient cycling (Zechmeister-Boltenstern et al. 2015). While marine microbial primary producers have a relatively narrow range of atomic C:N:P ratios around 106:16:1, known as the Redfield ratio (Redfield 1958), land plants vary more in their nutrient concentrations due to structural materials like lignin and cellulose and variations in nutrient concentrations between lifeforms such as trees vs. herbs, deciduous vs. evergreen, etc. Microbes generally have higher N and P concentrations (lower C:N and C:P ratios) than do macroscopic organisms, and since microbes are the primary decomposers in soils, stoichiometric ratios in soil organic matter are driven toward that of the microbes over time. This occurs because N and P tend to be retained in microbial biomass but C is respired away as CO_2. When the C:N and C:P of decomposing litter drop below

a threshold value, which depends on microbial CUE, N and P from the litter are in excess of microbial demand and are mineralized and released back into the soil. While the range of element ratios is limited by the nature of cellular life on Earth, there is also considerable variation in C:N:P among microbial groups, particularly between bacteria and fungi. Microbes are also capable of storing C (e.g., as neutral lipids, glycogen, and starch) and also P (typically stored as polyphosphate) when these nutrients are in excess of immediate needs. Therefore different plant litter types lead to variations in stoichiometry of soil organic matter and microbial biomass, especially in terms of P concentrations (Cleveland and Liptzin 2007; Fanin et al. 2013; Hartman and Richardson 2013; Xu et al. 2013). While these variations in microbial element ratios are presumably driven by plants, there is the possibility that changes in microbial community structure could reinforce and accelerate changes in the plant community through their stoichiometry, as discussed in the Plant-Microbe Interactions section below.

There is a strong but complex relationship among stoichiometric ratios, ecological strategies, and CUE in microbes. Faster-growing microbes tend toward higher N and P concentrations and lower CUE (Keiblinger et al. 2010; Zechmeister-Boltenstern et al. 2015; Sinsabaugh et al. 2016). Faster growth rates require more enzymes, ribosomes, and RNA synthesis, increasing the demand for N and P (Gillooly et al. 2005; Hartman and Richardson 2013). Therefore, CUE might decrease with increasing P availability, as C:P ratios decline (Sinsabaugh et al. 2016). On the other hand, under conditions of nutrient limitation (which would occur with high C:N and C:P ratios of soil organic matter), microbial respiration can become decoupled from growth, leading to low CUE (Schimel and Weintraub 2003; Manzoni et al. 2012). In marine and aquatic ecosystems, P limitation commonly reduces bacterial growth efficiency (Del Giorgio and Cole 1998). Soil microbial growth is generally considered to be limited by labile C, even in relatively nutrient-poor soils (Heuck et al. 2015). However, P can limit microbial biomass in some tropical soils (Cleveland et al. 2002), and nutrient additions can stimulate microbial nutrient uptake and activity without necessarily causing an immediate increase in biomass (Jonasson et al. 1996; Schimel and Weintraub 2003; Allen and Schlesinger 2004; Sistla et al. 2012). The effects of high C:N and C:P ratios of plant litter can sometimes have negative effects on CUE (Spohn and Chodak 2015). It has also been shown that social interactions within microbial communities, such as when a subset of microbes "cheat" by not contributing to extracellular enzyme production, lead to different outcomes than those predicted by stoichiometric theory (Kaiser et al. 2014). In summary, ecological stoichiometry is a promising approach for scaling from microbes to ecosystems, but the relationships are complex due to potentially opposing forces of nutrient limitation and ecological strategies.

3.2.4 Extracellular Enzymes

Soil extracellular enzymes are a major link between the microbial community and its impact on the ecosystem, particularly through litter decomposition. The majority of organic inputs to soil are polymers like cellulose, lignin, and proteins that require extracellular enzymes for their degradation, and so extracellular enzymes usually catalyze the rate-limiting steps in the C and N cycles. For example, it was shown that litter decomposition was limited by the activity of phenol oxidases in anaerobic peatlands due to a lack of oxygen required by these enzymes (Freeman et al. 2001). The production and turnover of enzymes along with their substrate specificity, turnover rate, and temperature responses shape ecosystem processes. Shifts in the microbial community can change the compliment of enzymes in the soil, altering patterns of decomposition and nutrient cycling. Soil feedbacks in plant invasions are an example where this seems to be important (Henry 2013) (also discussed in the following section). Similarly, the role of the microbial community in stabilizing soil C could depend on the enzyme profile produced by the community, for example, through the relative dominance of fungi or bacteria (Waring et al. 2013). Because extracellular enzymes represent a cost for cells in terms of both C and N, and because they control rates of decomposition, extracellular enzymes tightly integrate C and N cycling with microbial physiology (Schimel and Weintraub 2003; Burns et al. 2013). Enzymes that degrade cell walls are often regulated by N availability, and it is thought that the main microbial motivation for producing them is increasing their access to N bound in this litter. For example, N deposition can alter soil C storage by reducing production of extracellular enzymes in oak forests with low litter quality (Waldrop et al. 2004). However, this relationship between N availability and lignin degradation varies by biome and microbial community (Sinsabaugh 2010). Another argument for explicitly considering extracellular enzymes in ecosystem-scale processes is that they represent a semiautonomous entity in themselves: they can be functional in the soil matrix after the cells that released them have already died, adding to the complexity of pulse dynamics in soils, such as drying-rewetting or freeze-thaw events (Burns et al. 2013). Furthermore, climate change could bring about changes in the stability of extracellular enzymes in the soil.

One potential advantage to explicitly including extracellular enzymes in ecosystem models might be to more realistically model temperature responses and acclimation to climate change. Enzymes and the whole microorganisms that produce them do not share the same temperature envelopes: psychrophilic enzymes have higher temperature optima than the optimal growth temperature for the whole cell (Huston et al. 2000), and thermophilic enzymes can function at higher temperatures than thermophilic microbes can tolerate (Cowan 2004). This disparity between temperature optima of microbes and their enzymes is one factor in decoupling of respiration and growth that can occur at supraoptimal temperatures in soils (Pietikäinen et al. 2005). Not only is the maximum catalytic rate (V_{max}) of enzymes controlled by temperature, but the substrate affinity (K_m) is also sensitive to temperature (German et al. 2012). This cross-latitudinal study found that soil extracellular

enzymes had higher affinity for their substrates at low temperatures, partly offsetting decreases in V_{max}, and in the case of β-glucosidase, the K_m of enzymes from an Alaskan soil were more sensitive to temperature than those from Costa Rica. This demonstrates that adaptations for high activity at low temperatures can come with a cost in affinity as temperatures are raised.

One final consideration that could earn enzymes (both intracellular and extracellular) some respect in global models is the requirement for trace elements. Enzymes involved in the production and consumption of greenhouse gases, CH_4 and N_2O, require metals such as Fe, Ni, Cu, Zn, Mo, and W (Glass and Orphan 2012; Wang et al. 2013b), and several phenol oxidases involved in lignin degradation require Cu or Mn (Sinsabaugh 2010). It is established that trace element limitation is a major factor in oceans (Morel and Price 2003) and that alternative nitrogenase enzymes (the key enzyme in N fixation) use V in soils that are poor in Mo (Bellenger et al. 2014). Local limitations in micronutrients could provide surprises in how the soil environment constrains microbial activity.

3.2.5 Stress Tolerance

The capacity for soil microbes to deal with stresses, such as drought or extreme temperatures, will influence ecosystem responses to disturbance and climate change (Schimel et al. 2007). Evolutionary trade-offs, such as the one between rate and yield, can constrain how microbial communities respond to climate change (Wallenstein and Hall 2012). For example, the ecological strategies of soil microbes may also predict the resistance and resilience of the communities to global change, with communities dominated by K-strategists hypothesized to be more resistant to disturbance and those dominated by r-selected microbes to be more resilient (de Vries and Shade 2013).

Local adaptations of microbial communities can lead to unpredictable responses to climate. Microbial responses to climate change experiments tend to vary by ecosystem (Castro et al. 2010; Weber et al. 2011; A'Bear et al. 2014b; Lipson et al. 2014; Classen et al. 2015), showing that microbial communities vary widely in their resistance to disturbance and stress. In arid and semiarid ecosystems, the drought resistance of soil microbes (especially fungi and their extracellular enzymes) and of biological soil crusts can lead to surprising levels of biological activity under very dry conditions (Collins et al. 2008; Austin 2011).

Dry ecosystems are subject to pulse dynamics from drying-rewetting events that cause the soil microbial community and biomass fluctuate rapidly, leading to ecosystem losses of C and N that are not captured by standard models (Collins et al. 2008; Inglima et al. 2009; Dijkstra et al. 2012). During these windows of disequilibrium, the local characteristics of microbial communities can have large impacts on ecosystem function (Lawrence et al. 2009; Placella et al. 2012; Kuzyakov and Blagodatskaya 2015). For example, different phylogenetic groups responded at different rates to rain pulses in California grasslands (Placella et al. 2012). In this

study, the set of bacterial groups implicated in immediate, intermediate, and delayed responses was surprising, as the rapid responders included *Actinobacteria* and *Verrucomicrobia*, groups generally considered slow-growing and K-selected, while the delayed responders included *Proteobacteria* associated with rapid growth rates. The authors found that the rapid responders already had high ribosome levels in the pre-wet soils, indicating that the stress resistance of these microbes contributed to their ability to rapidly exploit these pulses, and speculated that the extracellular enzymes these bacteria are known to produce may have been stabilized in the soil matrix and instantly available for activity upon rewetting. Conversely the fast-growing, less stress-tolerant microbes represented a smaller, dormant pool before wetting and therefore required time to become active and grow. Spore formers (*Bacillus* spp.) showed an intermediate response, as they also required time to germinate but are adapted to rapidly respond to such pulses.

In arid and semiarid ecosystems, biological soil crusts can have major impacts on ecosystem functioning and responses to pulses (Austin et al. 2004; Delgado-Baquerizo et al. 2013). Collins et al. (2008) proposed a "fungal-loop" model for arid ecosystems in which a network of fungi connect plants and biological soil crusts, allowing interchange of fixed N and C. Biological soil crusts are sensitive to disturbance, including N deposition and climate change (Johnson et al. 2012). The continued loss of soil crusts could drastically alter processes such as N fixation, C storage, and plant community dynamics in these ecosystems (Bowker et al. 2014). Incorporating the spatial heterogeneity of biological soil crusts and the "resource islands" produced by patchy plant distribution in dry ecosystems would pose a challenge for ecosystem models but might be worth the effort in terms of improving understanding of processes in arid ecosystems (Austin 2011).

3.3 Microbial Community Interactions that Impact Ecosystem Processes

3.3.1 Microbial Food Webs

Protozoal grazers, soil animals, and viruses can alter outcomes of climate change experiments and alter temperature responses of microbial communities (A'Bear et al. 2014a; Crowther et al. 2015; Pelini et al. 2015). Top-down control of microbial biomass would have a big impact on modeling respiration and other microbial activities, as it is generally assumed that microbes are limited by supply of C or nutrients. For example, climate change models have assumed that elevated CO_2 will stimulate soil respiration and methanogenesis in the Arctic by increasing the flux of labile C to soil microbes (Melton et al. 2013), but in C-rich peat soils like those in the Arctic Coastal Plain of Alaska, C additions do not result in stimulation of methanogenesis (von Fischer et al. 2010) or respiration (Allen et al. 2009), and

bacteriophage may represent an important top-down control that limits microbial responses to increased substrate (Allen et al. 2009).

3.3.2 Competition and Synergism in Microbial Communities

There are cases where positive and negative interactions within microbial communities have impacts on larger-scale processes, and it may sometimes improve the predictive capabilities of ecosystem models to include these processes. Microbes compete for energy sources, mineral nutrients, and electron acceptors. In an individual-based model of social interactions within microbial communities, it was found that "cheaters" that benefit from extracellular enzymes produced by other microbes can lead to retention of N and accumulation of soil organic matter (Kaiser et al. 2015). Similarly, modeled changes in enzyme production activities depend on interactions among consortia of complimentary microbes that produce extracellular enzymes for acquiring different nutrients (C, N, or P) (Folse and Allison 2012). In anoxic environments, the presence of alternative acceptors can inhibit less thermodynamically favorable pathways. For example, ferric iron [Fe(III)] and humic acids can inhibit sulfate reduction and methanogenesis, though these processes can also coexist in environments where energy is plentiful (Lovley and Phillips 1987; Keller et al. 2009; Miller et al. 2015). Synergistic relationships also contribute to the coexistence of otherwise competing functional groups. For example, the Fe(III)-reducer, *Geobacter metallireducens*, can donate electrons from the fermentation of ethanol to the methanogen, *Methanosarcina barkeri*, in a process known as direct interspecies electron transfer (DIET) (Rotaru et al. 2014). There is a plethora of potentially competitive and synergistic interactions that influence the relative and absolute production rates of CO_2 and CH_4 in soils. Given that CH_4 has about 34 times the greenhouse warming potential of CO_2, when considered over a 100-year time span (Myhre et al. 2013), the relative fluxes of CH_4 and CO_2 will have a big impact on the dynamics of climate change over the next century. Hydrogenotrophic methanogens, which use H_2 gas, must compete with many other groups for this prized substrate. Acetate can sometimes accumulate in soils as the end product of fermentation and acetogenesis reactions, eventually leading to the establishment of acetoclastic methanogens that can exploit this pool (Hines et al. 2008). Why does it matter if a model includes these two different methanogenic pathways? The two functional groups probably respond differently to environmental factors such as temperature and pH and also to biological factors like competition for substrate or synergistic relationships with fermenters. Therefore, hydrogen- and acetate-dependent methanogens could respond differently to changes in C flux through the soil community driven by elevated CO_2 or warming. In a permafrost thaw gradient in Sweden, increasing methane fluxes were associated with a switch to more acetoclastic production, and these changes coincided with the abundance of *Candidatus Methanoflorens stordalenmirensis* (McCalley et al. 2014). Another warming study found changes in the functional groups responsible for pathways

leading to methane production (polysaccharide breakdown, fermentation, and methanogenesis), with different steps limiting the overall process at different temperatures (Tveit et al. 2015).

The majority of CH_4 that is produced in soils is thought to be oxidized to CO_2 by methanotrophs before it leaves the soil (Le Mer and Roger 2001). The existence of two rapid, nearly balanced processes that are subject to different controls could produce complex behavior, such as rapid spikes or crashes in net fluxes as the production and consumption become uncoupled. Until recently, all methanotrophic activity was mainly attributed to groups of bacteria within the *Proteobacteria* phylum (*Methylocystaceae*, *Methylococcales*; Table 3.1). However, acidophilic methane oxidizers within the *Verrucomicrobia* phylum have recently been described (Pol et al. 2007), and anaerobic oxidation of methane (AOM), originally discovered in marine environments, also plays a role in soils (Smemo and Yavitt 2011). The presence of alternative electron acceptors in methanogenic environments can lead to a reduction of CH_4 flux to the atmosphere due to the activity of AOM species or consortia. The presence of AOM could have the same impact as aerobic methanotrophs, except their activity would be harder to predict. Dissolved oxygen can be modeled relatively simply based on the water table height, but AOM relies on the presence of a variety of other alternative electron acceptors [e.g., nitrite, sulfate, Fe(III)] that are not as easily modeled.

Like CH_4, nitrous oxide (N_2O) is a powerful greenhouse gas that is subject to complex transformations in soils by diverse groups of microbes. By our current understanding, N_2O is produced by (1) facultative anaerobes (including bacteria, archaea, and fungi) that use either nitrite or nitrate as terminal electron acceptors in anaerobic respiration (including denitrification and dissimilatory nitrate reduction to ammonium, DNRA), (2) nitrifiers (chemoautotrophic bacteria and archaea that oxidize NH_4^+ for energy using oxygen), (3) anammox (anaerobic oxidation of ammonium using nitrite as the terminal electron acceptor, carried out by chemoautotrophic bacteria within the *Planctomyces* phylum), and (4) codenitrification (a process carried out by fungi and bacteria in which reduced N compounds, such as ammonium, hydroxylamine, or amino acids, react with oxidized forms of N such as nitrite or nitric oxide) (Giles et al. 2012; Long et al. 2012; Mothapo et al. 2015; Stein and Klotz 2016). The full denitrification pathway leads to the production of N_2, with intermediate products NO and N_2O emitted depending on the level of oxygen limitation in the soil. Some denitrifying microbes lack N_2O reductase, including most denitrifying fungi, and so have a truncated pathway that strictly leads to N_2O production rather than N_2 (Mothapo et al. 2015; Roco et al. 2016). Recent studies showed that N_2O can be consumed by a wider diversity of soil microbes than recently thought (Jones et al. 2012; Sanford et al. 2012). The diversity of microbes that produce and consume N_2O leads to complex controls over N_2O emissions from soils, as these groups may have different production efficiencies and respond differently to environmental controls such as pH, temperature, oxygen, and energy availability (Pan et al. 2013; Stieglmeier et al. 2014; Jiang et al. 2015; Mothapo et al. 2015).

Table 3.1 Examples of prokaryotic groups that can generally be assigned an ecological role based on phylogeny

Taxonomic group	Associated function
C cycling	
Methanosarcinales	Methanogenesis (from H_2, acetate or methanol)
Methanobacteriales, Methanopyrales, Methanococcales, Methanomicrobiales	Hydrogenotrophic methanogenesis
Methanosphaera	Methylotrophic methanogenesis
Methylocystaceae, *Methylococcales*	Methane oxidation
Methanosarcinales subgroups (ANME)	Anaerobic oxidation of methane (in consortia with various anaerobic bacteria)
NC10 (*Methylomirabilis oxyfera*)	Nitrite-dependent methane oxidation[a]
Methylacidiphilales	Low pH methane oxidation[b]
Cyanobacteria	Photosynthesis
Chromatiales, *Chlorobiaceae*, *Rhodospirillaceae*	Anoxygenic photosynthesis[c]
Clostridia, *Bacteroides*	Fermentation
N cycling	
Nitrosomonas	Ammonium oxidation
Nitrobacter	Nitrite oxidation
Thaumarchaeota	Ammonium-oxidizing archaea (AOA), important in acidic soils[d]
Planctomyces subgroup	Anaerobic ammonia oxidation (anammox)
Frankia, *Rhizobium*, *Bradyrhizobium*	Symbiotic N fixation[e]
Azotobacter, *Azospirillum*	Associative N fixation[f]
Other functions	
Desulfovibrionales, *Desulfobacterales*, *Desulfotomaculum*	Sulfate reduction
Thiobacillus, *Epsilonproteobacteria*[g]	Oxidation of inorganic S compounds
Geobacteriales	Reduction of Fe(III), other metals, humic substances
Gallionella, *Zetaproteobacteria*	Fe(II) oxidation
Dehalococcoides	Organohalide respiration
Dechloromonas, *Dechlorosoma* (*Azospira*)	Perchlorate and chlorate reduction[h]
Pseudomonas syringae, *Ralstonia solanacearum*, *Agrobacterium tumefaciens*, *Xanthomonas* spp., *Erwinia amylovora*, *Xylella fastidiosa*, *Dickeya* spp., *Pectobacterium* spp.	Plant pathogens[i]

[a]Only enrichment cultures have been studied
[b]The full physiological diversity of this clade is not known
[c]Contain some non-photosynthetic members
[d]Probably capable of other forms of chemoautotrophy
[e]Also commonly free-living in soils
[f]N fixation is a common trait among prokaryotes, but these groups commonly form associations with plant roots
[g]*Epsilonproteobacteria* also includes human pathogens
[h]Metabolically versatile group capable of other pathways
[i]Include nonpathogenic varieties

Microbial community interactions that influence soil processes depend on small spatial scales, such as redox gradients across soil horizons or soil aggregates, diffusion rates between extracellular enzymes and microbial colonies, and differing processes in the rhizosphere, bulk soil, and litter layer compartments. Incorporating this small-scale spatial heterogeneity into the understanding of processes at larger scales is a challenge but is probably worth the effort, at least in some cases (Faust and Raes 2012; Folse and Allison 2012; Giles et al. 2012; Schimel and Schaeffer 2012; Kaiser et al. 2015; Kuzyakov and Blagodatskaya 2015).

3.4 The Impact of Plant-Microbe Interactions on Ecosystem Processes and Global Change

3.4.1 Global Change and Nutrient Cycling

Soil microbes represent an important feedback in the growth responses of plants to global change by modulating the availability of nutrients (Lipson and Kelley 2014). Elevated atmospheric CO_2 stimulates photosynthesis rates in the short term, but plants in natural environments generally experience limited growth benefits from elevated CO_2 due to nutrient limitations (notably N in most temperate ecosystems). As a result of increased photosynthesis while growth is constrained by nutrient limitations, N concentrations generally decrease in plant tissues when grown under elevated CO_2. This lower quality litter can slow down N mineralization in soils, leading to progressive N limitation. Because of this effect, the stimulation of the terrestrial C sink under elevated CO_2 is expected to be finite, as plants run out of limiting mineral nutrients from the soil (Ciais et al. 2013). However, increased root growth and microbial activity can partly counteract progressive N limitation (Finzi et al. 2007). Elevated CO_2 generally increases plant allocation to roots and to the soil community. The increased roots help plants mine for nutrients more effectively, and increased "rhizodeposition" can stimulate N fixers and mycorrhizae, leading to increased nutrient acquisition. Increased flow of labile exudates from roots to soil can also stimulate N cycling rates through "priming" effects, in which heightened activity in the rhizosphere leads to increased mineralization of N from soil organic matter. And to make matters even more complicated, increased soil temperature generally speeds up N mineralization, partly compensating for progressive N limitation (Dieleman et al. 2012). In summary, the responses of plant growth to climate change depend on changes to the N cycle, which in turn are driven in opposing directions by microbial responses to elevated CO_2 and temperature. These biological feedbacks lead to large model uncertainties and are probably best resolved by studies that explicitly examine soil microbes and their interactions with plants and multifactorial climate change.

3.4.2 Soil Feedbacks and Plant Community Change

In climate change experiments, changes in plant communities tend to overwhelm effects of elevated CO_2 and temperature (Classen et al. 2015; Steinauer et al. 2015), and so when plant communities change as a result of direct human disturbance or climate change, the rules change completely for the soil microbes. Changes of plant communities to an alternative stable state are often facilitated by feedbacks through the soil microbial community (van der Putten et al. 2016). These can be manifested as changes in nutrient cycling or in a shift of the microbial community in terms of the mutualism-parasitism axis. For example, initial disturbance can allow the establishment of plants with higher litter N content, leading to a faster mineralization rate, further encouraging the invasion by weedy species (Liao et al. 2008; Castro-Díez et al. 2014). Or initial introduction of an exotic plant can favor the presence of microbes that are beneficial or neutral to the invading species and harmful to the natives (Sigüenza et al. 2006; Callaway et al. 2008). The microbial community, if left undisturbed and intact, could also function to limit the invasion of a new community by preferentially benefitting native species (Bozzolo and Lipson 2013; Abbott et al. 2015). Similarly, changes in plant ranges can be limited by the presence of suitable symbiotic microbes. Ectomycorrhizal fungi seem to be more subject to dispersal limitation than arbuscular mycorrhizae (Peay et al. 2010; Davison et al. 2015). For example, invasion by ectomycorrhizal trees into a heathland dominated by ericaceous shrubs is reportedly limited by the influx of mycorrhizal spores (Collier and Bidartondo 2009). Conversely, the maintenance of ectomycorrhizal fungi can help plant species maintain the trailing edge of their range as the climate changes (Lankau et al. 2015).

3.5 Relating Soil Microbial Community Structure to Ecosystem Function

The previous sections dealt with how the individualistic properties of soil microbial species and their interactions might influence ecosystems. But how are these microbial traits and interactions expressed when aggregated into complex communities with tens of thousands of species? Because of their complexity, soil microbial communities are generally described in broad terms, such as the relative proportion of major taxonomic groups (like phyla or classes), or by other coarse metrics like bacterial/fungal ratio. Given the premise that microbial species composition matters at larger scales, how can we know which species are important and how the general structure of communities influences ecosystem processes? One also needs to keep in mind that the relative abundance of microbes is not necessarily proportional to their importance in ecosystem functioning. For example, rare microbes can have very small but active populations due to rapid turnover from predation (Lynch and Neufeld 2015; Neuenschwander et al. 2015). And it might be expected that the

microbes that contribute most to CO_2 flux might be fast-growing r-selected types that respond quickly to resource pulses but otherwise have low populations most of the time. However, many studies have shown relationships between broad-scale microbial community structure and function, as detailed below.

3.5.1 Predicting Ecological Strategies from Taxonomy

The taxonomic structure of microbial communities varies greatly among soils of the world and is strongly influenced by soil properties (such as pH, texture, and organic and matter content) and by the plant community (Högberg et al. 2007a; Fierer et al. 2009; Caporaso et al. 2011; Chau et al. 2011; Legay et al. 2014; Docherty et al. 2015). Most descriptions of soil microbial communities are focused on bacteria and are based on the sequences of 16S rRNA genes, though a growing number of studies describe entire microbial communities using shotgun sequencing of the soil metagenome (Fierer et al. 2012). While there can be remarkable physiological diversity among closely related bacterial species (Jaspers and Overmann 2004; Hahn and Pöckl 2005), some general trends have emerged that link broad taxonomic groups with ecological strategies (Fierer et al. 2007; Philippot et al. 2010; Goldfarb et al. 2011; Evans and Wallenstein 2014). For example, the *Acidobacteria* phylum is very common in soils but has few cultured representatives, all of which grow quite slowly (Ward et al. 2009). This group appears to represent a K-selected (or oligotrophic) strategy, growing slowly on complex substrates derived from plant tissues and tolerating stresses. Some species within the *Acidobacteria* have also been implicated as rhizosphere dwellers (da Rocha et al. 2013). On the other extreme are groups such as the *Betaproteobacteria*, containing many cultured representatives and representing an r-selected (or copiotrophic) strategy, growing rapidly on labile substrates like amino acids and taking advantage of disturbances that increase resource availability, but with higher sensitivity to stress.

The ratio of bacteria to fungi is another broad index that is linked to soil processes. Fungi are generally associated with improving C sequestration in soils (Six et al. 2006; Fontaine et al. 2011; Waring et al. 2013). Filamentous fungi generally have an advantage over bacteria when growing in complex, high C:N substrates, especially those rich in lignin, and in low pH soils (Högberg et al. 2007a). Their nutrient requirements are lower, having a more flexible C:N ratio, and their extensive hyphal network allows them to exploit sporadic hotspots of resource availability in an otherwise resource-poor environment. The dominance of fungi is associated with nutrient retention in soils and slower mineralization rates (Allen and Zink 1998; Högberg et al. 2007b; Waring et al. 2013). High fungal/bacterial ratios are linked with lower biomass-specific respiration rates (Sakamoto and Oba 1994; Lipson et al. 2005; Six et al. 2006; Lipson et al. 2009). Fungi tend to have lower growth rates and turnover rates than do bacteria in soil (Rousk and Bååth 2011), which would generally lead to lower CO_2 production per unit biomass. However, this may not be universally true (Thiet et al. 2006). Fungi are physiologically diverse

and include a variety of ecological strategies, growth rates, and growth yields. There is increasing evidence that mycorrhizal fungi have direct and indirect effects on soil C storage. Mycorrhizal fungi, in addition to receiving C from their host plant, can also degrade soil organic matter, either in search of mineral nutrients or for supplemental energy (Talbot et al. 2008). There is also evidence that ectomycorrhizae stimulate C sequestration in soils by competing with saprotrophs for soil nutrients (Averill et al. 2014).

Soil bacteria and fungi generally respond differently to climatic variables. In a study of two soils in Sweden (an agricultural soil and a forest soil), fungal growth was more sensitive to warming than was bacterial growth (Pietikäinen et al. 2005). Additionally, fungal species have been noted as having individualistic phenological responses to past climatic variation, with saprotrophic and mycorrhizal groups associated with deciduous and evergreen trees responding differently to patterns in temperature and rainfall (Diez et al. 2013). These observations indicate that warming could lead to functional changes in fungal communities, with some species increasing vegetative growth and respiration due to delayed fruiting body formation. Several studies have shown differential effects of elevated CO_2 on bacteria and fungi, though the results vary by ecosystem (He et al. 2010; Anderson et al. 2011; Lipson et al. 2014).

3.5.2 *Assigning Ecological Roles Based on DNA Sequence Data*

Numerous techniques are now available to study the functional roles of uncultured environmental microbes, such as shotgun sequencing of soil metagenomes, PCR-based surveys of functional genes, stable isotope probing, fluorescent in situ hybridization and other advanced imaging techniques, single cell genomics, and other innovative approaches such as epicPCR, in which functional genes and 16S rRNA genes from the same cell can be linked (Spencer et al. 2015). However, many descriptions of soil microbial communities are based on 16S rRNA genes, and so it is convenient if conclusions can be drawn from these taxonomic data regarding the functional capabilities of the community. While many prokaryotic taxa are extremely physiologically diverse (e.g., most of the *Proteobacteria* classes), there are some taxonomic groups that share a reasonably coherent lifestyle (Table 3.1). While it is harder to make generalizations about the relationship between phylogeny and function at finer taxonomic scales, recent studies support the idea that there are consistent relationships between the phylogenetic placement of a bacterial operational taxonomic unit's (OTU) 16S rRNA gene and its ecological role (Langille et al. 2013).

3.5.3 Temperature Responses Versus Taxonomy

Because of the strong influence of soil chemistry on microbial community structure, no differences are detected between tropical, temperate, and arctic biomes when communities are compared at a broad (e.g., phylum-level) phylogenetic scale (Fierer and Jackson 2006; Fierer et al. 2012). However, temperature is clearly an important selective factor given the tight adaptations to ambient conditions reported in many studies (Bennett and Lenski 1993; Giardina and Ryan 2000; Bárcenas-Moreno et al. 2009; Salvadó et al. 2011; Rousk et al. 2012). In fact, the effect of temperature is so fundamental that cold-adapted species occur in nearly every major bacterial phyla (Margesin and Miteva 2011). This could explain the difficulty in detecting a clear temperature signature in overall community comparisons that are driven by phylum-level differences. However, when focusing on a narrow group of microbes, latitudinal patterns have been observed at a finer taxonomic scale (Rodrigues et al. 2009; Robador et al. 2015). Similarly, a correlation was observed between the diversity of *Betaproteobacteria* and temperature responses with changes in season and altitude (Lipson 2007).

Adaptations to temperature occur over the entire genome. For example, each enzyme must be adapted for ambient conditions by having the optimal flexibility for functioning at low temperatures or, conversely, high stability for functioning at higher temperatures (Feller and Gerday 2003). Although cold-tolerance genes have been found in plasmids (Dziewit and Bartosik 2014), it is unlikely that a single mobile element could transform a mesophile into a high functioning psychrophile capable of competing within a diverse community. Therefore temperature adaptation should leave an evolutionary signature on a taxonomic marker gene like 16S rRNA, as observed for other complex traits (Langille et al. 2013).

Bacterial genomes from extremely cold environments such as the Arctic, Antarctic, and permafrost zones show very clear signatures of cold adaptation, such as alterations in amino acid composition of the proteins, changes in membrane composition, and enhanced expression of cold shock proteins (Bakermans et al. 2012; Kuhn 2012). However, there is considerable variability among different species and environments (Grzymski et al. 2006; Ayala-del-Río et al. 2010). We are still years away from being able to predict the temperature responses of complex soil processes from metagenomic data, but in principle, all the information is there.

3.5.4 Emergent Properties of Microbial Communities: The Importance of Diversity in Ecosystem Functioning

To this point, we have only considered the constituent microbes that make up microbial communities and how their relative abundance might impact ecosystem processes. But biodiversity (including species richness and evenness) is a property of biological communities with important ecological consequences (McCann 2000),

and this appears to be true for microbial communities as well (Ferris and Tuomisto 2015). Diversity-function relationships are often nonlinear, with decreasing impacts of diversity at high levels of species richness. Given the high diversity of soil microbial communities, it would be expected that species-function relationships in soil communities would be fairly flat or require drastic reductions in diversity to see an effect in manipulative experiments. Consistent with this logic, relationships between soil microbial diversity and C cycling are found more frequently in experiments with low diversity levels, and it is often found that because of the high degree of functional redundancy in these communities, species composition matters more than species richness (Nielsen et al. 2011). Nonetheless it still appears that microbial biodiversity is important for the overall functioning of ecosystems and that even given the high functional redundancy within microbial communities, increased diversity can increase process rates (Nielsen et al. 2015). More diverse microbial communities are also more stable and resistant to invasion by pathogens (van Elsas et al. 2012).

The impacts of climate change on microbial diversity vary by ecosystem and microbial group (Nielsen et al. 2015). Elevated CO_2 increased fungal diversity in a semiarid shrubland (Lipson et al. 2014), but a similar effect was only seen in two of seven ecosystems in a different study (Weber et al. 2011). In an analysis of several long-term studies, drought stress and pressure on pinyon pine from competitors, herbivory, and parasites decreased the diversity of ectomycorrhizal fungi but did not tend to decrease their mutualistic benefit to the plant host (Gehring et al. 2014). Warming led to increased species evenness in the bacterial community (DeAngelis et al. 2015). Theoretically, more diverse communities should be more resistant to disturbances. Therefore, disturbances or land management practices that reduce soil microbial diversity could lead to increased vulnerability of microbial communities and ecosystems.

3.6 Integrating Microbial Diversity and Physiology into Ecosystem Models

It has been well recognized that microbial mechanisms dominate the biological aspects of soil biogeochemistry (Jenkinson and Ladd 1981; Staley et al. 1997; Schimel and Gulledge 1998; Falkowski et al. 2008). However, soil models do not always simulate the microbial roles on biogeochemistry in an explicit way (Schimel 2001). For example, first-order differential equations have been broadly used to describe transformation rates of soil carbon and nitrogen pools, with rate constants for these equations controlled by a variety of environmental factors such as soil temperature, moisture, soil pH, texture, etc. (Manzoni and Porporato 2009). It has been argued that these traditional soil models do simulate microbial mechanisms implicitly, as the microbial impacts are embedded in the decomposition rate constant, k (turnover rate of the pools) (Schimel 2001; Manzoni and Porporato 2009).

However, compared to microbial kinetics, the first-order differential equations lack feedbacks because they are developed based on a strategy of donor-controlled flow. The mechanistic controls (primarily physiological and structural feedback) from microbes are ignored.

3.6.1 Emergence of Microbial Models

Modeling microbial processes started as early as the development of the first soil organic matter model (Veen and Paul 1981; Van Veen et al. 1984; Jenkinson et al. 1987; Parton et al. 1987). When the early soil organic matter models were built, soil microbes were ignored. Later model evolution wherein the microbial pool was treated as a small labile pool without any feedbacks to either upstream litter or soil organic matter decomposition did not result in significant improvements of microbial representation (Jenkinson et al. 1987; Schimel 2001). For example, the VVV model (Veen and Paul 1981; Van Veen et al. 1984), Century model (Parton et al. 1987) and RothC model (Coleman and Jenkinson 1996) separate microbial biomass as an independent labile carbon pool, while none of the three models explicitly simulate the impact of microbial regulation on litter and soil organic matter decomposition. This lack of feedbacks has been identified as a potential uncertainty for projecting soil carbon dynamics with Earth system models (Wieder et al. 2015; Luo et al. 2016). Therefore, there is a strong call for developing a microbial modeling framework for use in Earth system models (DeLong et al. 2011; Treseder et al. 2012; Xu et al. 2014).

3.6.2 Classification of Microbial Physiology and Diversity Simulated in Selected Models

A number of microbial models have been developed (Allison et al. 2010; Allison 2012; Wieder et al. 2013; Sulman et al. 2014). Some are individual-based microbial models, emphasizing the societal and interactions between microbes (Kaiser et al. 2014, 2015); some are functional group-based microbial models to simulate trade-offs among different microbial functions (Wieder et al. 2013, 2015; Xu et al. 2015); some are enzyme-kinetics microbial models, emphasizing the dynamics of microbial processes in response to different substrate quality and environmental conditions (Allison et al. 2010; Wang et al. 2013a). In this section, we will review the state of the art of microbial models simulating microbial physiology and diversity and show the gaps which evidence need for future modeling efforts. Microbial models consider various microbial traits or functions. Categorized below are the primary aspects of microbial physiology and diversity that have been modeled (Table 3.2, Fig. 3.2).

Table 3.2 Microbial physiological functions represented in a group of selected microbial models

Microbial functions	Model representation	Models	References
Physiological limits	Ranges of microbial responses (e.g., pH, oxygen, redox, water potential)	CLM-Microbe, CORPS, MIMIC, Tang and Riley model, Manzoni's model	Manzoni et al. (2014), Sulman et al. (2014), Wieder et al. (2014), Tang and Riley (2015), and Xu et al. (2015)
Microbial growth	Monod, logistic growth	CLM-Microbe, Manzoni's model, MIMIC, MEND	Wang et al. (2013a), Manzoni et al. (2014), Wieder et al. (2014), and Xu et al. (2014)
Plant-microbe interaction	Rhizosphere, competition for nutrients	CORPSE, CLM-Microbe	Sulman et al. (2014) and Xu et al. (2014)
Stoichiometry	Dynamic elemental ratio (C:N:P:S)	Kaiser's model, SCAMPS, CLM-Microbe, GDM	Moorhead and Sinsabaugh (2006), Kaiser et al. (2014), Sistla et al. (2014), Xu et al. (2014), and Zechmeister-Boltenstern et al. (2015)
Microbial interaction	Competition, altruism (compete for space, nutrients)	DEMENT, Kaiser's model	Allison (2012), Kaiser et al. (2014), and Kaiser et al. (2015)
Microbial dormancy	Active and total biomass or microbial dormancy	MEND, Manzoni's model, CLM-Microbe	Manzoni et al. (2014), Xu et al. (2014), and Wang et al. (2015)
Community structure shift	Bacteria: fungi, K- and r-strategy	MIMIC, SCAMPS	Sistla et al. (2014) and Wieder et al. (2014)
Environmental control	Function of temperature, moisture, pH, etc.	MIMIC, GDM, CLM-Microbe, DEMENT, MEND, Kaiser's model, SCAMPS	Moorhead and Sinsabaugh (2006), Moorhead et al. (2012), Wang et al. (2013a), Kaiser et al. (2014), Moorhead et al. (2014), Sistla et al. (2014), Wieder et al. (2014), and Xu et al. (2014)

This section reviews a few of the typical microbial models developed over the past decade (it is not intended to be a comprehensive review).

3.6.2.1 Physiological Limits

All living organisms have limits for physiological functioning. The physiological limits could be soil temperature, soil moisture, soil pH, oxygen, substrate avail ability, etc. For example, the lowest currently reported temperature for soil microbial respiration is −39 °C, although it has been suggested that activity at lower temperatures is possible (Panikov et al. 2006). Models normally simulate microbial activities over a limited range of temperature and soil moisture. For example, −2 °C has been considered as a threshold for microbial activities in the microbial community

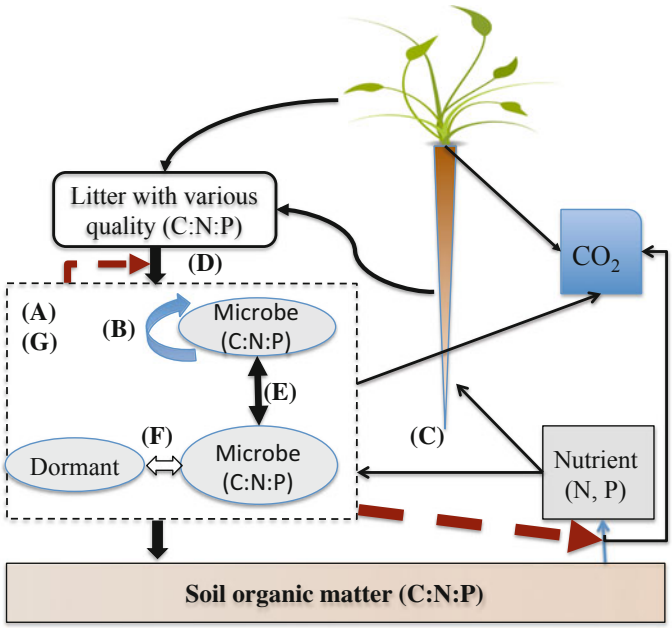

Fig. 3.2 Conceptual diagram showing microbial physiology and diversity in models (**A**) physiological limits, (**B**) microbial growth, (**C**) plant-microbe interaction, (**D**) stoichiometry, (**E**) microbial interaction, (**F**) microbial dormancy, and (**G**) microbial community structure; solid lines indicate flows and dashed lines indicate controls; the red dash lines represent microbial regulation of soil organic matter decomposition; the dashed rectangle represents the entire microbial population in soils, which is composed of different states and functional groups of microbes

land surface model, CLM-Microbe (Xu et al. 2014), which simulates the seasonality of microbial activities in response to changes in soil temperature and moisture. In addition, soil physical conditions provide limits for microbial physiology. For example, MIMIC model, CORPS (Sulman et al. 2014), and the model of Tang and Riley (Tang and Riley 2015) simulate the effects of physical protection on microbial carbon cycling. Manzoni's model simulates water diffusion and its impacts on microbial activity (Manzoni et al. 2014). All these physiological limits control the microbial activity to maintain microbes functioning well under favorable conditions while avoiding detrimental conditions. A good simulation of microbial physiological limits is fundamental in order for models to accurately capture the real dynamics of ecosystem functions.

3.6.2.2 Microbial Growth

Microbial growth is the fundamental component in microbial models designed to simulate microbial mechanisms and their controls on ecosystem functions. Normally microbial growth is a function of substrate and microbial uptake under the control of

environmental factors, simulated by Monod and Michaelis-Menten functions. Most microbial models simulate microbial assimilation of carbon. The CLM-Microbe model simulates microbial assimilation of soil organic carbon under the control of litter quality and microbial physiology and microbial biomass as a net balance between growth and respiration (Xu et al. 2014). The CUE parameter has been considered as an important factor controlling microbial carbon assimilation and carbon sequestration in global soils because it determines how much carbon is released as CO_2 versus how much carbon is used for biomass buildup (Manzoni and Porporato 2009; Wieder et al. 2013; Xu et al. 2014; Wang et al. 2013a, b). The environmental controls on microbial growth are another important aspect of microbial modeling, for example, warming impacts on microbial growth efficiency (similar to CUE) (Wieder et al. 2013; Xu et al. 2014) and moisture impacts on microbial activities (Manzoni et al. 2014). Microbial metabolic quotient (the biomass-specific microbial respiration rate) is another important parameter for simulating microbial activities that can benefit the performance of microbial models (Xu et al. 2017).

3.6.2.3 Plant-Microbe Interactions

Plant roots have strong impacts on microbial growth and uptake of nutrients. Of the developed microbial models, the CORPS model simulates plant impacts on microbial cycling of soil carbon (Sulman et al. 2014), and the CLM-Microbe model explicitly simulates root exudation. However, most microbial models are based upon a theoretical framework and have not been incorporated into real ecosystem models. Therefore, microbial models typically lack the important aspects of interactions with plants and plant roots. Plant-microbe interactions have a variety of ecological consequences (Kuzyakov and Xu 2013). For example, plant-microbe competition for nitrogen affects carbon sequestration of the ecosystem (de Vries and Bardgett 2012), and plant-microbe interactions facilitate plant diversity and production (Van Der Heijden et al. 2008). Considering the centrality of plant-microbe interactions to the biology of both plants and soil microbes, a larger investment in these phenomena is warranted in future microbial modeling development and application. The impact of roots on microbial activities is a particularly important mechanism models should represent.

3.6.2.4 Stoichiometry

Substrate quality (primarily expressed as C:N stoichiometry or lignin content) controls microbial activity. The C:N ratio has been explicitly simulated in SCAMPS (Sistla et al. 2014) and implicitly in CLM-Microbe (Xu et al. 2014). The GDM model uses a lignocellulose index (LCI) to simulate substrate quality impacts on litter decomposition. The LCI is well correlated with litter stoichiometry. The individual-based microbial model, such as Kaiser's model, also explicitly simulates microbial community dynamics and stoichiometry during litter decomposition

(Kaiser et al. 2014). Strong microbial homeostatic regulation has also been found for nitrogen, phosphorus, and sulfur (Zechmeister-Boltenstern et al. 2015; Sinsabaugh et al. 2016). We need to further advance our understanding with a framework that explicitly simulates both microbial dynamics in assimilating substrates with varying stoichiometry and how microbes respond to different qualities of substrates. The interplay of stoichiometry in litter and the microbial community under changing environmental conditions is additional critical information needed for better simulations of microbial physiology.

3.6.2.5 Microbial Community Interactions

Interactions within microbial communities are widely recognized as having important controlling effects upon microbial activity (Faust and Raes 2012). For example, microbial interactions lead to evolutional separation of generalists and specialism for enzyme production (Nam et al. 2012), cheaters and producers of enzyme production (Travisano and Velicer 2004). Yet, these interactions have not been well simulated in most microbial models. There are a few approaches used in microbial models to simulate different groups of microbes. For example, the MIMIC model uses the r- and K-strategies for separating the microbial groups, as suggested by Fierer in a previous concept paper (Fierer et al. 2007). The GDM model simulates interactions among three guilds of microbes (groups of microbes that exploit the same resources, see Moorhead and Sinsabaugh 2006). Kaiser's model simulates societal interaction among individual microbes (Kaiser et al. 2015). The Decomposition Model of Enzymatic Traits (DEMENT) model simulates trade-offs between different functional groups (Allison 2012). Although multiple microbial groups or traits have been simulated to a certain degree in some microbial models, the representation of microbial community interactions in models is far from complete. Given the importance of microbial interactions in ecosystem functions and microbial evolution (Faust and Raes 2012), more effort should be invested to modeling microbial interactions and their impacts on ecosystem functions.

3.6.2.6 Microbial Dormancy

The majority of microbial biomass in soils is in an inactive state during most of the year. Because only a small portion of the microbial biomass is active, the ecosystem functions are carried out by this active microbial biomass. The active microbial biomass and dormant biomass should be differentiated, and this separation has proved to be important for better simulating microbial processes (Wang et al. 2015). Over the past years, few microbial models have been developed to simulate the dormant microbial biomass either as an independent pool or as a season over certain time period. Both the MEND model and Manzoni's model have separated active versus dormant microbial biomass from the total microbial biomass pool (Manzoni et al. 2014; Wang et al. 2015). In a different approach, the

CLM-Microbe model simulates temporally separated active microbial biomass and its impacts on litter mineralization as a seasonality component of microbial functioning (Xu et al. 2014). Both approaches have been proved to be robust in simulating microbial biomass and their contribution to carbon cycling.

3.6.2.7 Community Structure

The Guild Decomposition Model (GDM) is among the first to simulate the dynamic of microbial community shift during litter decay (Moorhead and Sinsabaugh 2006). The MIMIC model simulated two microbial functional groups (MICr and MICk) to represent r- and K-strategists (Wieder et al. 2014). The SCAMPS model is a mechanistic microbial model explicitly simulating the separation of bacteria- and fungi-like microbes and the interplay dynamics of these two groups of microbes (Sistla et al. 2014). Moorhead's model is based on guilds, which represent the microbial groups with different traits (Moorhead and Sinsabaugh 2006). Xu's functional group-based methane model (incorporated in CLM-Microbe) also simulates different microbial functional groups and their dynamics in response to substrate and environmental conditions (Xu et al. 2015). These models consider the dynamics of different microbial functional groups, representing the microbial community structure.

In summary, microbial physiology and community structure have been simulated to a certain degree, and some convincing results have been obtained. While much knowledge has accumulated, it is important to note that modeling microbial physiology and diversity is still in its infancy. More effort is particularly needed in the areas of microbial interactions, community structure shifts and their associated changes in microbial functions, ecological stoichiometry of phosphorus beyond carbon and nitrogen, and microbial interactions with plants. All these aspects are beneficial for model improvement in simulating terrestrial microbial biogeochemistry in the context of climate change. We anticipate that the investment of modeling microbial processes in theoretical and applicable ways will pay off with significant contributions to the robustness of Earth system models in one or two decades.

3.7 Conclusion

The broad range of topics relevant to connecting soil microbial communities to ecosystem function underscores the need for interdisciplinary studies. In particular, it is profitable for soil microbiologists and ecosystem modelers to work together, as this can inform microbiologists how to tailor their studies to make them immediately helpful to modelers, while helping modelers become aware of the importance of mechanisms they may not have considered. The field of environmental microbiology has been revolutionized by modern molecular and isotopic techniques. As computers become more powerful, modelers will be less hesitant to build increasing complexity

into their models. It is likely that this field is already, or will soon become, limited only by the level of communication among scientists studying the same processes at multiple scales and the imagination of these researchers.

Compliance with Ethical Standards

Conflict of Interest David A. Lipson declares that he has no conflict of interest. Xiaofeng Xu declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Chapter 4
Environmental Systems Biology Approach to Bioremediation

Terry C. Hazen

Abstract Pollution is everywhere. Microbes are also everywhere, and many have the ability to degrade environmental contaminants. Understanding how these microbial communities work to degrade environmental contaminants will enable us to use these microbes to clean up the pollution. Understanding, monitoring, and controlling the environment with biological processes, i.e., an environmental systems biology approach to bioremediation, answer the need which is everywhere. By using an environmental systems approach to bioremediation, we make sure we know of any "fatal flaws" in the approach, get a much better handle on life-cycle cost analysis, and can grade an engineered solution into a natural attenuation solution. The whole is greater than the sum of its parts. By using an environmental systems biology approach to bioremediation and cross-linkage of systems at all levels providing multiple lines of evidence involving environmental observations, laboratory testing, microcosm simulations, hypothesis refinement, field testing and validation, and multiple iterations of this circle, we will be able to make new theories and paradigms for bioremediation of contaminated environments.

4.1 Introduction

Pollution is everywhere (Fig. 4.1). Microbes are also everywhere, and many have the ability to degrade environmental contaminants. Understanding how these microbial communities work to degrade environmental contaminants will enable us to use these microbes to clean up the pollution. Understanding, monitoring, and controlling the environment with biological processes, i.e., an environmental systems biology approach to bioremediation, answer the need which is everywhere. By using an environmental systems approach to bioremediation, we make sure we know of any "fatal flaws" in the approach and get a much better handle on life-cycle cost analysis.

There are three million parts in a Boeing 777 aircraft provided by more than 900 suppliers from 17 countries around the world. Completed genomes provide

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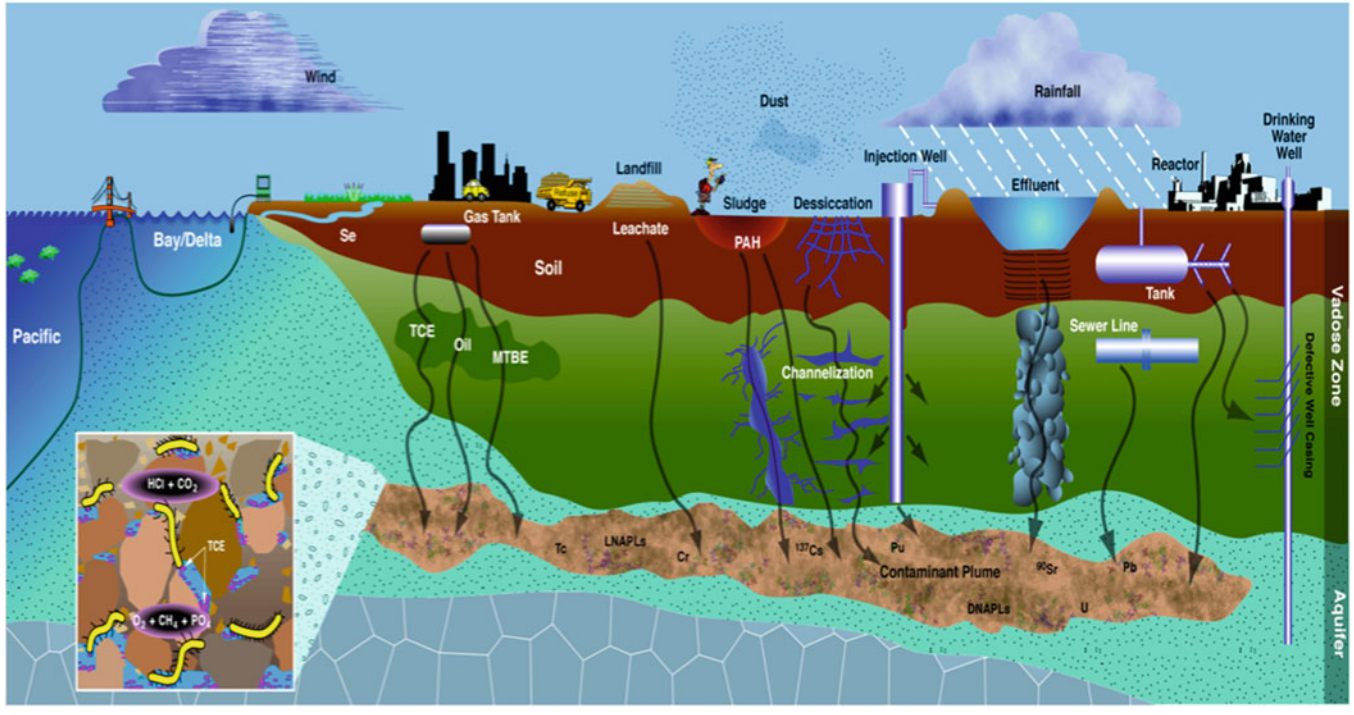

Fig. 4.1 Environmental systems biology (selenium (Se), trichloroethylene (TCE), methyl tert-butyl ether (MTBE), technetium (Tc), light nonaqueous phase liquids (LNAPL), polyaromatic hydrocarbons (PAH), chromium (Cr), cesium (Ce), plutonium (Pu), strontium (Sr), lead (Pb), uranium (U), dense nonaqueous phase liquids (DNAPL))

"parts lists" for many microbes, although the genomic sequence is little more than the blueprint for each part (protein) in the organism. Having a blueprint for the parts of a 777 jet gives few clues as to how each part is made, how it assembles into devices and systems, and much less how it flies which is its essence. For an organism the parts list (genome sequence) does not tell us even the shape of the parts or their function and at a higher level the principles that govern the ability of this large monolith to "fly." The genome sequence is really only a blueprint for each part. The order in which they are made and how that synthesis is controlled is in the parts list, but there is no instruction manual. Genes, proteins, metabolites, and multimolecular assemblies ("molecular machines") interact in an intricate labyrinth of pathways and networks to create, sustain, and reproduce the system we call the living cell—complexity well beyond the engineering and essence of a 777. Systems biology will transform biology from an empirical and descriptive science to a more quantitative and predictive science enabling us to manipulate and use living systems and their components.

Using an environmental systems biology approach to bioremediation requires that we consider interactions at different levels, e.g., the ecosystem (de Lorenzo et al. 2016), community, population, cell, genomics, transcriptomics, glycomics (Kay et al. 2010), lipidomics, fluxomics, proteomics, metabolomics, and phenomics (Hazen and Sayler 2016) (Fig. 4.2). This type of full-spectrum perspective is necessary for successfully using an environmental systems biology approach to bioremediation, because, like the 777 jet, the "whole is greater than the sum of its parts." Engineering success requires that all of the parts function together, and so we must take measurements at various levels in the systems being studied and then build models that interact at those various levels in the systems approach (Fig. 4.3). Systems biology can be a powerful approach to explain environmental phenomenon. However, this methodology intrinsically relies on multiple methods, each of which carries its own advantages and limitations. In order to accurately describe the system in question while not over-interpreting the data produced by these methods, a careful

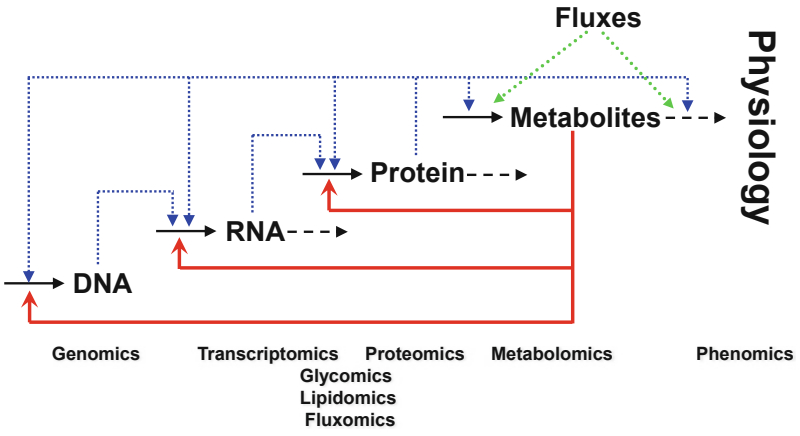

Fig. 4.2 The Omics!

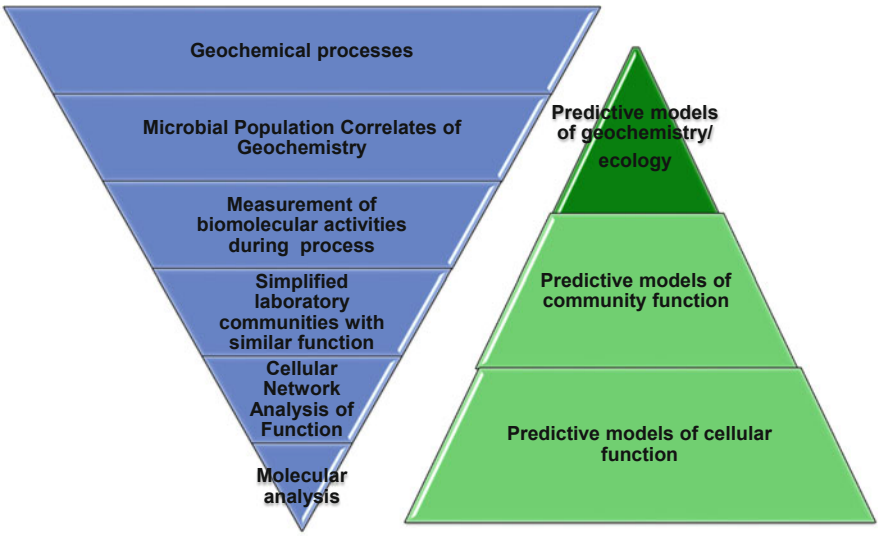

Fig. 4.3 Environmental systems biology

study of the biases of the methods employed in systems biology is warranted (Hazen et al. 2013).

4.2 Geochemical Processes

Setting up an environmental systems approach to bioremediation requires that first we have either a testable hypothesis or a (question) regarding the system to be tested. Examples include:

1. Chromium-contaminated aquifer impacting river (Zhang et al. 2015; Faybishenko et al. 2008)
2. Chlorinated-solvent-contaminated aquifer (Hazen 2010b)
3. Release of CH_4 and CO_2 from either agricultural soil or rain forest (Yao et al. 2018)
4. Biodegradation of an oil spill in the ocean (Atlas and Hazen 2011; Hazen et al. 2010)
5. Biodegradation of oil in soil (Hazen et al. 2003)
6. Combinatorial saccharification of lignocellulose (Woo et al. 2014)
7. Flowback fluid from hydraulic fracking (Trexler et al. 2014)
8. Memory response in bioremediation (Hazen 2018)
10. Landfill biodegradation rates and production of CH_4 (Borglin et al. 2004)

To clarify the boundary system, size must be measured using such assessment approaches. Google Map (GIS, GPS), vessel characteristics (size, e.g., reactor, test

tube fermenter), sampling equipment, and we must obtain any required permits. Knowledge of the system type is also necessary, i.e., whether it is soil, seawater, freshwater, glass, plastic, metal, plant species and strain, animal species and strain as well as operational taxonomic units (OTU), and the involved bacteria, archaea, fungi species or strain, and OTU. The dynamic parameters must also be defined and considered, e.g., direction and rates for (1) physical and chemical characteristics (current, wind, temperature, pH, redox, conductivity, alkalinity, nutrients (nitrogen, phosphorus, carbon, and metals), isotope chemistry, geology (seismic and radar tomography, water availability), (2) meteorological characteristics (rainfall, weather), (3) hydrological characteristics including climate hydraulic residence time, (4) biological characteristics (species, health, sex, age of plant or animal, pretreatment), and (5) whole environment respiration (CO_2/CH_4 flux).

A prime example of biogeochemical effects upon bioremediation would be the emphasis that competing terminal electron acceptors will control how low the redox potential can go and what metals can be either reduced or halorespired in the case of perchloroethylene/trichloroethylene (PCE/TCE) (Fig. 4.4). Presence of certain chemical species in groundwater can also be superb indicators of the dominant terminal electron-accepting (TEA) processes and the relative redox conditions. Many times, bioremediation has been applied to sites that have initially high nitrate, sulfate, or iron, thus resulting in a "stall" which stopped the progression to redox conditions that would allow dehalorespiration of PCE/TCE in groundwater. For bioremediation, monitoring carefully TEA concentrations offers better control of

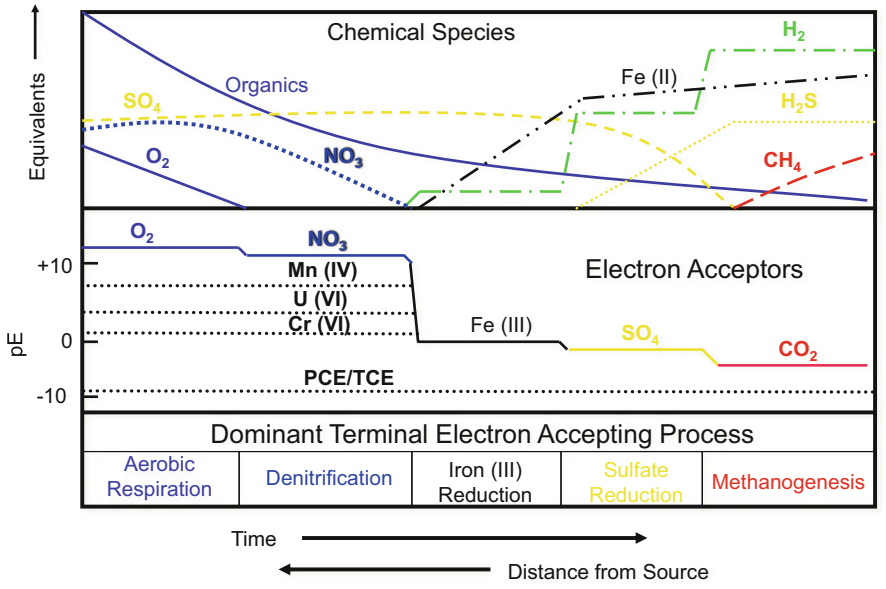

Fig. 4.4 Critical biogeochemistry (sulfate (SO_4), oxygen (O_2), nitrate (NO_3), manganese (Mn), uranium (U), chromium (Cr), perchloroethylene/trichloroethylene (PCE/TCE), iron (Fe), hydrogen (H_2), hydrogen sulfide (H_2S), methane (CH_4), carbon dioxide (CO_2))

electron donor additions and avoids reasons for "stalls" in expected bioremediation processes in situ.

4.3 Microbial Population and Community Correlates with Geochemistry

At this level we must determine key factors that impact communities and populations, a process which includes identifying the stresses and survival pathways (Fig. 4.5). For bioremediation practices, we must analyze the involved communities and their populations so as to better understand the structure and functional relationships and how they control the relevant geochemistries and contaminant degradation. This may be especially important in terms of understanding "fatal flaws," including life-cycle cost analysis, and understanding transitioning to natural attenuation in a treatment train. Measurements can include metagenomes, 16S rRNA, clone libraries, PhyloChip®, GeoChip®, phospholipid fatty acids (PLFA), and proteogenomics. This is, in addition to the other techniques for measuring

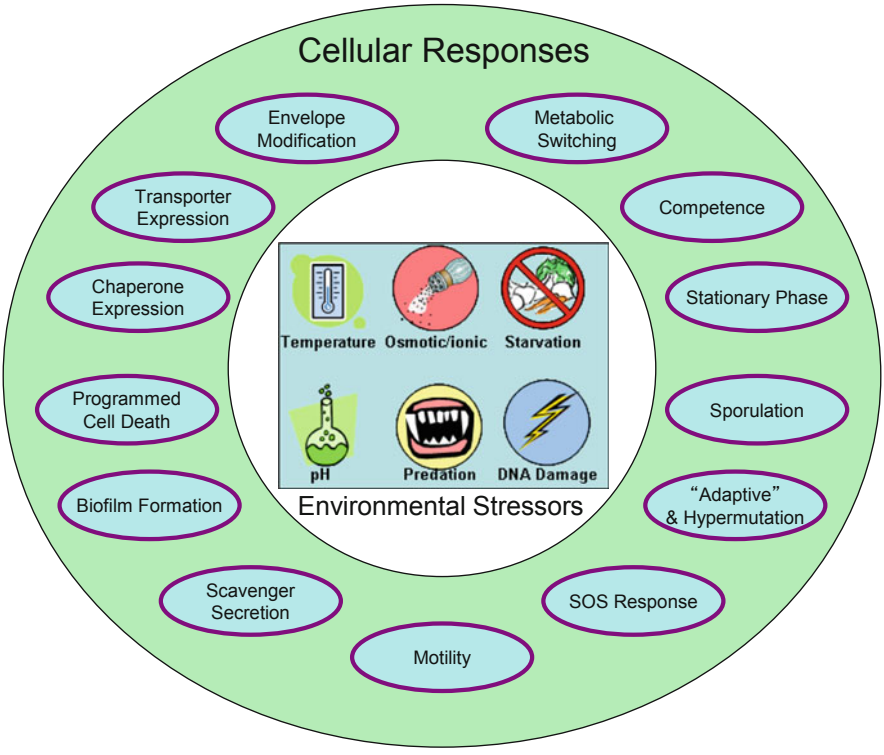

Fig. 4.5 Relevant stress responses

populations and activity, direct counts, fluorescent antibodies, live versus dead cell, fluorescent in situ hybridization (FISH), and enzyme activity probes (EAP).

Prior to the widespread availability of next-generation sequencing, environmental diversity classification was done by amplifying the 16S rRNA gene from DNA extracted from environmental samples that contained prokaryotes. With the current ability to instead sequence all genetic material contained in a sample was born a new possibility of contemporary metagenomics. Whole genome-based metagenomics aims to answer the same questions that 16S-based classification methods did; however there is potentially much more information contained in whole genome sequence information that is not present in 16S sequence information. While metagenomic data can be useful in describing an intangible component of nearly every environment, it should not be used without an understanding of its limitations and biases. It is implied that metagenomic sequences are subject to the same biases that all high-throughput sequencing is subject to. The reader is directed to a review of these biases in (Kircher and Kelso 2010). Outside of sequencing bias, other biases present in metagenomics come from sampling and filtering through the methods chosen for data analysis, which can skew or misrepresent sequence data. In addition to these biases, metagenome data is limited because it is often fragmented and incomplete. Rarely are whole genomes extracted from metagenome data, contiguous sequences are rarely longer than 5 kb (Thomas et al. 2012), and much of the sequence data is from organisms that are themselves not sequenced, uncultured and possibly unculturable, and therefore scarcely characterized.

4.3.1 16S rRNA

We have used 16S rRNA as a standard analytical marker ever since nucleotide sequencing became relatively cheap and fast for determining community structure. The 16S rRNA gene is believed to be highly conserved and has been used for decades as identification at different taxonomic units from Phyla to OTU. So, 16S rRNA analysis does in effect shows the relative community structural profile and has been used to indicate changes in diversity, evenness, and relative densities of certain groups. This trait can be particularly important to see if certain biodegraders are present and also to know if the community structure changes in ways that are either advantageous or detrimental to bioremediation. It cannot tell you anything about the activity, so even if you see that a group of biodegraders is present by 16S rRNA analysis, it does not tell you the group is metabolically active. In addition, 16S rRNA may not be highly conserved in some environments which would lead to erroneous conclusions. However, 16S rRNA analysis has been used to predict geochemistry for bioremediation (Smith et al. 2015).

4.3.2 PhyloChip®

PhyloChip® arrays are a technique which can be used to measure the presence versus absence as well as relative abundances of known prokaryotic species within a sample. The chip was developed by Affymetrix and uses a microarray consisting of 25mer groups which are single-stranded oligonucleotides called probes. Each probe is designed to be complementary to a certain region on the 16S rRNA gene of a particular species. The 16S rRNA gene codes for an RNA component of the small ribosomal subunit. It is a convenient taxonomic marker since it is present in all bacteria and archaea and contains conserved regions allowing for easy design of polymerase chain reaction (PCR) primers, as well as variable regions which allow for discrimination between species. PhyloChip® contains over one million different probes and allows discrimination of over 50,000 OTUs. PhyloChip® has been used to quantify relative abundances of microbial species in deepwater samples from the Gulf of Mexico, with analysis of those results showing that there was indeed a difference in the relative abundances of the microbial populations in samples collected from within an oceanic oil plume versus those which were collected outside of the oil plume. Plume samples were shown to be enriched for taxa (all of which were *Gammaproteobacteria*) known to degrade hydrocarbons. These species could be potential targets for bioremediation (Hazen et al. 2010).

4.3.3 GeoChip®

The GeoChip® functional gene microarray approach is based upon using deoxyribonucleic acid (DNA) microarray evaluations which contain oligonucleotide probes. These gene probes are focused on the biogeochemical cycles of certain metals and important nutrients including carbon, nitrogen, phosphorus, and sulfur. They can also detect resistance to antibiotics, virus, energy production, and the ability to degrade organic contaminants. The GeoChip® is also able to detect gyrB-based phylogenetic markers which represent useful knowledge when trying to detect slow-growing bacteria such as mycobacterium. Advantages to the GeoChip® are that it works for microorganisms not only from environmental origin such as soil, water, and air but also from human and animal sources. Another advantage is that prior knowledge of the microbial community being sampled is not necessary. It is also possible to detect low-abundance microorganisms, which helps to prevent annotation bias. The usage process is relatively quick, with the ability to receive data nearly every day from either DNA or ribonucleic acid (RNA). While the GeoChip® does have advantages, there are also some prevalent weaknesses. For instance, the GeoChip® cannot detect novel gene families because the GeoChip® is only capable of detecting those genes present on the probes. In addition to providing a small pool of information from which to draw information, the information for these functional gene array probes was developed using regions of genes which have

been well conserved throughout time on the 16S rRNA sequence. Problems could arise from focusing only on conserved genes due to natural variations and divergence in gene families, allowing some families to then be missed during probing. Other problems may stem from cross-hybridization problems introduced by match-mismatch probe sets or fluorochrome labeling skewing biases. GeoChip 5.0® contains 167,044 probes which are able to cover 395,894 coding sequences and 1500 gene families. It has been used to show functions that were inhibited during the Deepwater Horizon oil spill and predict geochemistry in a mixed waste site (Lu et al. 2012; He et al. 2018).

4.3.4 Phospholipid Fatty Acids

PLFAs are a main component of cell membranes, and their analysis can be a useful tool for microbial community analysis. These lipids generally degrade quickly upon cell death, allowing their analysis to only target viable cells, unlike many other microbiology techniques. However, PLFA does come with its own wide set of biases associated with its measurements. The PLFAs are measured and profiled with either a gas chromatography (GC) or a gas chromatography combined with mass spectrometry (GCMS) after they have been extracted and purified. Since this is a direct extraction method representing the fatty acids which doesn't include an intermediate step like PCR or culturing, its usage represents an advantage over several other techniques. Chromatography peaks representing the fatty acids are easily analyzed with proper equipment, without requiring other steps unlike nucleic acid techniques that necessitate sequencing. Fortunately, PLFA analysis is very sensitive and easily reproducible, with the benefit of its being fast and comparatively inexpensive. However, PLFA analysis can require large amounts of sample in order for the obtained data to be statistically significant, and with certain techniques, like fingerprinting, PLFA may require up to ten times more sample size than would analyses based upon examination of fatty acid methyl esters (FAME), which is another type of fatty acid analysis tool. There are four main types of analyses that can be done with the aid of PLFAs: total biomass, physiological indicators, fingerprinting, and taxonomic biomarkers. When examining the Deepwater Horizon spill plume, it was seen that PLFA data supported the 16S rRNA pattern analysis results and provided additional biomass measurements (Hazen et al. 2010).

4.3.5 Functional Gene Clone Libraries

Functional gene clone libraries are a method that can help elucidate gene functionality based upon nucleotide sequence data, as described. This technique is often employed at the population level of systems biology, particularly when the sequence of a gene of interest is known while the corresponding function remains cryptic.

Advantages of this technique include its culture independent nature, which bypasses the need for painstaking microbial isolation. For example, a gene can be PCR-amplified and cloned into a plasmid, such as pUC19, and then transformed into a model heterologous host such as *Escherichia coli*. In doing so, the functionality of the gene can be assayed in vivo independent of isolating the organism. Another advantage is the ability to selectively engineer a regulated promoter to drive the transcription of a gene, thereby providing much needed control of expression in relation to phenotype. A commonly used regulated promoter in *E. coli* is the T5-lac promoter. Limitations of this technique include the fact that functional heterologous expression of a gene in a foreign host may be considered a fortuitous event, at best. For example, some particular gene of interest may require a specific chaperone in order to produce key folds in the protein structure that are critical for enzymatic activity. If the heterologous host lacks the genes encoding for these chaperones, then the resulting protein may not fold correctly and thus yield no enzymatic activity. Even if protein folding takes place independently of chaperones, the enzyme in question may require additional cofactors that would need to be supplied in order for the process to function, such as vitamin B_{12}. For example, the *Dehalococcoides* solely rely on reductive dehalogenases to perform organohalide respiration yet lack the ability to produce the vitamin B_{12} essential to reductive dehalogenase function (Yan et al. 2013). If the heterologously expressed enzyme is indeed functional, the level of its activity may not be reflective of that which occurs in the gene native organism. This can occur because the activity of a gene may be regulated by possibly a single or even multiple regulatory networks in the native host and that regulation perhaps absent in the foreign host. Additionally, expression of the target gene may prove toxic to foreign hosts, which may compromise host cell viability. These limitations must be acknowledged and addressed when employing functional gene cloning in systems biology.

4.4 Measurement of Biomolecular Activities During Process Operations

Analyzing DNA, RNA, and proteins at the cellular level to understand cellular effects in terms of bioremediation can be effective in determining biomolecular activities during a critical remediation process. Measurements in addition to the ones listed in Sects. 4.2 and 4.3 include quantitative PCR (qPCR), stable-isotope probing, enzyme activity probes, metabolomics, proteogenomics, and fluxomics.

4.4.1 Stable-Isotope Probes

Stable-isotope probes (SIP) have been applied in numerous environmental studies (Madsen 2006), and many commercial laboratories offer analytical services (Microbial Insights Inc. (MI), Knoxville, TN). Pombo et al. (2002) used carbon-13 labeled acetate ($CH_3COO–$) which was injected and sequentially extracted from a groundwater zone in order to investigate its fate and transformation in the environment. Carbon-13 was measured in dissolved inorganic carbon (DIC) in groundwater and in phospholipid fatty acids (PLFA) in planktonic microbial biomass. The relative abundance of carbon-13 in DIC was significantly higher than were background levels in groundwater following injection of carbon-13 labeled $CH_3COO–$. This suggested that organic $CH_3COO–$ was transformed to inorganic carbon in the form of bicarbonate ($HCO_3–$). The relative abundance of carbon-13 in PLFA was also significantly higher than background levels in planktonic microbial biomass. This suggested the transformation of $CH_3COO–$ was, to some degree, microbial mediated. Pombo et al. (2002) clearly demonstrated that the fate and transformation of a carbon-13 labeled substrate can be investigated in an environmental system.

4.4.2 Enzyme Activity Probes

Enzyme activity probes (EAPs) are chemicals employed to detect and quantify the activities of specific microorganisms in environmental samples (e.g., soil, water, or sediment). These serve as alternative or surrogate substrates for the protein catalysts (enzymes) which are responsible for the metabolic activities of microorganisms. These surrogate compounds can be transformed by target enzymes into distinct and readily detectable products. Most enzymes cannot function well outside of cells due to ensuing rapid degradation or inactivation of the enzymes. There is a strong relationship between the transformation rate of EAPs and the number of active cells that possess the active form of enzyme. Moreover, enzymes are very selective, and EAPs can only be transformed by specific enzymes. Therefore, EAPs can be used to estimate the numbers of microorganisms which are capable of biodegrading a certain contaminant. They can also be used to determine in situ biodegradation rates of specific contaminants, such as chlorinated solvents.

4.4.3 Metabolomics or Metabolite Expression

Metabolomics or metabolite expression involves using a hydrophilic interaction chromatography technique coupled to tandem mass spectrometry (MS/MS) detection and capillary electrophoresis-mass spectrometry (CE-MS) methods for assaying amino acids, nucleosides, nucleotides, organic acid acetyl-CoAs (CoAs), redox

cofactors and metabolic intermediates of glycolysis, the tricarboxylic acid (TCA) and pentose phosphate pathways, etc.

4.4.4 Proteogenomics

Proteogenomics—the concept of "proteomics" was first coined to describe the complete protein complement expressed by a genome. The definition was specified later to be the protein complement of a given cell at a specified time, including the set of all protein isoforms and protein modifications. Proteomics-based methods have been used in discovery, quantification, and validation of protein-protein interactions. The types of large-scale experiments performed in the field of proteomics require specialized tools, and each tool may be suited to only a particular type of experimental design or question asked. Traditionally, proteomic analyses of complex protein samples involve the resolution of proteins using two-dimensional gel electrophoresis followed by the identification of resolved proteins by use of mass spectrometry. Bias can be caused in both the protein separation and identification processes. This technique has been used for studying contaminated sites (Jiao et al. 2011).

4.4.5 Fluxomics

Fluxomics studies the rates of metabolic reactions within a biological entity, including how those rates change, and connects them with dynamic physiology. This quantitative approach integrates in vivo measurements and estimates of reaction rates with stoichiometric network models to allow the determination of carbon flux through cellular networks in central metabolism. The combination of cellular network fluxes, termed the fluxome, represents a unique cellular phenotype. This approach can identify metabolic interactions leading to the development and rational design of cellular functions. This can be applied to make informed genetic modifications of industrial organisms and further analyze the resulting changes to optimize the metabolic network that results from those modifications. Two techniques deployed in fluxomics are ^{13}C flux analysis and flux balance analysis (FBA). The latter consists of utilizing the stable isotope of carbon ^{13}C to trace the partitioning of carbon through different pathways followed by either MS or nuclear magnetic resonance analysis (NMR) for identification of the labeled compounds. The former technique uses the stoichiometry of metabolic reactions in conjunction with many biological, chemical, and thermodynamic parameters to create a constrained model of metabolic flux. In ^{13}C isotope labeling, substrates like glucose can be labeled and fed to an organism, distributing the ^{13}C throughout the metabolic network, and the label then reach a steady state in the metabolite pool. Specific labeling patterns in metabolic intermediates can arise as a function of an organism's unique flux

distribution. This is followed by the use of either MS or NMR to measure these patterns and to construct the network flux distribution. Transitioning to the computational side with FBA, this technique consists of constructing models that constrain flux by observed cellular input and output measurements and by the stoichiometric, energy, and mass balance of metabolic reactions. Once constructed, these models can serve to predict fluxes and identify those that may either optimize or enhance a certain desired characteristic (Tang et al. 2007).

4.5 Simplified Laboratory Communities with Similar Function

Microrespiration and mesocosm studies are also an effective technique to determine biodegradation rates or mineralization rates. By mimicking environmental conditions as closely as possible and using samples from the environment that are extremely fresh, this technique can provide a reasonable approximation of the biodegradation or mineralization rates. Unfortunately, since it requires that samples be transferred to laboratory, the "bottle effect" can be enormous, as shown on a comparison of biodegradation of oil on ship studies with lab studies (Liu et al. 2017). It also can sometimes not represent the actual biodegradation rates because electron donors and electron acceptors may not be added to the samples in the right proportions to mimic what actually is going on in the environments (Baelum et al. 2012).

All of the methods used in Sects. 4.2–4.4 can be used in producing simplified lab communities and can be cross-referenced using the systems approach to link with other methods described in Sects. 4.2–4.4. Environmental systems approaches to bioremediation studies often require multiple lines of evidence cross-linked at different system levels (Fig. 4.6).

4.6 Cellular Network Analysis of Function

Phenomics, i.e., phenotype expression and physiology, are integral to understanding cellular network analysis of function. These measurements can include phenotypic microarrays, real-time analyses using Fourier transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), proteogenomics, PLFA, microbial isolation, FISH, EAP, and fluxomics.

4.6.1 High-Throughput Phenotypic Microarray

High-throughput phenotypic microarray (PM) systems are available, such as the OmniLog® by Biolog® Inc., of Hayward, California. Each PM consists of a 96-well plate that contains different variations of a cell culture medium and dye to test a particular phenotype or cell function. It can process up to 50 96-well plates (total of 4800 reactions at a time). Biolog® Inc. has over 1920 (20 plates) phenotypic tests available for gram-negative, gram-positive, yeast, filamentous fungi, and mammalian cells. These 20 different plates can detect respiration with different carbon sources, nitrogen sources, phosphorus and sulfur sources, nutrient supplements, peptide nitrogen sources, osmolytes, pH gradient, antibiotics, chemical sensitivity, and different metals. Furthermore, the user can also create a tailored plate with a colorimetric assay of choice, such as a particular combination of contaminant and electron donor. These systems can be used with isolates or mixed cultures or environmental samples under both aerobic and anaerobic conditions (Borglin et al. 2012).

4.6.2 Fourier Transform Infrared Spectroscopy

FTIR is a routinely used method of infrared spectroscopy and produces a unique molecular fingerprint of a sample based upon the absorption and transmission of infrared light through the chemical bonds contained in the sample. This makes FTIR very useful for identifying unknown materials, determining the quality of a sample, and quantifying the amount of components in a sample. Recent applications of FTIR include biological questions, especially those related to systems biology and bioremediation. Valuably, FTIR may be used as a high-throughput screening technique to identify and classify key small molecules, including metabolites in real-time for examining living bacteria on rocks and soil during active microbial exposure to contaminants (Baelum et al. 2012; Hazen et al. 2010).

All of the methods used in Sects. 4.2–4.5 can be used in producing simplified lab communities and can be cross-referenced using the systems approach to link to measurements in Sects. 4.2–4.5. Environmental systems approaches to bioremediation require multiple lines of evidence cross-linked at different systems levels (Fig. 4.6).

4.7 Molecular Analysis

Ecogenomics, definable as studies of genomes in an environmental context, can be done at the molecular level using 16S rRNA microarrays for community analyses, metagenome sequencing, annotation of sequences for environmental context,

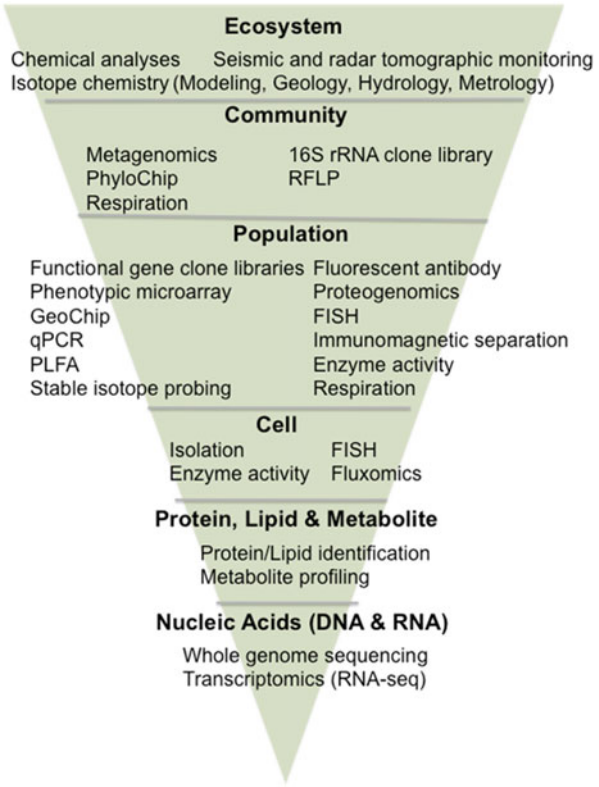

Fig. 4.6 Measurements at different system levels

transcriptomics-gene expression, mRNA expression arrays of one organism or functional group, real-time PCR analyses, protein/lipid identification, metabolite profiling, whole genome sequencing, and transcriptomics (RNA sequencing). Although Sects. 4.2–4.6 give examples of these techniques, it is important to realize that many of these techniques have inherent biases that can be multiplicative and illustrate the reason that conclusions must be considered carefully (Hazen et al. 2013). Figure 4.7 demonstrates where these biases occur and how they might affect the final bioinformatics analysis and conclusions (Hazen et al. 2013).

4.8 Predictive Models of Cellular Function

Predictive models of cellular function can involve annotation of sequences, comparative genomics, integration from biomolecules to ecosystems, and bioinformatics. Ecogenomics has been defined as the study of genomes in an environmental

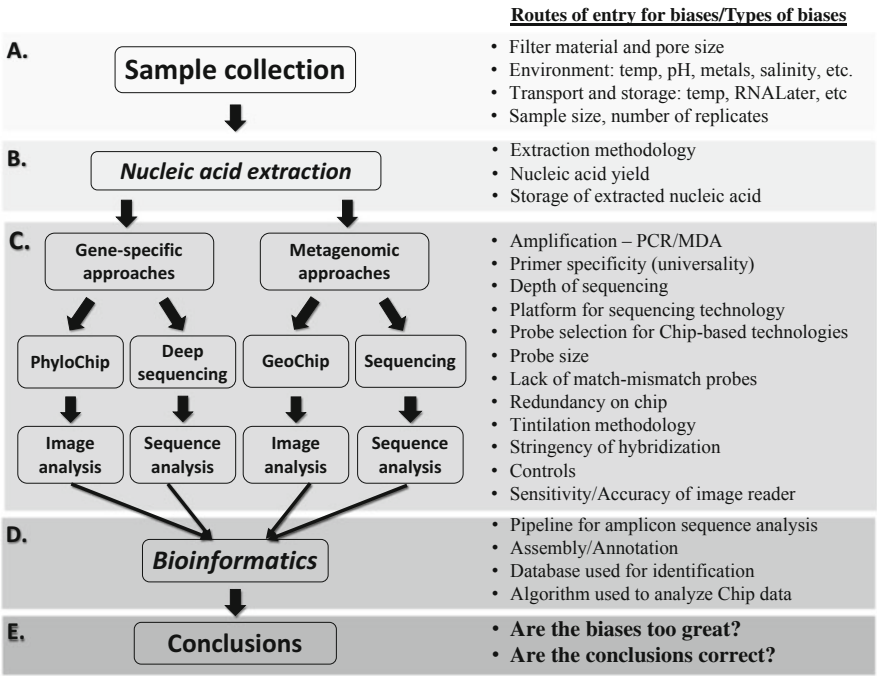

Fig. 4.7 Biases that can be introduced in a genomic pipeline (stabilization solution that stabilizes and protects cellular RNA (RNA later), polymerase chain reaction/multiple displacement amplification (PCR/MDA)) (after Hazen et al. 2013)

context, and Fig. 4.8 illustrates the associated conceptual integration for environmental systems biology (Fig. 4.8) (Deutschbauer et al. 2006).

> Genomics, functional genomics, proteogenomics and systems modeling allows for the analysis of community population structure, functional capabilities, and dynamics. The first step is to obtain DNA extracted from an environmental sample, either after cloning the DNA into a library or by direct sequencing. After the DNA sequence has been assembled, the computational identification of marker genes allows for identification and phylogenetic classification of members of the community and enables the design of probes for subsequent population structure experiments. The assignment of sequence fragments into groups that correspond to a single type of organism (a process called 'binning') is facilitated by identification of marker genes within the fragments, as well as by other characteristics such as G+C content bias and codon usage preferences. Computational genome annotation, consisting of gene prediction and assignment of their possible function using characterized homologs and genomic context, allows for description of the functional capabilities of the community. Knowledge of the genes present also enables functional genomic and proteomic techniques, applied to extracts of protein and RNA transcripts from the sample. These latter studies inform systems modeling, which can be used to interpret and predict the dynamics of the ecosystem and to guide future studies. (Deutschbauer et al. 2006)

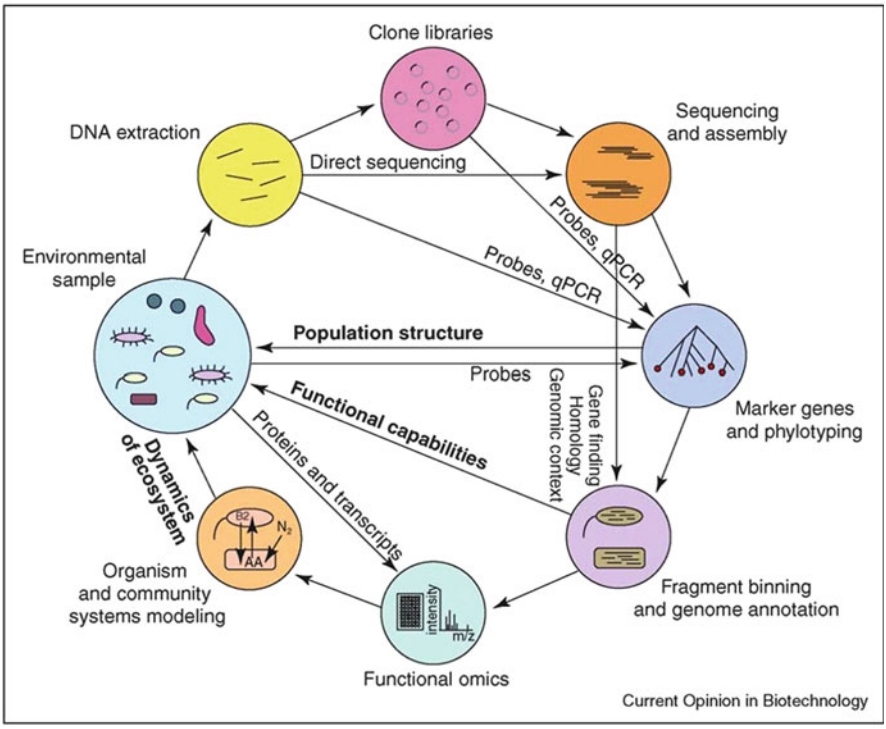

Fig. 4.8 Environmental systems biology using ecogenomics—using a variety of molecular techniques to provide multiple lines of evidence for microbial structure and function at organism, population, and community level (after Deutschbauer et al. 2006)

4.9 Predictive Models of Community Function

Stress response techniques can be used to monitor process control pathways for the purpose of achieving more effective bioremediation (Fig. 4.5) (Hazen et al. 2006). "Although the microbial stress response has been the subject of intensive laboratory investigation, the environmental reflection of the laboratory response to specific stresses has been little explored. However, it is only within an environmental context, in which microorganisms are constantly exposed to multiple changing environmental stresses, that there will be full understanding of microbial adaptive resiliency. Knowledge of the stress response in the environment will facilitate the control of bioremediation and other processes mediated by complex microbial communities" (Hazen et al. 2006). Biostimulation through the addition of nutrient amendments to contaminated environments has recently started to focus on specific stressors that could affect biodegradation and biotransformation processes. Holmes et al. (2004) monitored the nifD gene for nitrogen fixation during acetate stimulation of organic- and nitrogen-poor subsurface sediments. Although nifD expression decreased 100-fold after the addition of ammonium, it had no effect on rates of

toluene degradation or Fe(III) reduction. Thermodynamic analysis of Cr (VI) exposure to sulfate reducers has also been shown to induce an inhibition of growth and energy production that is similar to oxidative stress responses (Chardin et al. 2002). This suggests that commonality in stress responses might provide strategies that can be used to maximize biodegradation and biotransformation processes in situ against specific contaminants without increasing biomass of the target organism. Bioaugmentation (the addition of living cells) for the biodegradation of carbon tetrachloride has also been shown to benefit not only from nutrient balance but also from pH adjustments to avoid pH stress (Dybas et al. 2002). It has been demonstrated that by adding a combination of alkali, acetate, and phosphorus to aid a carbon tetrachloride degrader in a biocurtain strategy, biodegradation of carbon tetrachloride in groundwater passing through the biocurtain could be sustained at 100%.

4.10 Predictive Models of Geochemistry and Ecology

The success of any bioremediation application will be highly dependent on careful advance planning of the overall project, including consideration of the characterization, analysis, and monitoring that are to be done before and during the field deployment. The overall planning of the remediation needs to consider a number of steps from conceptual modeling to demobilization and report writing. For any field remediation, the first step is to form a conceptual model of the contaminant plume in the environment and how that environment effects that plume. The uncertainties in that conceptual model will provide the defining drivers for the characterization and monitoring needs. For example, characteristics of an aquifer will have a profound impact on its remediation strategy. The largest part of the expense of any remediation project is the characterization and monitoring. For example, hydraulic conductivities can have a severe effect on your ability to deliver nutrients to the subsurface and can be the most limiting part of the environment. Fortunately, new advances in geophysics and hydraulic push technology such as Geoprobe® have enabled us to characterize sites in a fraction of the time and cost. Once we have established the hydrology and basic geochemistry at a site and used that data to refine our conceptual model, a base line characterization of the microbiology is essential to establish that the right microorganisms are present, that they can be stimulated, and that no undesirable reactions associated with the stimulants or daughter products from the stimulation will occur. This usually requires some treatability and soil compatibility studies as well as monitoring of microbial community structure and function to establish the base conditions prior to stimulation. For example, some metals like arsenic actually increase solubility under the same redox potentials that precipitate chromium and uranium.

Perhaps the best documented and most widely used model for hydrocarbon bioremediation has been the BIOPLUME® model (Borden and Bedient 1986). This model, now in its forth version, uses a series of simultaneous equations to

simulate growth, decay, and transport of microorganisms, oxygen, and hydrocarbons. Rifai et al. (1987) later modified this model (BIOPLUME II®) to incorporate the USGS two-dimensional method of characteristic model (Konikow and Bredeheoft 1978). The original BIOPLUME® model was used to simulate PAH biodegradation at a Texas Superfund site (Borden and Bedient 1986). BIOPLUME II® has been used to model biodegradation of aviation fuel at the US Coast Guard Station at Traverse City, Michigan (Rifai et al. 1988), and to characterize benzene biodegradation over 3 years in another shallow aquifer (Chiang et al. 1989; Choi et al. 2009). Travis and Rosenberg (Travis and Rosenberg 1997) used a numerical simulation model to successfully predict aerobic bioremediation of chlorinated solvents in the groundwater and vadose zone using methane biostimulation at the US Department of Energy's Savannah River Site near Aiken, South Carolina. Their model also used a series of simultaneous equations for microbial growth, and nutrient limitations, in addition to modeling contaminant, microbe, and nutrient transport. Their model predicted the amount of TCE that was biodegraded during a 14-month, full-scale demonstration and was validated by five other methods (Hazen et al. 1994). Other models that are in use these days are BIOSCREEN® (USEPA 2018b), BIOCHLOR® (USEPA 2018a), REMChlor® (USEPA 2018c), REMFuel® (USEPA 2018d), and Matrix Diffusion Toolkit® (GSI_Environmental_Inc 2018). Models like these are becoming increasingly important as our need to understand the terrestrial subsurface "black box" of bioremediation increases in response to increased emphasis on intrinsic in situ bioremediation as a final solution (Hazen 2010a, b, c).

Intrinsic bioremediation is developing rapidly as an important alternative or end-game approach for many contaminated environments. This strategy of a monitored natural attenuation (MNA) through characterization, treatability studies, risk assessment, modeling, and verification monitoring of contaminated environments was first proposed by John Wilson of the US Environmental Protection Agency's Robert S. Kerr Lab in the early 1990s, following which the development and regulatory acceptance of this strategy have increased exponentially. Certainly, much of this rapid deployment of intrinsic bioremediation has been due to the crushing financial burden that environmental cleanup represents and our need to use more risk-based cleanup goals for the thousands of new contaminated sites identified every year. The MNA strategy carries with it a burden of proof for (1) risk to health and the environment and (2) a model that will accurately predict the unengineered bioremediation of the environment (Hazen 2010a, b, c).

One of the most recent models to use a more environmental systems biology approach was the Structured Learning in Microbial Ecology (SLiME) model. In developing the SLiME model, we used data from our 100-well survey campaign at the Department of Energy's (DOE) Oak Ridge-contaminated field site from groundwater and also from similar measurements from the *Deepwater Horizon* spill response phase of the deepwater plume (Smith et al. 2015). Although these two sites represented completely different contaminants, knowledge of the microbial community structure enabled predictions for the contaminate concentrations at both sites (Fig. 4.9).

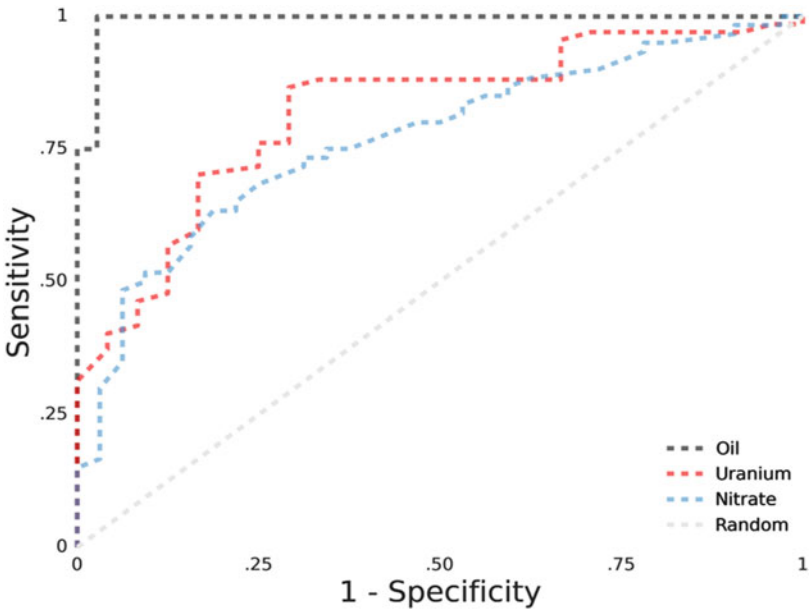

Fig. 4.9 Structured Learning in Microbial Ecology (SLiME) identified biological features associated with the presence of oil, uranium, and nitrate, i.e., predicting contaminants using microbial community structure in situ (after Smith et al. 2015)

4.11 Conclusion and Research Needs

Once all of the above aspects have been thoroughly considered, then a *Field Test Plan* can be developed for the given bioremediation. Environmental systems biology *Field Test Plans* must incorporate the following components: (1) hypotheses to be tested and why, (2) boundaries of environment system, (3) measurements to be made, (4) sampling (protocols, holding times, storage, etc.), (5) resources needed (costs, equipment, people, time, etc.), (6) the Field Test Plan summarizing the best possible scenario of 2–5 to accomplish 1 (including permits, safety, protocols, responsibilities, budgets, priorities, expected outcomes, reports and publications, data management plan, and science of opportunity). After these components have been considered, then (7) feedback to the Field Test Plan involves mobilization, implementation, demobilization, final analyses, and use of models for the preparation of reports and publications (Hazen and Sayler 2016). One campaign for which we used an environmental systems biology approach was at DOE's Oak Ridge-contaminated field site that was studied for over 2 years, and the project illustrated the successful management of a large-scale environmental systems approach (Smith et al. 2015) (Fig. 4.10).

By using an environmental systems biology approach to bioremediation and linked systems at all levels, providing multiple lines of evidence involving

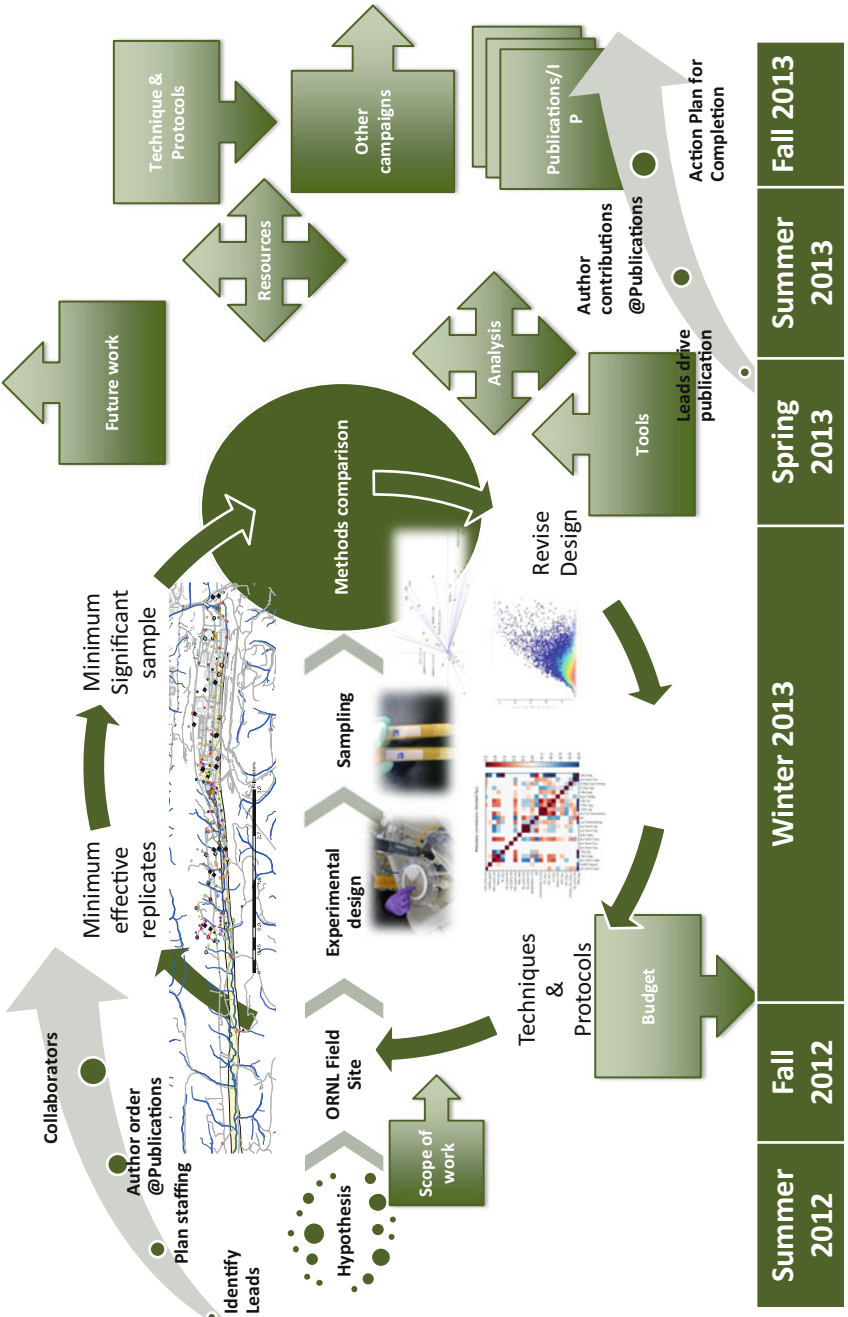

Fig. 4.10 100-well survey timeline

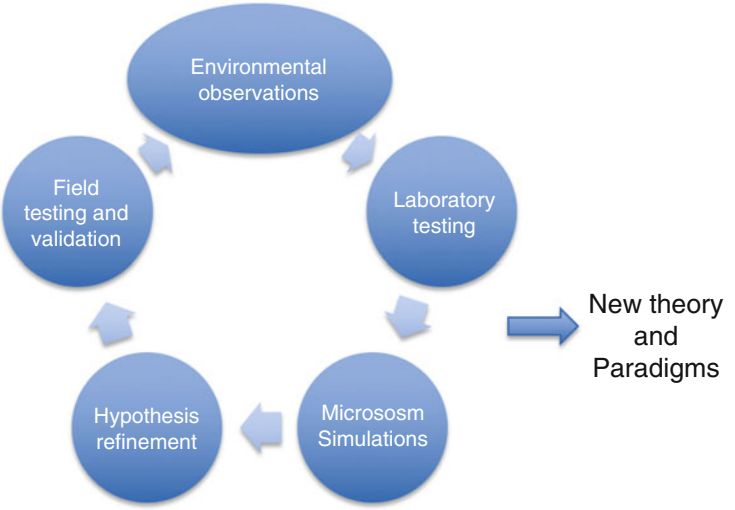

Fig. 4.11 Iterations for using an environmental systems biology approach to bioremediation

environmental observations, laboratory testing, microcosm simulations, hypothesis refinement, field testing and validation, and iteration of this circle, we will be able to make new theories and paradigms for bioremediation of contaminated environments (Fig. 4.11).

Compliance with Ethical Standards

Conflict of Interest Terry C. Hazen declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by the author.

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Chapter 5
Systems and Methods for Studying Microbial Processes and Communities in Landfills

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Abstract The objective of this chapter is to review research on the microbiology of landfills. This chapter focuses on anaerobic reactions that dominate waste decomposition in engineered landfills and begins with a brief description of the major components of a sanitary landfill followed by a discussion of MSW (municipal solid waste) composition. The processes by which cellulosic substrates are converted to CH_4 and CO_2 are described. Systems for studying landfill processes (including testing setups and sampling) are then discussed, followed by traditional and molecular methods that have been used to investigate the microbial ecology of landfills.

5.1 Introduction

To provide a context for discussing landfill microbiology, we first describe the structure of engineered landfills that receive mostly municipal solid waste (MSW). Biosolids derived from wastewater treatment and MSW are the major anaerobically degradable fractions of landfilled wastes. In the USA, non-hazardous waste landfills (i.e., those permitted to receive MSW) may also receive other wastes that do not extensively biodegrade but may affect the chemical and physical environment in which biodegradation occurs. These wastes include ash from the combustion of various fuels (e.g., MSW, coal), construction and demolition (C&D) waste, and a wide variety of non-hazardous industrial wastes, such as auto-shredder residue, foundry sands, and off-specification as well as out-of-date consumer products. The mix of wastes actually received at an MSW landfill is likely to vary with time as landfills are often designed with capacities to receive wastes for 10–100 years. In this respect, landfills represent a unique microbial ecosystem in which a wide variety of substrates are continuously buried.

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A complex series of biological and chemical reactions begins with the burial of MSW in a landfill, and landfills represent an active anaerobic ecosystem with methane (CH_4) and carbon dioxide (CO_2) as the major end products. Methane is recovered in commercial quantities for beneficial use from about 645 landfills in the USA (US Environmental Protection Agency 2018a) and is collected and flared at many additional sites. Methane that is not collected is released through the landfill cover where a portion is oxidized and the balance is released to the atmosphere. In 2016, US landfills were estimated to emit 107 Tg (million metric tons) of CO_2 equivalents (CO_2e), making landfills the third and fourth largest sources of anthropogenic methane in the USA (US Environmental Protection Agency 2018b) and globally (Ciais et al. 2013), respectively.

The objective of this chapter is to review research on the microbiology of landfills. This chapter focuses on anaerobic reactions that dominate waste decomposition in engineered landfills and begins with a brief description of the major components of an engineered landfill followed by a discussion of MSW composition. The microbial processes by which cellulosic substrates are converted to CH_4 and CO_2 are then described. Systems for studying landfill processes (including testing setups and sampling) are then discussed, followed by traditional and molecular methods that have been used to investigate the microbial ecology of landfills.

5.1.1 Description of an Engineered Landfill

Landfills in the USA must meet criteria set by both Subtitle D of the Resource Conservation and Recovery Act (Solid Waste Disposal Facility Criteria 40 CFR § 257–258 1991) and related state regulations. Modern landfills are designed to isolate waste from the environment by use of a composite liner (clay plus geomembrane) on the bottom and sides, a leachate collection system to capture contaminated water, and a gas collection and control system at all but the smallest landfills.

As a highly engineered facility, landfills are not just open excavations into which waste is haphazardly discarded. Instead, waste is placed within landfills according to a specific fill plan, the smallest unit of which is the daily cell. A daily cell is sized to fit the average amount of waste in 1 day and is typically 2–3 m tall, with a working face oriented to minimize wind, and sized to fit the number of operating vehicles, with approximately 3 m allocated to each. At the end of each day, the cell is covered to prevent windblown refuse and the attraction of disease vectors. Daily cells are organized into larger cells, which themselves are organized into multiple vertical lifts constituting the active portion of the landfill. Systems of a similar complexity are used to manage landfill gas and leachate, ensure geotechnical stability, and provide final cover for closed cells.

One consequence of the highly structured nature of waste placement is that landfills are not only heterogeneous due to the nature of the waste itself but are also heterogeneous due to the discrete nature of the cells. In contrast to natural soils, which generally receive carbon inputs primarily through plant and animal activities

at or near the surface, landfills may contain organic materials stratified throughout the profile (which may be as deep as 80 m) as a result of the continuous burial of solid wastes. One benefit of this is that records of cell histories can allow researchers to estimate the waste age based on sampling depth and location. A further consequence is that the distribution of organic carbon within a landfill is stratified and can increase in depth (Gomez et al. 2011).

5.1.2 *Refuse Composition*

An estimate of the composition of MSW discarded to landfills in 2015 (after materials are removed for recycling and composting) in the USA is presented in Table 5.1.

Paper, yard waste, and food waste, the biodegradable components of MSW, include three major organic components: cellulose, hemicellulose, and lignin. Cellulose, a polymer of glucose, and hemicellulose, a C-5 polymer, are the principal biodegradable components of MSW (Barlaz et al. 1989), while lignin is recalcitrant under methanogenic conditions (Colberg 1988) and interferes with the decomposition of cellulose and hemicellulose by physically impeding microbial access to these degradable carbohydrates. The cellulose, hemicellulose, and lignin fractions in residential MSW have been reported to range from ~30–50, 6–11, and 10–25%, respectively (Barlaz 2006). Lipids, starch, and proteins originate in food waste, which is expected to increase in percentage over time, as the amount of paper produced decreases and the amount recovered for recycling increases. Similarly, in low- and middle-income countries, there is also a greater fraction of food waste (and thus lipids and proteins) discarded, largely owing to the effects of income on goods purchased, retained, and recovered (Tchobanoglous et al. 1993).

Table 5.1 MSW composition landfilled in the USA in 2015 (US Environmental Protection Agency 2018c)

Material	Tons (thousands)	Percent by weight
Food	30	22
Plastics	26	19
Paper and paperboard	18	13
Rubber, leather, and textiles	15	11
Metals	13	10
Yard trimmings	11	8
Wood	11	8
Glass	7	5
Other	6	5

5.2 Anaerobic Biological Processes in a Landfill

The decomposition of MSW to CH_4 and CO_2 in landfills is a microbially mediated process that requires the coordinated activity of several trophic groups of bacteria (Fig. 5.1) (Zehnder and Mitchell 1978; Brock et al. 1994).

Initially, polymers (i.e., carbohydrates, fats, and proteins) are hydrolyzed, yielding soluble sugars, amino acids, long-chain carboxylic acids, and glycerol. Fermentative microorganisms then convert these hydrolysis products to short-chain carboxylic acids, ammonia, CO_2, and H_2. Acetate and alcohols are also formed. Next, fatty acid-degrading bacteria oxidize products like propionate and butyrate to acetate, CO_2, and H_2. Oxidation of propionate and butyrate is only thermodynamically favorable at very low H_2 concentrations (Zehnder and Mitchell 1978). Thus, these bacteria only function in syntrophic association with an H_2 scavenger such as a methanogen or a sulfate reducer. Typically, sulfate concentrations in landfills are minimal and CH_4 is the major electron sink. The terminal step in the conversion of complex polymers to CH_4 and CO_2 is carried out by the methanogenic archaea. The most common methanogenic substrates are acetate (aceticlastic methanogenesis) and CO_2 plus H_2 (hydrogenotrophic methanogenesis). Should the activity of the fermentative organisms exceed that of the fatty acid degraders and methanogens, there will be an imbalance in the ecosystem. Carboxylic acids and H_2 will accumulate and the

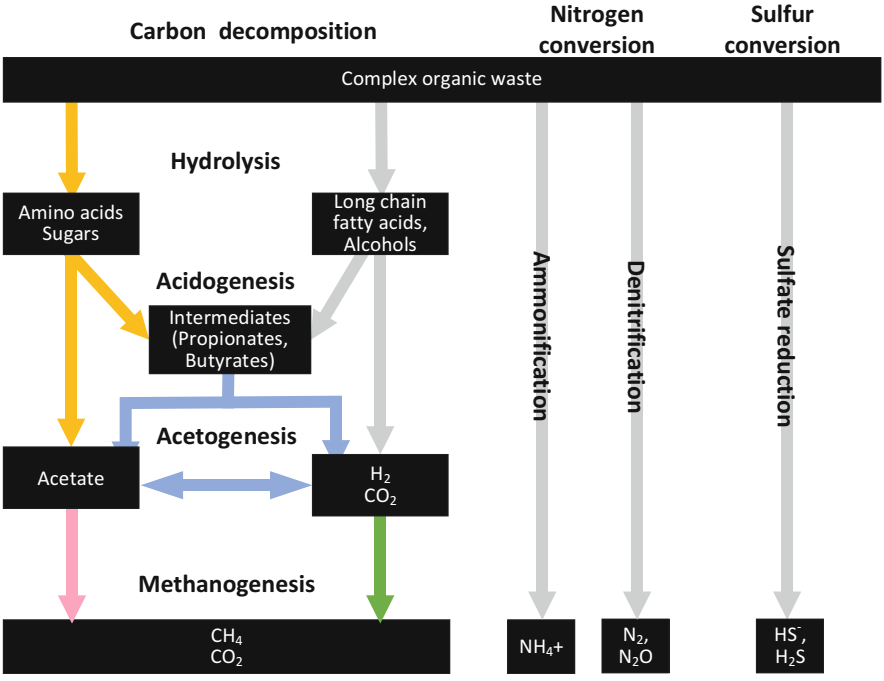

Fig. 5.1 Anaerobic biological processes in a landfill

pH of the system will decrease, inhibiting methanogens, most of which have a pH optimum around 7 (Zinder 1993).

Most of the above processes are mediated by bacteria and archaea. The presence of anaerobic protozoa in refuse excavated from landfills has been reported (Finlay and Fenchel 1991), and many of the protozoa contained symbiotic methanogens that utilize H_2 released by the host's hydrogenosomes. The dominant protozoan isolated from the samples was the ciliate, *Metopus palaeformis*. A later study showed no evidence that protozoa were stimulating refuse decomposition through enhanced nutrient recycling (Finlay et al. 1993)

5.2.1 Phases of Refuse Decomposition

Four sequential refuse decomposition phases have been defined by chemical and microbiological characteristics (Fig. 5.2): aerobic, anaerobic acid, accelerated CH_4 production phase, and decelerated CH_4 production (Barlaz et al. 1989). All of the trophic groups required for methanogenesis (hydrolytics, fatty acid degraders, and methanogens) are present in fresh refuse; it is their relative abundance which varies with phase.

In the aerobic phase (phase 1), both O_2 and NO_3 are consumed with soluble sugars serving as the carbon source for microbial activity. In the anaerobic acid phase (phase 2), carboxylic acids accumulate and the pH decreases because of an imbalance between fermentative and acetogenic and methanogenic activity. There is limited cellulose and hemicellulose decomposition in phase 2. The methanogen population begins to increase and CH_4 is first detected. In the accelerated CH_4

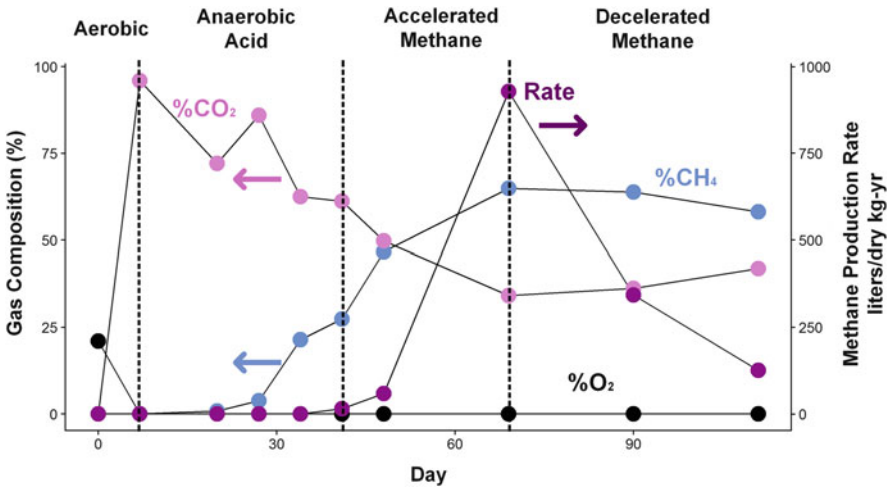

Fig. 5.2 Phases in refuse decomposition in a landfill (replotted from Barlaz et al. 1989). Phase 1—aerobic; phase 2—anaerobic acid; phase 3—accelerated methane; phase 4—decelerated methane

production phase (phase 3), the CH_4 production rate increases to some maximum value, carboxylic acid concentrations decrease, the pH increases, there is little solids hydrolysis, and the populations of hydrolytic, fatty acid-degrading bacteria and methanogens increase. The accumulated carboxylic acids are the principal substrates supporting CH_4 production in phase 3. In the decelerated CH_4 production phase (phase 4), the CH_4 production rate decreases, the fatty acid-degrading population increases, carboxylic acids are depleted, and there is an increase in the rate of cellulose plus hemicellulose hydrolysis (Fig. 5.2). While acid utilization limits CH_4 production in phases 2 and 3, solids hydrolysis limits CH_4 production in phase 4. These phases were originally derived from lab-scale reactors (Barlaz et al. 1989), and samples excavated from a landfill confirmed the relationship between H_2, pH, and carboxylic acid concentrations expected based on this four-phase description (Gurijala and Suflita 1993). Buried waste will in theory continue to decompose until no more biodegradation occurs and the landfill becomes aerobic, perhaps over geologic time, if at all.

Several points need to be made with respect to applying this description of refuse decomposition to full-scale landfills. First, the time required for the onset of each phase may be significantly longer than the times shown in Fig. 5.2 owing to such variable factors as the biodegradability and heterogeneity of wastes. Second, gas and leachate samples from landfills often reflect a composite of refuse in several different states of decomposition, and because the dominant taxa vary with phase, the microbial communities detected would also be a composite of the different microbial populations dominant in each phase. Third, the presence of NO_3 would stimulate denitrification, which would inhibit methanogenesis. Fourth, in the presence of sulfate, electrons would be diverted from CH_4 production to sulfate reduction. Sulfate reduction and CH_4 production have been shown to occur concurrently, with three to nine times more organic carbon degraded through methanogenesis than SO_4 reduction (Fairweather and Barlaz 1998) because of the abundance of degradable carbon. Others have shown that SO_4 inhibited CH_4 production and that inhibition of SO_4 reduction resulted in increased CH_4 production (Gurijala and Suflita 1993).

5.2.2 Factors Limiting Decomposition in Landfills

A number of factors influence the onset and rate of CH_4 production in landfills, including moisture content and flow, pH, particle size, inoculum addition, nutrient concentrations, and temperature (Barlaz et al. 1990). Of these factors, moisture content and pH appear critical; high moisture promotes both nutrient and microbial transport and neutral pH encourages methanogen growth. Providing these conditions via recycling neutralized leachate enhances the onset and rate of CH_4 production in laboratory-scale tests (Barlaz et al. 2010), and this technique, now referred to as bioreactor landfills, has been demonstrated at field scale. The microbiology of such environments has attracted the attention of researchers (Sang et al. 2012).

5.3 Systems for Studying Decomposing Refuse and Landfill Microbiology

5.3.1 Experimental Systems

The most representative experimental system is a field-scale landfill containing several tons of well-characterized refuse (Hilger and Barlaz 2007); it is also the most expensive and difficult to experimentally control. Lab-scale reactors embody the opposite trade-off, sacrificing verisimilitude for increased affordability and experimental control while also allowing more practical sampling and, potentially, accelerating experiments via rapid degradation (Fig. 5.3).

5.3.1.1 Landfills

Samples from working and closed landfills have been used to detect microbial communities as they occur in the field. Soil cores have been used to observe microbial community differences arising from not only depth (Sawamura et al. 2010) and geography (Song et al. 2017) but also due to environmental gradients, such as hydrocarbon pollution within an uncontrolled dumping ground (Gomez et al. 2011). Beyond measuring diversity, cores have also been used to search for populations with the genetic potential to perform metabolic processes not yet directly observed in landfills, such as anaerobic methane oxidation (Dong et al. 2015).

5.3.1.2 Test Cells

When greater experimental control is needed, test cells are sometimes used. Test cells may be a landfill subsection or a separate excavation in the same area which may or may not be adjoined to the landfill proper. The leachate from multiple

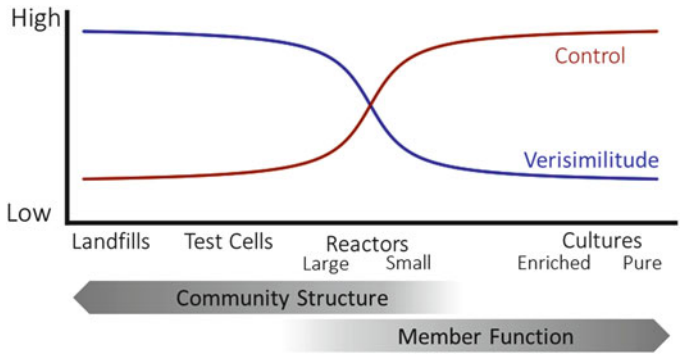

Fig. 5.3 Trade-offs in choice of experimental system

15 kiloton test cells was shown to contain anaerobic cellulose-degrading fungi previously only observed within mammalian guts (Lockhart et al. 2006). Leachate from test cells has also been used to test primers specific to cellulose-degrading *Clostridium* subgroups (I, III, IV, and XIV) (Van Dyke and McCarthy 2002). Because test cells are often used to evaluate operational decisions, it is possible to correlate those decisions with microbial community changes, such as the shifts associated with aeration (Hale Boothe et al. 2001) or adding cellulose-rich layers in a low-level radioactive waste test pit (Field et al. 2010).

5.3.1.3 Lab-Scale Reactors

Lab-scale reactors are relatively affordable and can be located in a controllable environment, making them particularly useful when multiple, reproducible samples are required or when manipulating specific experimental factors. Reactor designs and sizes are variable and have been extensively reviewed (Fei et al. 2014). Reactor volume is the major differentiating factor and influences many other reactor parameters (e.g., particle size, sampling methods, homogeneity). In general, larger reactors, which can be loaded without reducing waste component sizes, may more accurately represent landfill homogeneity and avoid the sometimes undesirable effects of shredding, such as altered leachate and chemical transport. Conversely, smaller-scale reactors, which require shredding of waste, tend to minimize local environmental effects, produce more homogenous samples, and can degrade waste at an accelerated rate.

Lab-scale reactors are useful experimental systems for studying both the products of microbial activity (e.g., methane and carbon dioxide) and the microbes themselves. The methane yields, extents, and decomposition rates (de la Cruz and Barlaz 2010) of many substrates in landfills have been estimated using reactors, including wood (Wang et al. 2011), paper (Wang et al. 2015), food waste (Lopez 2015), and biodegradable plastics (Weaver 2013). Further, landfill reactors have been used to correlate biological activity with chemical mobilization. For example, comparing abiotic and biotic reactors suggests microbial activity significantly increases poly- and perfluoroalkyl substance (PFAS) releases into landfill leachate (Allred et al. 2015; Lang et al. 2016). Similarly, both solid and leachate reactors were used to elucidate the biological role of arsenic mobilization (Ghosh et al. 2006; Cortinas et al. 2008).

Direct studies of the microbes within landfill reactors have been used to determine the factors that influence landfill ecology. Landfill simulation reactors were among the first systems used to elucidate the different decomposition stages within a landfill and the microorganisms associated with each stage (Barlaz et al. 1989). Since then, reactors have been continually used to refine our understanding of landfill microbiology, such as by comparing competing methanogenic and sulfur-reducing populations (Fairweather and Barlaz 1998), relating specific microbial clades to degradation of specific substances, such as cellulose degradation by *Clostridium* (Burrell et al. 2004) and *Fibrobacter* (McDonald et al. 2012). Further work has also

incorporated dynamic changes in microbial populations, such as determining the influence of initial hemicellulose and cellulose concentrations on methanogenic population shifts (Bareither et al. 2013) and by suggesting that a reaction front attributable to acid-tolerant methanogens, such as *Methanosarcina barkeri*, is responsible for initiating methane generation (Staley et al. 2011a), rather than only by spreading out from initially neutral microniches, as was previously hypothesized (Martin 2001).

5.3.2 Sampling for Microbial Analysis

5.3.2.1 Leachate Versus Solid Samples

Regardless of the experimental system employed, representative sampling for microbial analysis is critical (Hilger and Barlaz 2007) and should be considered when deciding whether to collect solids or leachate. Leachate, which is more easily collected and processed, is often erroneously assumed to be representative and chosen for sampling. One argument for leachate sampling is that leachate has ostensibly pervaded the entire system and therefore could be presumed representative of the entire landfill. However, leachate samples are biased and favor planktonic biomass (Burrell et al. 2004; Li et al. 2009). Planktonic biomass experiences different selection pressures than attached biomass; one example is the differences in nutrient availability caused by the varying solubilities of carbon sources and adsorption of nutrients onto solids (Fei et al. 2015). These different selection pressures lead to different community compositions (Staley et al. 2011b), and a sample biased toward either planktonic or attached biomass will likely not represent the entire microbial community.

A second argument for leachate samples is that while leachate may not represent the total microbial community, the microbes contained in the sample may be correlated with the status (e.g., acidic, methanogenic, aged) of the landfill. When all of the waste is the same age, as in a lab-scale reactor, leachate composition may be used to infer waste status (Fei et al. 2015). In a landfill however, waste is laid down in layers and is therefore heterogeneous in age as well as in size and composition. In that case, inferences are confounded by layers of older waste essentially treating leachate which has passed through overlying newer waste layers.

There has not yet been a direct comparison between solid and leachate microbial communities from the same landfill samples using high-resolution culture-independent methods. Given the heterogeneity of landfills, meta-analyses that results derived from various methods, including different high-throughput sequencing technologies, applied to leachate and solid samples from separate landfills, are not conclusive.

Multiple lab-scale reactor experiments suggest that leachate samples do not adequately represent the entire microbial community. A 454 pyrosequencing metagenomics study of lab-scale solid and leachate samples showed similar trends between archaea at the family and functional (i.e., aceticlastic vs. hydrogenotrophic)

levels (Fei et al. 2015), indicating that leachate samples were appropriate for determining the overall state of refuse degradation. However, the communities were sufficiently different, even at the large and relatively diverse family level, limiting the validity of inferences about the actual archaeal population of the landfill. Bacterial populations have also been analyzed using 454 pyrosequencing (Bareither et al. 2013) and by that technique have been more clearly shown to differ between leachate and solids. Those results agree with earlier work using terminal restriction fragment length polymorphism (T-RFLP) (Staley et al. 2011b, 2012) and fluorescence in situ hybridization (FISH) probes (Burrell et al. 2004) which also indicated the differences between leachate and solids microbial populations.

5.3.2.2 Collecting Samples

Solid samples from full-scale landfills are often taken through geotechnical coring and have gone as deep as 40 m (Chen et al. 2003a; Field et al. 2010; Krishnamurthi and Chakrabarti 2013). Other studies often take advantage of excavation being performed for other reasons and have included the use of backhoes (Chen et al. 2003b), which increase the volume of solids available, but presumably at the cost of lowered spatial resolution (particularly vertical) and inability to sample far beyond 3 m deep. Leachate is generally either collected at some location from a preexisting leachate collection system (often at the point where leachate enters collection ponds) or in containers sunk into gas collection vents (Lockhart et al. 2006). In some instances, leachate can be collected as it "seeps" from poor cover (Huang et al. 2003; Zhu et al. 2007).

As with a landfill, lab-scale reactor leachate samples are easier than solids to obtain but may not appropriately support the research goals. Solid sampling is generally preferable and either incorporates destructive sampling of replicate reactors (Staley et al. 2011a) or nondestructive coring (Fei et al. 2014).

5.3.2.3 Preserving Samples

Samples for nucleic acid-based analysis, such as DNA sequencing, are generally immediately stored on ice when possible and then frozen. The major differentiating factors are whether freezing takes place at −20 °C or −80 °C, if flash freezing is performed, if a preservative (e.g., polyethylene glycol) is added, and if anaerobic conditions are maintained during sampling. As landfill microbiology studies expand into other "omics," further preservation steps will become necessary. Examples include preventing RNA degradation through a preservative for transcriptomic work and preventing the loss of volatiles for metabolomic analysis. Landfill researchers may be able to avoid common pitfalls by borrowing methods developed for similar complex environments, such as soils and sediments.

5.3.2.4 Processing Samples

Nucleic acids can either be extracted directly from samples or extraction can be preceded by indirect methods which concentrate cells separated from the sample matrix. Direct methods are simpler, but the small sample size excludes large waste components and results are sensitive to the level of heterogeneity. In contrast, indirect methods such as use of solutions to separate cells from particles require more labor but concentrate genetic material, essentially allowing a larger sample size less sensitive to heterogeneity and likely producing more representative results. Additionally, early studies assumed that indirect extraction methods would be biased based on cell adhesion (He et al. 2009). A later study critically evaluated multiple direct and indirect solid extraction techniques and compared them on the basis of DNA yield and bias using pure culture spikes and T-RFLP fingerprinting (Staley et al. 2011b). Based on the results, an indirect method was recommended in which cells were concentrated via centrifugation of a filtrate produced by homogenizing samples in a phosphate buffer followed by hand squeezing through a nylon bag. Despite the evidence showing that indirect methods are preferable, direct methods are more commonly performed. Heterogeneity is sometimes addressed by quartering and mixing large samples prior to subsampling a small volume based on the assumption that it produces microbial samples which are more representative (Chen et al. 2003b).

After cell extraction, the nucleic acid must still be extracted and purified, with special attention paid to removal of PCR inhibiting substances, such as humic acids. In one study, various extraction methods were compared for DNA yield and purity, with the recommendation to use bead-beating lysis and proteinase K treatment followed by SDS-based extraction and modified Sephadex G-200 spin column purification (He et al. 2009). Every processing method produces unavoidable bias, and while the best way to counteract bias is through pooling extracts from multiple techniques (Staley et al. 2011b), such an approach is often impractical. As such, reporting the details of the extraction process is essential for guiding researchers, who may be comparing results between studies.

5.4 Traditional Methods for Microbial Analysis

Previous reviews have examined the microbial analysis of landfills using traditional methods, and a comprehensive analysis of the results will not be covered here. These methods include culture-dependent methods to quantify the levels of microorganisms, such as most probable number (MPN) (Barlaz et al. 1989; Qian and Barlaz 1996; Fairweather and Barlaz 1998; Mori et al. 2003; Barry 2008; Sawamura et al. 2010) and CFU (colony-forming units) (Hale Boothe et al. 2001; Sawamura et al. 2010; Krishnamurthi and Chakrabarti 2013). Microscopy-based methods include acridine orange counts (Ladapo and Barlaz 1997) to visualize DNA or RNA,

visualization with DAPI (a DNA intercalating dye) to stain DNA, use of autofluorescence (Finlay and Fenchel 1991; Mori et al. 2003), and electron microscopy (Finlay and Fenchel 1991; Westlake et al. 1995; McDonald et al. 2012).

Other traditional approaches are focused on assessing and quantifying microbial activity, such as measurement of microbial biomass and activity (Bogner et al. 1995), enzyme assays (Pourcher et al. 2000), and determining methane production rate (Fairweather and Barlaz 1998). The production or removal rates of other compounds have also been used extensively to assess activity in landfills. Examples include non-methane lignin products (Kim et al. 2009; de la Cruz et al. 2014), perfluoroalkyl substances (PFASs) (Allred et al. 2015), non-methane organics in the gas (Staley et al. 2006), alkylbenzenes and phenol (Wang and Barlaz 1998), and hydrogen sulfide (Sun et al. 2016).

5.5 Molecular Approaches for Analysis of Landfill Microbiology

Nucleic acid (DNA and RNA)-based approaches for analyzing the microbial communities in environmental samples, which have revolutionized environmental microbiology in the last few decades, have also heavily influenced landfill research. Most applications of molecular methods answer the question of "who's there?": these are microbial surveys that profile community composition, often described by the community membership, individual species abundance, and community diversity and evenness. By themselves, surveys add little to our understanding of how waste decomposes in landfill. However, surveys can be foundations leading to informative hypotheses (de los Reyes III et al. 2015; Hugerth and Andersson 2017) linking landfill performance to microbial populations, environmental conditions, and operation. Knowledge gained from the integration of thorough microbial surveys and hypothesis-driven experiments may lead to important ecological insights for the development and optimization of landfill microbiota. Below we summarize some of the key advances in landfill microbiology using these techniques. We discuss available molecular and computational strategies to assess microbiome dynamics, distribution, and ecology of the landfill biosphere and provide examples of hypothesis-driven characterizations and analyses of important community members—those who play important roles in landfill cellulose metabolism.

5.5.1 Surveying Microbial Populations

Community surveys of landfill microbiomes have been performed using terminal restriction fragment length polymorphism (T-RFLP) (Staley et al. 2012; Kong et al. 2013), while recent advances of high-throughput molecular techniques have allowed

more massive community profiling using 454 pyrosequencing (Bareither et al. 2013; Köchling et al. 2015; Song et al. 2015a, b, c; Fei et al. 2015) and Illumina Miseq (Dong et al. 2015; Stamps et al. 2016). As an example finding of such surveys, genera identified by 454 pyrosequencing were mapped to the classic anaerobic digestion pathway diagram (Köchling et al. 2015). Other integrations of molecular tools for targeted microbial surveys include qPCR and SEM (McDonald et al. 2012; Ransom-Jones et al. 2014), FISH and DNA-SIP (stable-isotope probing) (Li et al. 2009), FISH and clone library sequencing (Burrell et al. 2004), and GeoChip-based metagenomic and functional gene arrays (Lu et al. 2012).

Targeted surveys may focus on different functional or phylogenetic groups or attempt to answer a specific question by a careful selection of samples. For example, because methanogens are sensitive to low pH and oxygen, how they are transported to the landfill is of interest. A T-RFLP comparison of communities on individual components of fresh refuse showed that while up to a third of the microbes in fresh refuse may contribute to the final community composition and that leachate transport of some organisms throughout the landfill is limited and biased toward planktonic organisms, the final methanogenic population is not determined by the dominant inoculating population (Staley et al. 2012). An example study focusing on functional genes used the nitrite reductase genes nirS and nirK to determine the identity and prevalence of denitrifying organisms in landfills (Fang et al. 2010).

5.5.1.1 Landfill Biosphere Exhibits a Unique Microbiome Structure

Landfill microbiomes have been shown to harbor a distinct taxonomic and ecological diversity. A notable 16S rRNA (ribosomal RNA) metagenomic survey was recently performed using leachate samples from 19 landfills distributed throughout the USA (Stamps et al. 2016). The scale and consistency of the study allowed researchers to determine that while landfill microbiomes differ from each other, they differ even more from other methanogenic environments, both natural (e.g., peat bogs) and built (e.g., anaerobic digesters), and are a microbially unique environment. Furthermore, the microbial data were taken in concert with a USGS (US Geological Survey) national survey (https://toxics.usgs.gov/) of leachate chemical composition, allowing researchers to correlate major environmental factors to their influence on the microbial community. In addition to the expected waste age and moisture content (specifically evapotranspiration rate), chloride, barium, and miscellaneous household chemical concentrations were strong influencers of biodiversity within landfills. On a more specific scale, microbiomes from environments where cellulose degradation occurred were examined and revealed a unique community identified exclusively in landfill sites within the cellulolytic phylum *Fibrobacter* (Ransom-Jones et al. 2014).

5.5.1.2 Linking Landfill Microbiome Dynamics to Community Function and the Environment

Different landfill sites can have different biotic and abiotic traits that are specific to the local environment and waste characteristics. Microbial surveys can be integrated with measurements of various environmental traits and functional parameters for the purpose of correlating community dissimilarities either to environmental conditions or to certain microbial functions. For example, Song et al. (2015a) demonstrated the influence of precipitation and landfill age on community structure in a bacterial community survey. In another study, it was shown that nitrate concentration, total phosphorus, and conductivity primarily drove the archaeal community dissimilarity (Song et al. 2015b). Other landfill surveys have explored associations between the rates of methane generation, specimen volume reduction, and DNA concentration in the leachate (Fei et al. 2015); landfill age and the abundance of specific taxa (Köchling et al. 2015); depth and age of landfill cover soils in relation to methane oxidation activity and community structure (Kong et al. 2013); seasonal differences in ambient air temperature and bacterial microbial community richness (Staley et al. 2012); community shifts during initial and stable methanogenic phases (Song et al. 2015c); and changes in pH and initiation of methanogenesis (Staley et al. 2011a).

Another way to disentangle the dynamic relationship between community composition, function, and environmental traits is to perform a co-occurrence or microbial network inference analysis (Faust and Raes 2012). Co-occurrence patterns between community members have been used to infer species interactions and identify ecological roles of core populations in the microbiome assemblies of soil (Barberán et al. 2012), wastewater (Ju and Zhang 2015), marine (Steele et al. 2011), and human gut environments (Goodrich et al. 2014). Although not yet explored for landfill surveys, this co-occurrence approach has been used to reveal possible associations between communities and environmental characteristics of their habitats (Gilbert et al. 2012; Coutinho et al. 2015).

5.5.1.3 Identification of Rare Taxa in Landfills

The dynamics, distribution, and ecological contribution of rare taxa, those present at low relative abundance, have been recognized in a variety of environments, such as marine water and sediment (Sogin et al. 2006), soil (Elshahed et al. 2008), and bacterioplankton (Vergin et al. 2013). The presence of rare biosphere members in landfill microbiomes was first addressed in a high-throughput microbial survey by Köchling et al. (2015), despite undersampling. They profiled the rank-abundance distributions of both dominant and rare populations and compared a variety of diversity indices between the entire communities and the low-abundance subpopulations. The results revealed the presence and ecological significance of more diverse subgroups of microorganisms within landfill habitats compared to the abundant populations. In an archaeal community survey applying principal component and

cluster analyses, nonabundant taxa contributed to the community dissimilarity, although the same approaches showed that the abundant taxa were more responsible for the discrimination of bacterial communities (Song et al. 2015a, b).

The rare biosphere members can provide a recruitment pool of functional potentials as a microbial seed bank. Rare members can become dominant populations in response to availability of specific niches and are often associated with the maintenance of species diversity and community stability. Rare biosphere members may be recruited to fulfill the role of keystone species, exerting a disproportionately large ecological influence on community dissimilarity or on some specific function (Galand et al. 2009; Lynch and Neufeld 2015). The rare biosphere components can also exhibit disproportionate abundance dynamics where only some rare taxa conditionally became dominant after a disturbance (Shade et al. 2014). Thus far, the unique community structure or metabolic network associated with members of the rare populations has not been fully understood in landfill environments.

5.5.2 Relating Microbial Populations to Function

Important linkages within the microbial ecology of landfills can be found by combining an identification method with a functional method, generating strong correlations between function and identity, accompanied by using specific methods which directly relate function and identity (de los Reyes III et al. 2015).

5.5.2.1 Attributing Cellulose Degradation to Specific Taxa

Cellulose is a major (40–50% by weight) component of municipal refuse (Bookter and Ham 1982), and the hydrolysis of cellulose is often a rate-limiting step in MSW degradation (Barlaz 2006). Consequently, a majority of landfill experiments asking "Who, specifically, is eating what, when?" have focused on cellulose degradation. Early targeted molecular surveys using temporal temperature gradient electrophoresis (TTGE) fingerprinting and sequencing of 16S rRNA amplified with primers specific to *Clostridium* groups known to degrade cellulose have shown that clostridia were commonly found in landfill leachate, suggesting that *Clostridium* may be a significant genus associated with cellulolytic activity within landfills (Van Dyke and McCarthy 2002). FISH probes targeting related *Clostridium* subgroups showed clear spatial correlations with cellulose particles, continuing to highlight the key role of *Clostridium* in cellulose degradation in landfills (Burrell et al. 2004). The proximity of *Clostridium* to cellulose particles was again demonstrated using FISH by Li (2009). In the same study, *Clostridium* was directly associated with the degradation of cellulose, along with *Acetovibrio* (like *Clostridium*, a genus in the *Firmicutes* phylum) via DNA-SIP.

In other anaerobic environments such as the rumen, the major taxa associated with cellulolytic activity not only include the *Clostridium* cluster IV *Ruminococcus*

but also fungi from the order *Neocallimastigales* and bacteria from the genus *Fibrobacter* (McDonald et al. 2012). While early studies targeted *Clostridium* specifically, based on earlier culture-dependent work (Westlake et al. 1995), they did not rule out other taxa. Anaerobic fungi, such as *Neocallimastigales*, have neither been widely looked for nor observed in landfill samples. Primers specific to *Neocallimastigales* were developed and used in landfill leachate (Lockhart et al. 2006). However, the occurrence of that fungal genus was only observed at one landfill and its presence was attributed to an indigenous population. This explanation agrees with subsequent work in which *Neocallimastigales* specific primers failed to hybridize in a slot-blot test of landfill leachate (McDonald et al. 2010) and were observed in only 3 nested PCR amplifications covering 22 sites from 5 landfills (McDonald et al. 2012).

A similarly expanded search to determine the role of *Fibrobacter* in landfills has produced evidence that they participate significantly in cellulose degradation. It was hypothesized that the lack of *Fibrobacter* detected in landfills was an artifact of experimental methods, particularly underrepresentation in clone libraries. When a *Fibrobacter*-specific 16S rRNA nested PCR primer was developed, *Fibrobacter* was detected in multiple leachate samples, albeit generally in lower abundance than in the gut, as determined by qPCR (McDonald et al. 2008). A subsequent analysis of samples from multiple environments, including landfills, using *Fibrobacter*-specific primers also determined that *Fibrobacter* was found in many non-gut communities (Ransom-Jones et al. 2014).

5.5.2.2 Linking Degradation of Other Substrates to Specific Taxa

Substrates other than cellulose present a problem in that the material of interest is often soluble. While using a FISH probe can provide strong spatial evidence that certain microorganisms are involved in degrading large, insoluble cellulose particles, this is difficult to achieve in situ for dissolved species. Instead of single-substrate enrichments, stable-isotope methods have also been used, often using incorporation of $\delta^{13}C$ into the DNA of metabolically active taxa. The advantage with stable-isotope probing is that there is less culture bias and samples may be kept in the environmental matrix. These methods can be used to both to determine which organisms are metabolizing a substrate and to trace the relative activity of parallel metabolic pathways. Li (2009) traced the use of $\delta^{13}C$-labeled acetate and glucose and suggested that much of glucose assimilation was not directly due to cellulose degradation, as cellulose degraders may not be directly making glucose but rather the glucose assimilation represented production of cellodextrin intracellularly by cellulose hydrolysis.

5.5.2.3 Methanogenic Pathway Shifts and Initiation of Methanogenesis

Preferential isotope use in different metabolic pathways allows researchers to look at the isotopic ratio in products to determine the relative utilization of parallel pathways. In a landfill system, this is most pronounced for the production of methane. Qu et al. (2009) used isotopic ratios to observe that although the methanogenic pathway in landfill reactors shifted toward hydrogenotrophic metabolism, the dominant methanogenic family remained unchanged after significant methane production began, with *Methanosarcinaceae* prevailing over an initially heavy *Methanosaetaceae* population. *Methanosarcinaceae* is a known mixotroph while other methanogens are either obligately aceticlastic or hydrogenotrophic, suggesting that, at least under the conditions of the experiment, metabolic versatility is selected over specialization.

With regard to initial sites of methanogenesis, a widely held hypothesis that methane is initially produced in micropockets of neutral pH (Martin 2001) was tested using destructive random sampling of reactor slices. Measurements of micro-pH were unable to detect pockets of neutral pH prior to methanogenesis. However, RNA clone library analyses suggested that an acid-tolerant organism, *Methanosarcina barkeri,* initiated methane production and created expanding areas of pH neutralization, allowing other methanogens to begin methanogenesis (Staley et al. 2011a).

5.6 Summary

The use of molecular methods, such as DNA- and RNA-based analysis, has provided new insights into various aspects of landfill microbial ecology. The power and decreasing cost of metagenomic analysis has made survey-type studies accessible to landfill researchers. However, such studies may be of limited value: hypothesis-driven experiments, intended to address specific questions, should be the focus of future work. Such experiments require more in-depth analysis and the choice of the most appropriate microbial analysis methods. In particular, combinations of microbial methods, integration with chemical and physical measurements, and rigorous experimental designs offer the most promising avenues for advancing research on the microbiology of landfills. Ultimately, such advances should not only extend our knowledge of the unique landfill microbiome but also impact landfill design and operation.

Compliance with Ethical Standards

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Conflict of Interest Joseph E. Weaver declares that he has no conflict of interest. Ling Wang declares that she has no conflict of interest. Francis L. de los Reyes III declares that he has no conflict of interest. Morton A. Barlaz declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Chapter 6
Microbial Community Dynamics During the Composting Process of Animal Manure as Analyzed by Molecular Biological Methods

Nozomi Yamamoto and Yutaka Nakai

Abstract Composting is a useful technique that transforms livestock manure into stable organic fertilizer. In composting, the biodegradation of substrates is conducted by microbial communities of bacteria, archaea, and fungi. Bacteria are assumed to play an important role in the decomposition of organic substances. However, only a few studies have tracked bacterial communities throughout the composting process. Furthermore, the role of archaea in composting remains to be fully elucidated. To uncover the dynamics of these bacterial and archaeal communities, a variety of molecular biological methods like PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) and clone library were utilized. A clone library constructed from bacterial 16S rRNA genes showed that the structure of the bacterial community changed dynamically with compost processing time. At first, phyla Firmicutes and Bacteroidetes were dominant. Phylum Firmicutes maintained abundance for 20 days, indicating that these bacteria may be active under high temperatures. In the final compost, the library consisted of sequences belonging to the phyla Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria.

A clone library constructed from archaeal 16S rRNA genes showed that the archaeal community was mainly comprised of methane-producing archaea (methanogen) and ammonia-oxidizing archaea (AOA). During first 2 days, it was revealed that fecal methanogens could survive the early stage of composting. Thermophilic *Methanosarcina* spp. were present throughout the process, indicating that they may adapt to environmental changes such as high temperatures. Detecting AOA-like sequences showed that AOA could be actively involved in the nitrification of composting systems. Furthermore, the abundance of AOA varies markedly with the raw materials and composting technique used.

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6.1 Overview of Microorganisms in Composting Process

The annual production of livestock manure is estimated to be 1.1 billion tons in the USA (USEPA 2013), 1.4 billion tons in the EU (Lyngsø et al. 2011), and 83 million tons in Japan (MAFF 2015). Animal manure is a major portion of total wastes; in Japan, animal manure accounts for about 20% of the total industrial waste. Fresh manure applied directly to land can increase the risk of excessive nutrient load and transmission of pathogens and has an offensive odor when utilized as a soil fertilizer (Asano et al. 2007; Bernal et al. 2009; Mackie et al. 1998; Pell 1997). To solve these problems, composting is the most effective technique for the treatment of solid manure. Via composting, the easily degradable organic matter is mineralized, the offensive odor from short-chain fatty acids is reduced, plus pathogen levels typically are reduced and plant seeds are destroyed, resulting in a more desirable and biologically stabilized material (Bernal et al. 2009; Haga 1999). These changes are the result of biological action. During the composting process, microorganisms such as bacteria, archaea, fungi, and protozoa form huge and diverse communities and play important roles in the decomposition (Ryckeboer et al. 2003). Therefore, composting has been described as "a spontaneous biological decomposition process of organic materials in a predominantly aerobic environment" (Bernal et al. 2009). The technique is generally conducted over a period of 1–6 months and can be divided into several stages according to the temperature variation (Godden et al. 1983; Insam and de Bertoldi 2007). Various environmental factors affect the progression of the composting process (Diaz and Savage 2007). Each stage is described briefly as follows:

1. Initial, medium-temperature stage (25–40 °C): Easily degradable organic substrates such as sugar, amino acids, proteins, and lipids are degraded aerobically. Organic carbon is transformed into carbon dioxide and heat is generated. The temperature starts to increase because of the heat from decomposition.
2. High temperature stage (35–65 °C): The temperature rises above 60 °C and occasionally reaches up to 80 °C. Most of the degradable organic matter is decomposed during this stage. The moisture content is reduced through evaporation. Carbon dioxide and organic acids are produced within the aerobic and anaerobic regions, respectively. During this stage, mixing the materials regularly is needed to maintain aerobic conditions, and the production of short-chain fatty acids leads to an offensive odor. In addition, ammonia is generated from organic nitrogen compounds and volatilized by the increased pH. It is believed that prokaryotes play the major role in decomposition during this stage. The composting process needs to be maintained at 55 °C for 2 weeks or 65 °C for 1 week for sanitization, as proposed by the European Commission (Böhm 2002).
3. Cooling stage: The degradation rate is reduced owing to the lack of easily degradable substrates; therefore, the temperature also drops. However, persistent substances like cellulose and lignin begin to decompose. Fungi are the principal participants in the decomposition of non-degraded organic matter (Insam and de Bertoldi 2007).

4. Maturation stage: The compost has low concentrations of organic matter but has accumulated inorganic and humic substances that are not able to degrade further.

Of all the living microorganisms in compost, the bacterial community has the largest and most complex population (Ryckeboer et al. 2003), assumed to be a major component of the biomass and a major contributor to the overall metabolism during composting. The biomass of bacteria is estimated to be 10^9–10^{13} cells/g fresh weight during the medium-temperature stage and 10^8–10^{12} cells/g fresh weight during the high temperature stage (Ryckeboer et al. 2003). The structure and diversity of the bacterial community change dramatically to adapt to the environmental changes during the composting process, such as rising temperature, water evaporation, and aerobic versus anaerobic conditions (Ryckeboer et al. 2003; Schloss et al. 2003). To answer fundamental questions such as "which microorganisms survive in compost?" or "how do environmental parameters affect the microorganisms in compost?," it is necessary to track the changes in the structure and diversity of the bacterial community during composting. On the other hand, the archaeal community has been considered a minor component of the prokaryotic compost microbiota because archaea are usually either oligotrophic, thermophilic, or hyperthermophilic (Insam and de Bertoldi 2007). However, some reports have shown that archaea contribute to methane production during the initial stage of composting (Jäckel et al. 2005; Thummes et al. 2007a, b). Moreover, the novel archaeal group known as ammonia-oxidizing archaea (AOA) (Könneke et al. 2005; Leininger et al. 2006; Treusch et al. 2005) has been found to oxidize ammonia in various environments such as seawater and soil. Therefore, it is necessary to focus on the archaeal community in compost as well.

Analytical methods are vital to deal with the spatially and temporally heterogeneous populations present during the composting process. This chapter shows one example of how the bacterial and archaeal community structures change during the composting of animal manure.

6.2 Analytical Procedures for Studying Microbial Communities in Compost

Researchers have generally studied bacterial compost communities using traditional culture-dependent approaches. A wide variety of bacterial strains were isolated from composting materials. Some isolates have an ability to remove offensive odors from compost. For example, anaerobic indole- and skatole-degrading (Kohda et al. 1997), sulfur-oxidizing (Asano et al. 2007), and ammonia-assimilating bacteria (Sasaki et al. 2004) have been isolated and assessed for their usability. Other reports have shown that a thermophilic *Bacillus licheniformis* strain isolated from compost produced a bacteriocin-like substance, which inhibited the growth of pathogenic *Listeria monocytogenes* (Abdel-Mohsein et al. 2011). However, it is possible to detect only about 8.5% of the total microbes in the compost with these culture-

dependent methods; therefore, these methods are no match for culture-independent techniques (Gong et al. 2005). Table 6.1 lists published studies that used molecular biological methods to examine the prokaryotes contained in compost. Phospholipid fatty acid analysis (Klamer and Bååth 1998) and quinone profiling (Tang et al. 2004) are biochemical methods that focus on the cellular components of the prokaryotes in the composting materials. For instance, Tang et al. (2004) identified various quinone species and correspondingly made estimations regarding the microbial community contained in cattle manure during a composting process. The phylum Proteobacteria were dominant during the initial stage, followed by the genus *Bacillus* during the high temperature stage and then the phylum Actinobacteria in the final stage. However, these techniques need to be combined with other methods because these techniques are considered insufficient alone for use in phylogenetic classification (Kunihiro et al. 2014). Today, polymerase chain reaction-based approaches targeting prokaryotic 16S ribosomal RNA are widely used to unveil microbial communities. Among them, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analysis and clone library construction are well utilized. The former is advantageous for visualizing the community structure by analyzing DNA band patterns in polyacrylamide gel (Muyzer et al. 1993). The technique of DGGE is useful for imaging microbial communities from multiple samples because one DGGE band represents one species. The latter can both identify species as well as estimate the dominance of each species within the community (Giovannoni et al. 1990).

6.3 The Structure of the Bacterial Community During Composting of Cattle Manure

It has been thought that bacteria play an important role throughout the composting process because of the existence of a wide variety of bacterial species with varying characteristics. Currently, almost all such results are obtained by DGGE of 16S rRNA gene fragments.

6.3.1 Relationship Between Differences in Microbial Community and the Type of Waste Being Composted

In reports studying the composting of artificial substrates representing household waste and food waste, it was demonstrated that either aerobic *Bacillus* spp. (Schloss et al. 2003) or *Lactobacillus* (Ishii et al. 2000) in the phylum Firmicutes, or members of the phylum Proteobacteria (Danon et al. 2008), dominated during the initial stage. After the temperature increased, *Bacillus* spp. were the main bacteria detected in all the studies (Blanc et al. 1999; Ishii et al. 2000; Pedro et al. 2001; Schloss et al. 2005).

Table 6.1 A list of published papers about prokaryotic community analysis of composting processes

Target	Analysis method	Raw materials	Compost size	References
Bacteria	PLFA analysis	Swine manure	Lab-scale	Klamer and Bååth (1998)
	Quinone profile	Cattle manure and sawdust	Lab-scale	Tang et al. (2004)
	ARDRA	Spent mushroom substrate	Field-scale	Ntougias et al. (2004)
	ARISA	Synthetic food waste	Lab-scale	Schloss et al. (2003)
	Oligonucleotide microarray	Sewage sludge	Field-scale	Franke-Whittle et al. (2005)
	rRNA gene probe	Synthetic food waste	Lab-scale	Schloss et al. (2005)
	PCR-SSCP	Horse manure and plant waste	Field-scale	Peters et al. (2000)
	PCR-SSCP	Organic waste	Field-scale	Fracchia et al. (2006)
	PCR-DGGE	Cattle manure	Field-scale	Maeda et al. (2009), Maeda et al. (2010)
	PCR-DGGE	Cattle manure and sawdust/straw	Field-scale	Green et al. (2004)
	PCR-DGGE	Food waste	Field-scale	Pedro et al. (2001)
	PCR-DGGE	Garbage	Lab-scale	Haruta et al. (2002), Ishii et al. (2000), Takaku et al. (2006)
	PCR-DGGE	Garbage and cattle manure	Lab-scale	Asano et al. (2010)
	PCR-DGGE	Garbage and plant waste	Lab-scale	Halet et al. (2006)
	PCR-DGGE	Household waste	Lab-scale	Jarvis et al. (2009)
	PCR-DGGE	Litter	Field-scale	Dilly et al. (2004)
	PCR-DGGE	Marine animal	Lab-scale	Niisawa et al. (2008)
	PCR-DGGE	Multiple	Field-scale	Sasaki et al. (2009), Yamamoto et al. (2009)
	PCR-DGGE and clone library	Sewage sludge and plant waste	Field-scale	Danon et al. (2008)
	PCR-DGGE and RT-PCR	Multiple	Field-scale	Kowalchuk et al. (1999)
	PCR-DGGE, FISH, and real-time PCR	Garbage	Lab-scale	Hemmi et al. (2004)
	Clone library	Cattle manure	Field-scale	Yamada et al. (2008)

(continued)

Table 6.1 (continued)

Target	Analysis method	Raw materials	Compost size	References
	Clone library	Chicken litter	Field-scale	Lu et al. (2003)
	Clone library	Garbage and plant waste	Field-scale	Blanc et al. (1999)
	Clone library	Swine manure	Field-scale	Guo et al. (2007)
	Clone library	Swine manure and plant waste	Field-scale	Cho et al. (2008)
	Clone library	Synthetic food waste	Lab-scale	Dees and Ghiorse (2001)
	Real-time PCR	Cattle manure	Field-scale	Yamada et al. (2007)
	Metagenome	Animal manure and plant waste	Field-scale	Martins et al. (2013)
Archaea	SSCP and clone library	Multiple	Field-scale	Thummes et al. (2007a, b)
	PCR-DGGE	Rice straw	Field-scale	Cahyani et al. (2004)
	PCR-DGGE and clone library	Cattle manure	Field-scale	Yamamoto et al. (2010), Yamamoto et al. (2011)
	PCR-DGGE and real-time PCR	Multiple	Field-scale	Yamamoto et al. (2012)
	Clone library	Organic waste	Field-scale	Thummes et al. (2007a, b)
	Clone library	Swine manure and plant waste	Field-scale	Lee et al. (2010)
	Clone library	Multiple	Field-scale	de Gannes et al. (2012)

PLFA phospholipid fatty acid, *ARDRA* amplified rDNA restriction analysis, *ARISA* automated ribosomal intergenic spacer analysis, *SSCP* single-strand conformation polymorphism, *FISH* fluorescence in situ hybridization, *PCR-DGGE* polymerase chain reaction-denaturing gradient gel electrophoresis, *RT-PCR* reverse transcription polymerase chain reaction

During the final stages, actinomycetes appeared, suggesting the possibility that hard-to-degrade substances may become biodegradable at this point (Ishii et al. 2000; Dees and Ghiorse 2001; Sundh and Rönn 2002; Danon et al. 2008). However, these findings are likely to depend on which bacteria were present in the raw materials. *Clostridium* spp. and members of the phylum Bacteroidetes are dominant in cattle manure as compared with household wastes (Ozutsumi et al. 2005). Therefore, the structure of the bacterial community during the composting of cattle manure is assumed to be different from the bacterial community when household waste and artificial substrates are used as the raw material. There are several studies that tracked the bacterial community throughout the composting process using the different types

of animal manure. Green et al. (2004) analyzed the community structure of bacteria in cow-dung compost using PCR-DGGE and reported that the phyla Bacteroidetes and Proteobacteria were dominant in the samples they analyzed. Sasaki et al. (2009) analyzed four different composting materials using PCR-DGGE. They described that different bacterial groups dominated during the initial stage owing to differences in the raw materials, but the phyla Firmicutes and Bacteroidetes formed the major components of the community as determined by analyzing four different composting materials with PCR-DGGE. Typically, PCR-DGGE can identify bacterial information in only as many as ten bands per sample, and while that number is equivalent to the most common types of bacteria in the compost, it is not sufficient to clarify changes in the minor components of the bacterial community. To reveal details of changes in the structure of the microbial community during composting, attention must also be paid to the bacterial groups that are less dominant. Clone libraries are an alternative method for examining community structure and enable the detection of a larger number of species. Yamada et al. (2008) were able to sequence 100 clones per sample obtained during the high temperature and final phases and reported the dominant bacterial groups. Hence, cloning is considered the most suitable technique for analyzing compost microbiota. However, there are few other reports on cattle-manure compost.

6.3.2 *Yamamoto Study Project with Cattle Manure and Sawdust*

As a model case, we have examined the structure of the bacterial community during field-scale composting of cattle manure (Yamamoto et al. 2014). The raw materials (cattle manure and sawdust) were mixed and piled with daily aeration for about 30 days as an initial treatment stage followed by an additional stage consisting of 84 days without daily mixing. The temperature reached 76.1 °C within 5 days and stayed at >50 °C for 23 days. The temperature did not drop during the second stage of composting. The moisture content was about 70% at the beginning of the process and decreased consistently to reach 41.8% by the end of the process (Fig. 6.1). The materials were considered to be composted when the final stage was reached. Total DNA was extracted from five samples at different time points during composting. Then, approximately 600 bp of bacterial 16S rRNA genes were amplified by PCR. The PCR products were cloned with plasmids and competent *Escherichia coli*. Approximately 200 clones were sequenced in all.

The results from our study showed that almost all clones were similar to uncultured environmental sequences, which is a reminder that the microbiology of compost remains murky. The clone library constructed using the day 0 sample consisted mainly of members of the phyla Firmicutes and Bacteroidetes (Fig. 6.2a). Clones grouped under these phyla are related to the orders Clostridiales and Bacteroidales, respectively, suggesting that they both originated from the cattle

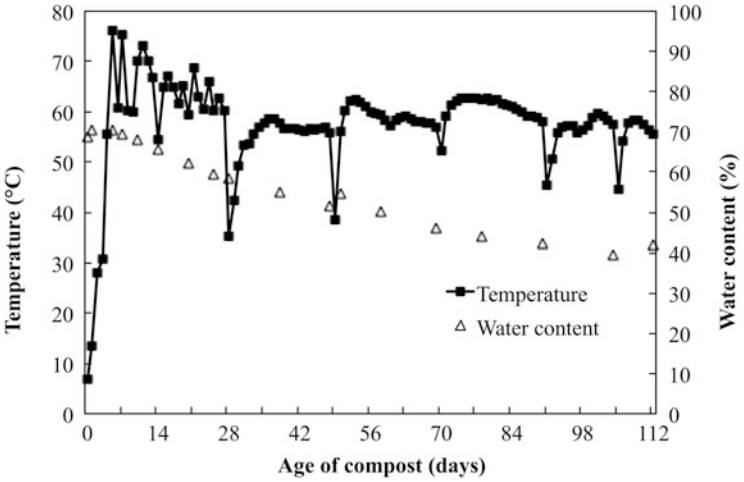

Fig. 6.1 Changes in temperature and water content during the composting process for analysis of bacterial community

manure (Ozutsumi et al. 2005). On days 5 and 20, corresponding to the high temperature stage, Bacillales-related clones (related to the phylum Firmicutes) were present. Particularly, the presence of the *Ureibacillus* spp. indicated that these bacteria can be active under high temperatures in compost, as reported by other researchers via the identification of novel isolates (Weon et al. 2007). The presence of members of the genus *Bacillus* has been shown to be very important for decomposition of organic matter. It is believed that the more *Bacillus* spp. dominate during the early stages the faster the composting progresses. As composting progressed, the dominance of the members of the phylum Firmicutes decreased, whereas that of Actinobacteria and Proteobacteria increased. At the final stage, the library presented a diverse and complex bacterial community, consisting of Proteobacteria (32.7%), Bacteroidetes (26.5%), Firmicutes (18.4%), Actinobacteria (12.2%), and others (10.2%). In terms of phylogenetic composition within each phylum, some groups such as the order Clostridiales maintained their proportion throughout the process, whereas others were detected only from the second stage onward. Interestingly, the members of the phylum Bacteroidetes had disappeared by the end of the first stage (day 28) and reemerged later. Clones involved in the later stages were grouped into the orders Sphingobacteriales and Flavobacteriales and not Bacteriales. This result suggested that composting materials reached stable phase at this stage when focusing on the phylogenetic change in the members of phylum Bacteroidetes throughout the process. Furthermore, the members of the orders Rhizobiales and Xanthomonadales in the phylum Proteobacteria or the order Acidimicrobiales in the phylum Actinobacteria may be responsible for the degradation of persistent organic matter remaining in the final stage (Fig. 6.2b).

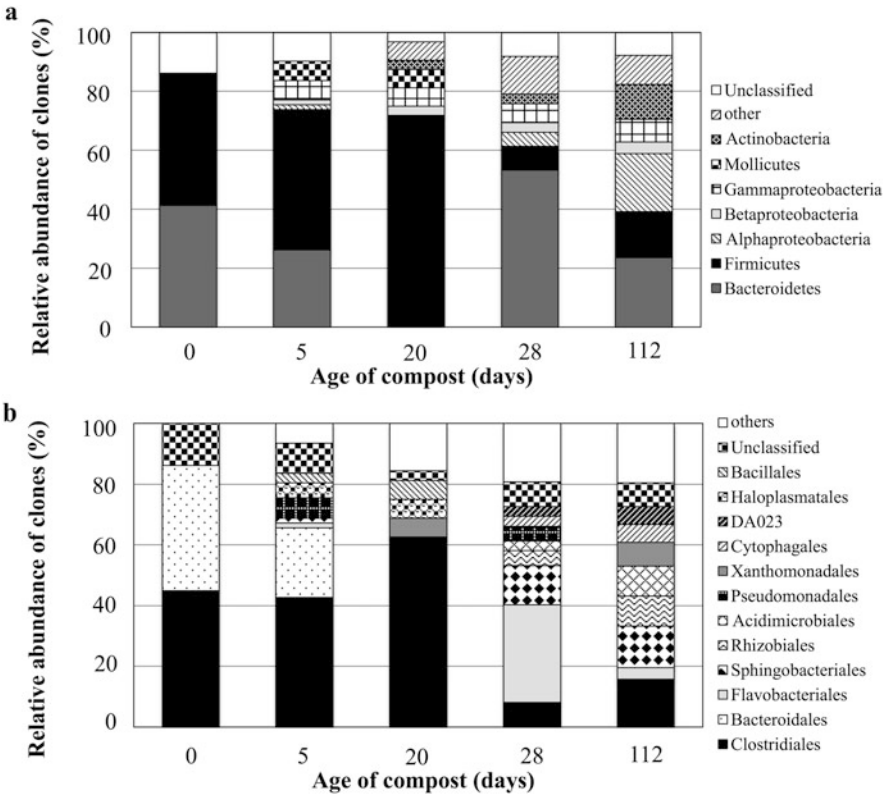

Fig. 6.2 Changes in bacterial community constructed from cloned 16S rRNA genes at (**a**) phylum level or (**b**) order level

6.3.3 Summarizing the Observed Community Dynamics of Nutrient Metabolism

The information about the dynamics in bacterial communities suggests a novel approach for either controlling or monitoring biologically complicated systems. RNA-based analysis is also required to determine the important contributors of nutrient metabolism during each stage of the composting process. Previous studies have used reverse-transcription PCR and DGGE to analyze methylotrophs and ammonia-oxidizing bacteria recognized as functional bacteria in compost and those studies reported a diverse community structure (Halet et al. 2006; Kowalchuk et al. 1999). In addition, there is a possibility that sequencing only several hundred clones would not be sufficient to detect all of the bacteria when composting wastewater with its high microbial diversity (McGarvey et al. 2004). Because it is clear that compost is not homogeneous and has high microbial diversity, metagenomic analysis using a next-generation sequencer may be the most powerful

method for understanding the entire microbial community. Thus, in order to cover all aspects of microbial ecology in composting, it is necessary to use a combination of several DNA analysis methods.

6.4 The Structure of the Archaeal Community During the Composting Process

Generally, archaea have been recognized as organisms that mainly survive in extreme environments. Considerable methane emissions have been measured in compost (Beck-Friis et al. 2000; Fukumoto et al. 2003; Hao et al. 2001), suggesting that methane-producing archaea (methanogens) live actively in compost. Hao et al. (2001) reported that a compost pile made with cattle manure released methane gas at rates of up to 1.67 gCH_4-$C/m^2/h$ without turnover and 0.83 gCH_4-$C/m^2/h$ with turnover. Particularly, it has been observed that methane gas generation is enhanced in the lower regions of the composting pile. Amon et al. (2001) demonstrated that farmyard manure produced more methane gas in winter than in summer. As for the type of methanogen present in compost, the thermophilic *Methanobacterium* spp. has been isolated from mushroom compost (Derikx et al. 1989). In a 145-day study on rice straw composting system, Cahyani et al. (2004) estimated that 10^9 cells of methanogen were present per gram dry weight (g/DW) of composting material, and authors also detected relatives of rice cluster I archaea during the high temperature stage. Although finding a diversity of methanogens in cattle manure has been reported (Gattinger et al. 2007), there are few studies that specifically identify those methanogen species which are present during the composting process. In addition, it should be noted that the archaea known as AOA can oxidize ammonia in natural environments such as seawater, freshwater, various soils, and hot spring (Hatzenpichler et al. 2008; Könneke et al. 2005; Leininger et al. 2006; Treusch et al. 2005). A number of studies pertaining to the AOA community have shown that AOA can live in a wide range of habitats from moderate to high temperatures. The AOA have also been detected at artificial sites such as rice fields (Fujii et al. 2010) and activated sludge (Sonthiphand and Limpiyakorn 2011; Wells et al. 2009; Zhang et al. 2009). Although the function of the archaeal community in compost has been elucidated, some studies perhaps surprisingly have reported no archaea detected in compost. Therefore, due to these contrasting findings, it remains difficult to determine the importance of the archaeal community in composting (Dees and Ghiorse 2001; Takaku et al. 2006). Until recently, there was no sequencing information available concerning the archaeal community during the composting of cattle manure, although the archaeal 16S rRNA gene copy number was determined to be 10^5–10^7 copies/gDW using real-time PCR (Yamada et al. 2007). This means that the archaeal species that make up the archaeal community in cattle-manure compost are currently unknown.

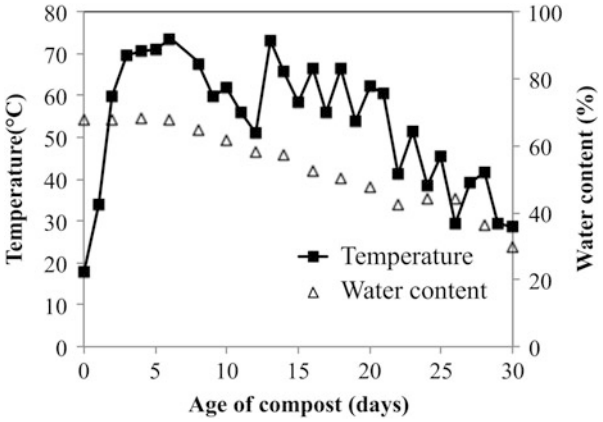

Fig. 6.3 Changes in temperature and water content during the composting process for analysis of archaeal community

Both ammonium content and temperature change extensively during composting, and thus results obtained from studying compost are likely to be relevant for understanding the biology of the archaeal community. Therefore, we collected six samples to analyze the temporal changes of archaeal community in cattle manure composting (Yamamoto et al. 2011). Changes in the physical and chemical parameters during the process are shown in Fig. 6.3. The treatment was performed over 30 days with the temperature reaching a maximum of 77.9 °C and maintained at >60 °C for 18 days. The moisture content declined throughout from about 67 to 30%.

The archaeal community was evaluated by sequencing 322 clones with about 1400 bp target fragments in total (Table 6.2). All clones belonged to 1 of the 14 operational taxonomic units (OTUs), which have a high homology between both the uncultured archaeal sequence and the cultured strain, allowing the classification of OTUs at the species level (Fig. 6.4). Our constructed clone library was likely to reflect the lower diversity within the archaeal community throughout the process. With the exception of one clone, detected OTUs were related either to methanogens or to AOA, indicating that methanogens and AOA were the dominant archaea during the composting of cattle manure. This result differed from previous studies that have found no AOA in composting materials (Maeda et al. 2010; Yamada et al. 2007). It has been suggested that the microenvironment is either anoxic or anaerobic in cattle manure compost (He et al. 2000); therefore, there is a high possibility of dominance by methanogens during treatment. Further studies are needed to assess the impact of compost as a fertilizer for soil by analyzing the microbial changes as well as the chemical changes. On days 0 and 2, the dominant OTUs were methanogens generally closely related to uncultured sequences from groundwater, animal rumen, and animal manure. On subsequent days, these OTUs decreased in abundance, possibly because of high temperatures. After day 2, the most dominant group showed a high homology with uncultured thermophilic *Methanosarcina* spp. Thummes et al. (2007a, b) detected the same cluster in compost made from organic waste; therefore, it seems likely that this thermophilic *Methanosarcina* spp. can adapt to the composting environment. Both OTU13 and

Table 6.2 List of archaeal operational taxonomic units (OTUs) obtained from cattle manure compost (Yamamoto et al. 2011)

OTU identification number	Closed relatives in database	Accession number	Phylogenetic group	Homology (%)	Number of clones in sample					
					Day 0	Day 2	Day 6	Day 16	Day 24	Day 30
1	Clone: HDBW-WA03	AB237736	*Methanocorpusculum*	99	25	10				3
2	Clone: MANA01	AB114309	*Methanosarcina*	99	11	15	13	14	39	18
	Clone 1B	GQ328818	*Methanosarcina*	99–100		1				2
	Clone: SA42	AB494252	*Methanosarcina*	98–99						6
3	*Methanosarcina mazei*	AB065295	*Methanosarcina*	99–100	4	1	1	3	2	2
	Methanosarcina mazei strain MT	AY260432	*Methanosarcina*	98–100	1		3			3
	Methanosarcina barkeri str. CM1	AJ002476	*Methanosarcina*	99	1					
4	*Methanomicrococcus blatticolus*	AJ238002	*Methanomicrococcus*	96–98		10				
5	*Methanoculleus palmaeoli*	Y16382	*Methanoculleus*	97				1		
6	*Methanoculleus thermophilus* strain DSM 2832	AJ862839	*Methanoculleus*	99					1	
7	Clone 3	DQ402011	*Methanosphaera*	99	1					
	Clone 16	DQ402024	*Methanosphaera*	97	1					
	Clone 22	DQ402030	*Methanosphaera*	98	1					
	Clone 24	DQ402032	*Methanosphaera*	97		1				
8	Clone M7	AB034184	CA11	97	3					
9	Clone DF86	AY816986	CA11	96–97	1	5				
10	Clone 25	DQ402033	CA11	95	1					
11	Clone TX4CA_19	EF690574	Halobacteriaceae	97					1	
12	*Candidatus* Nitrososphaera gargensis	GU797786	*Candidatus* Nitrososphaera	98	11	3	15	23	35	23
13	Clone SCA1145	U62811	Uncultured Crenarchaeota	99–100	2					1
14	Clone SCA1175	U62819	Uncultured Crenarchaeota	99			4			
Total number of clones					63	46	36	41	78	58

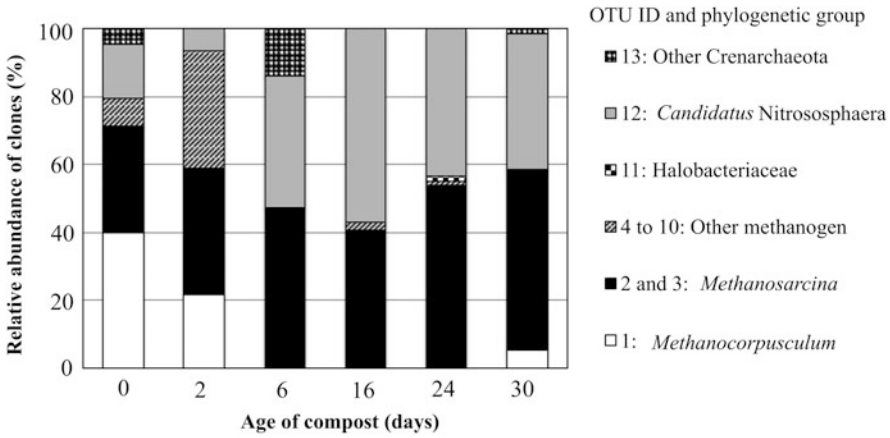

Fig. 6.4 Changes in archaeal community constructed from cloned 16S rRNA genes (Yamamoto et al. 2011)

OTU14 showed homology with uncultured sequences obtained from the soil environment (Bintrim et al. 1997).

This study (Yamamoto et al. 2011) was the first to show the presence of AOA-like organisms throughout the composting process, particularly from days 6 to 30. Almost all of the clones identified as AOA were closely related to *Nitrososphaera gargensis* with high homology (98%). *Nitrososphaera gargensis* is an AOA isolated from hot springs that can grow under moderate thermophilic conditions (Hatzenpichler et al. 2008). Similarly, AOA detected in the compost samples must have been able to adapt to the inherent temperatures and ammonium concentration, and therefore those AOA may have the ability to oxidize the ammonia in compost despite the low diversity of AOA during composting.

6.5 The Diversity of AOA in a Variety of Animal Manure Composts

Insight gained from the archaeal 16S rRNA genes detected in the compost indicated that not only ammonia-oxidizing bacteria (AOB) but also AOA may play an important role in nitrification during composting (Yamamoto et al. 2010). The DGGE analysis confirmed presence of archaeal *amoA* genes representing just one or two species throughout the composting process (Yamamoto et al. 2011). A variety of AOA species can be detected from soil samples according to the soil type or chemistry; therefore, the diversity of soil AOA seems to be relatively high at least in comparison with the manure compost that we studied. Identical AOA species have been sequenced from activated sludge in several wastewater treatment plants, showing that specific AOA live in wastewater environments regardless of either

the ammonium concentration or treatment method. Composting maintenance procedures such as aeration, turnover, and processing time vary in different facilities; however, it is unclear whether these environmental factors affect the composition of AOA communities. Therefore, we researched the structure of AOA communities in a variety of animal composts (Yamamoto et al. 2012). Samples were collected from composts of cattle, chicken, and swine manure. Archaeal *amoA* sequences were amplified and DGGE analysis was conducted. Only a few *amoA* sequences were amplified using fresh manure, swine manure compost, and chicken manure compost, whereas four to six sequences were detected using cattle manure composts. We hypothesized that the difference in ammonia concentration among the animal manures was the most influential factor for the presence of AOA.

Those sequences which we examined were divided into three groups: one group was phylogenetically related to *Candidatus* Nitrososphaera gargensis (group NG) and the others were closer to AOA sequences from either hot springs or wastewater (Fig. 6.5). The number of AOA cells relative to AOB cells in sample was estimated by quantifying each *amoA* gene copy using real-time PCR (Fig. 6.6). Real-time PCR confirmed that archaeal *amoA* gene copy numbers were greater than bacterial gene copy numbers in the end product at facility H, which treats swine manure. Overall, the AOA/AOB ratios ranged from 0.06 to 10.54. This large range may have been caused by differences in the operating conditions of the composting process, such as the addition of finished compost. In cattle manure compost, the abundance of archaeal *amoA* genes was clearly lower than that of bacterial genes. Across all compost materials analyzed, there were only a few samples where AOA dominated over AOB, indicating that the presence and abundance of ammonia oxidizers was determined by unknown factors other than manure type. These results showed that AOB, as compared with AOA, were more widely distributed in animal manure compost.

Our results suggest that both AOB and AOA are actively involved in the nitrification of composting systems. In addition, the concentration of ammonium and the temperature may be factors that control the AOA community in compost. The ammonium concentration is assumed to be the main factor controlling the levels of AOA in compost because the AOA community has low diversity and abundance in activated sludge with high levels of ammonium (Wells et al. 2009). Furthermore, the growth of AOA has been found to be inhibited in enrichment cultures which contained high concentrations of ammonium (Hatzenpichler et al. 2008). Other parameters such as pH and temperature also influence the diversity and structure of AOA communities, as described by Nicol et al. (2008) and Tourna et al. (2008). To determine the absolute influence of environmental factors on the AOA communities in compost, further study is required using the same materials under different operating conditions. Studies are also needed to evaluate ammonia oxidation activity and the diversity of AOA and AOB under various chemical parameters to measure the contribution of each ammonia oxidizer to the ammonia oxidation process during composting. For this purpose, there has been an attempt to isolate AOA strains from compost by Oishi et al. (2012). In their study, Oishi et al. (2012) seeded liquid culture media with fermenting cattle manure compost and by so doing succeeded at isolating an archaeon related to *Nitrososphaera gargensis*. The study which we

report here suggests that cattle manure is the best material for supporting growth of AOA during composting.

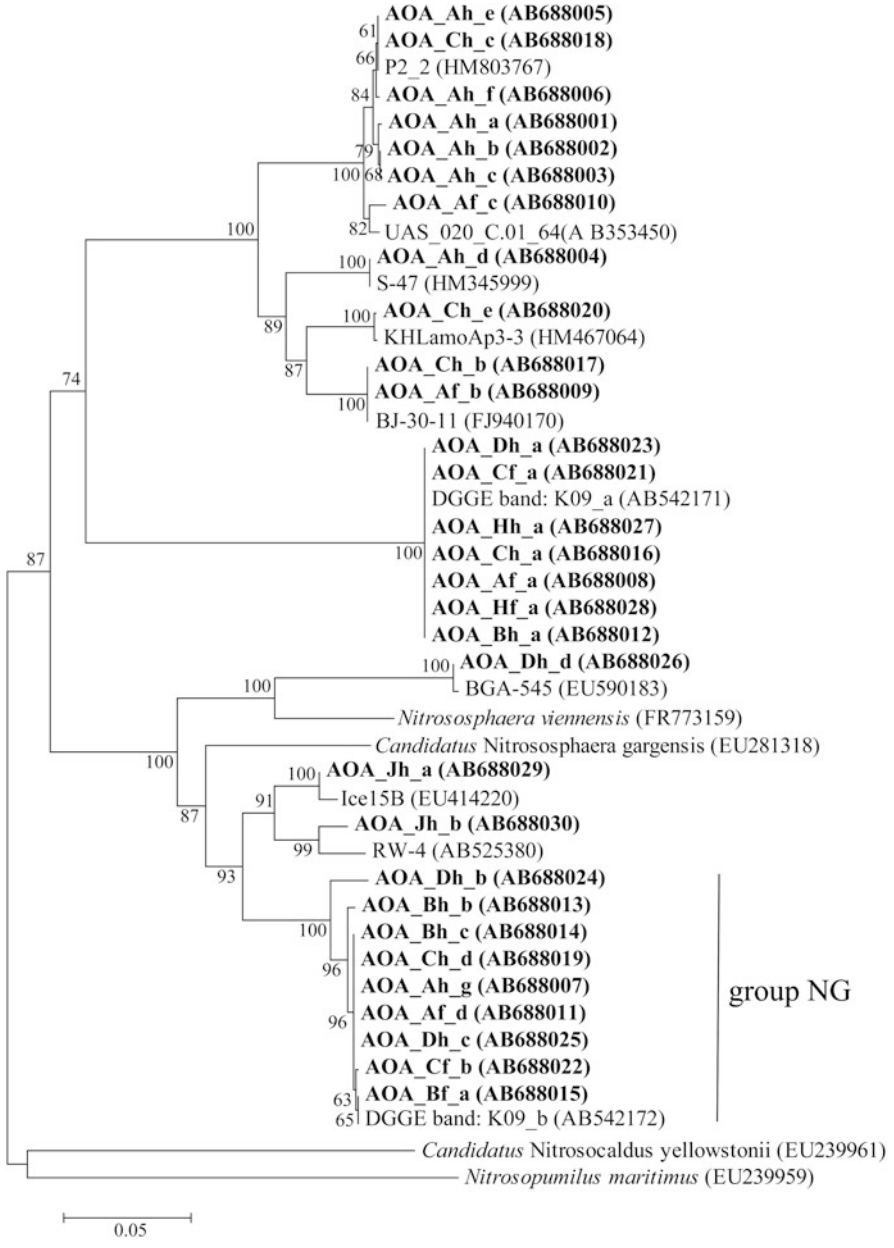

Fig. 6.5 Phylogenetic tree of the archaeal *amoA* sequences obtained from composting materials (Yamamoto et al. 2012)

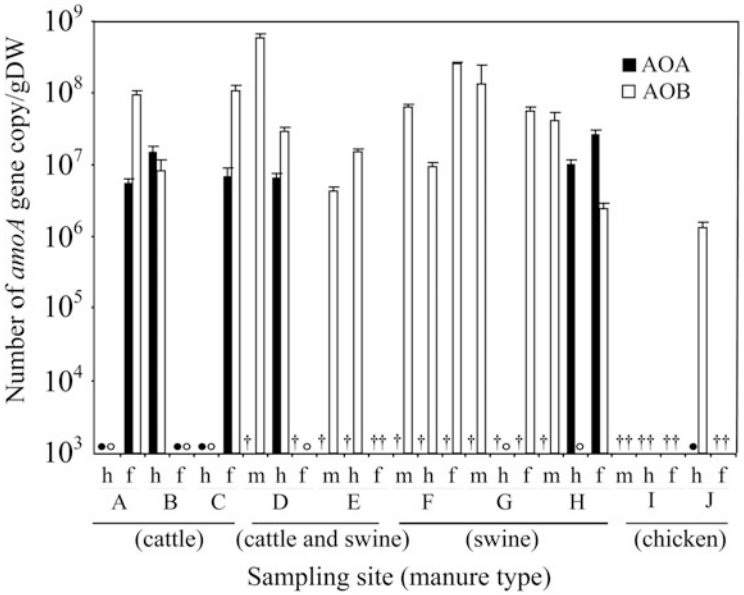

Fig. 6.6 *AmoA* gene copy numbers for AOA and AOB. Capital letter indicates sampling facilities. Samples were obtained from each facility at fresh manure (m), the high temperature stage (h), and the end of the composting (f). Samples with dagger did not perform real-time PCR. The sample with gene copy number below the detection limit was represented as closed circle (AOA) and opened circle (AOB) (Yamamoto et al. 2012)

6.6 Conclusion and Future Study

Many research studies using culture-independent techniques partially reveal the pattern of changes which occur in bacterial communities during the composting process. In addition, some studies underline the importance of investigating archaeal communities to help with understanding the microbiology of compost. It is expected that new insights like the existence of novel organisms will be gained by comparatively studying various samples originating from different composting materials. However, the metabolic activity of the bacterial and archaeal communities detected in former studies has not been analyzed well.

One of the most powerful solutions to these shortfalls of knowledge may lie with usage of the next-generation sequencer, which enables obtaining large amounts of either DNA or RNA sequences. Some researchers have already succeeded in identifying novel enzymes such as cellulase, esterase, glycoside hydrolase, and poly(DL-lactic acid) depolymerase by analyzing metagenomic sequences from compost (Allgainer et al. 2010; Kang et al. 2011; Kim et al. 2010; Lämmle et al. 2007; Mayumi et al. 2008; Pang et al. 2009). The technique of pyrosequencing gives even greater chance to discover hidden resources like enzymes derived from uncultured

organisms in compost. This technique was used to study rare species presented in compost for risk assessment of biosolids treatment (Bibby et al. 2010).

Pyrosequencing of 16S rRNA genes in conjunction with the next-generation sequencing approach has provided sequences which grouped into *Mycobacterium*, accounting for less than 0.1% of total sequences (Martins et al. 2013). They analyzed about three million readings that targeted not only 16S rRNA genes but also protein-coding genes in an effort to understand the biomass degradation mechanism of two individual zoo composts. Each of those two composts had a demonstrably characteristic bacterial community, which mainly consisted of the orders Xanthomonadales, Pseudomonadales, Clostridiales, Burkholderiales, Bacillales, and Lactobacillales. Their research also indicated that cellulose and hemicellulose decomposition was performed by bacterial enzymes from members of Clostridiales and Actinomycetales. There is also a very strong possibility that a number of microorganisms with unique functions remain unknown. Using various molecular biological analyses, we predict that in the future the microbial ecology of composting will be revealed to a greater level of understanding.

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Compliance with Ethical Standards

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Conflict of Interest Nozomi Yamamoto declares that she has no conflict of interest. Yutaka Nakai declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Chapter 7
Effects of Land Use and Restoration on Soil Microbial Communities

Vicky L. McKinley

A nation that destroys its soil destroys itself.
Franklin D. Roosevelt (1937)

Abstract Humans depend upon soil-based ecosystems for many essential activities including agriculture, grazing, and forestry, as well as for other uses such as recreation, mining, drilling, and development. All of these land uses have the potential to affect soil quality and viability. The North American Dust Bowl catastrophe of the early twentieth century focused attention on agricultural soil conservation. Later in the twentieth century, more holistic approaches to ecosystem reclamation and restoration were developed, driven by research documenting the close ties between aboveground and belowground communities of producers, consumers, and decomposers. The important roles of soil microbial communities in soil formation, biogeochemical cycling, nutrient retention, plant–microbe interactions, and soil food webs are now widely recognized as essential to good soil quality, essential ecosystem services, and maintenance of biodiversity. This chapter broadly reviews the progress made toward understanding the effects of various land uses on soil microbial communities, recent developments in our understanding of the roles of soil microbes in facilitating ecosystem reclamation and restoration following anthropogenic degradation, and the implications of these processes for carbon cycling and climate change mitigation.

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7.1 Introduction

Although humans rely upon soils to produce 99.7% of their food (FAO 2015a), until recently the concept of soil as a living, metabolizing ecosystem with its own biodiversity was not appreciated from an agricultural land management or policy standpoint. In the early twentieth century, soil conservation efforts focused on erosion control following the North American Dust Bowl, and midcentury the "Green Revolution" turned to increasing production through intensive irrigation, fertilization, and chemical pest control. Little notice was taken of soil microbes, however, aside from the advantages of planting legumes (used to increase soil fertility as early as the ancient Egyptian era) whose roots contained nodules filled with symbiotic nitrogen-fixing bacteria as first documented by Martinus Beijerinck in 1888 (Beyerinck 1888). Intensive agricultural practices are increasingly recognized as unsustainable, damaging to soil ecosystems, and exacerbating changes in the climate (FAO 2015a).

By the 1980s global attention and policies began to focus increasingly on soil productivity and conservation, leading to the creation of the 1982 World Soil Charter (FAO 1982) and the updated 2015 World Soil Charter (FAO 2015c) comprised of nine major principles summarizing our understanding of soils and a robust set of guidelines for soil conservation action by individuals, the private sector, the science community, governments, and international organizations. Publication of the *World Atlas of Desertification* (Thomas and Middleton 1997) followed, along with the formation of the Global Soil Partnership, with its five "Pillars of Action" for promoting soil sustainability by harmonizing soil research, monitoring methods and indicators (Montanarella 2015).

The late twentieth and early twenty-first century also brought the application of ecological concepts to agriculture and forestry, including a gradual awareness of the roles of soil microbial communities in the development of soils and soil quality (Buscot and Varma 2005), and their direct enhancement of plant growth (Khan et al. 2009). Following the publication of the *European Atlas of Soil Biodiversity* (Artz et al. 2010), attention turned toward expanding these efforts to a global scale. Status of the World Soil Resources (FAO/ITPS 2015), the first major global assessment of soils, soil ecosystem services, and soil change, was published as part of the United Nations "International Year of Soils" to galvanize action and provide a benchmark upon which to measure progress in sustainable soil management. In 2016 the Global Soil Biodiversity Initiative (GSBI) and Joint Research Centre (JRC) of the European Commission released the *Global Soil Biodiversity Atlas* (Orglazzi et al. 2016) as a framework for global biodiversity assessment.

Despite the international progress made in recognizing the importance of soil biota for global sustainability, many of the ecological relationships in soils are incompletely understood, with the soil still often thought of as a "black box" of processes and interrelationships. Determining the nature of these processes, and how human land use and activities may affect them, is an active and critical area of research with profound implications for human sustainability. Soils are now widely

recognized as being critical to global food security, water storage and purification, energy production (biomass and ethanol), biodiversity protection, and climate change abatement (FAO 2015b). And, as the climate changes due to increased levels of greenhouse gases in the atmosphere, the need to understand the resilience and adaptability of various soil microbial communities will become even more crucial (FAO/ITPS 2015). The United Nations, through its 2030 Agenda for Sustainable Development, has realized that management of food, forestry, livelihoods, and natural resources must be integrated through careful planning in each country and region if the growing human population is to be sustained while poverty and hunger are eliminated.

As the understanding of ecosystems and biodiversity has progressed, the benefits of reclaiming and restoring terrestrial ecosystems and their soils have become apparent. Early ecosystem restoration efforts starting in the late nineteenth century (Allison 2012) focused largely on reassembling the plant communities (seeding or planting native species and reducing invasive species) in terms of both the choice of restoration techniques (e.g., passive field abandonment, herbiciding, controlled fire or grazing, regrading the terrain, adding soil amendments, and restoring windbreaks or hydrology) and evaluating the progress and effectiveness of the projects by measuring erosion control and plant cover, biomass, and diversity. If soils were considered at all during restoration, it was usually in terms of their suitability for sustaining the desired plant species. Although native cultures had successfully managed small-scale agriculture in a sustainable manner for centuries in much of the world, including North America (Pretty and Shah 1997), the invention of the self-scouring steel plow led to large-scale soil disturbance in the late nineteenth century. The Soil Erosion Service (SES) was established in 1933 under the US Department of the Interior to prevent the types of soil loss experienced during the Dust Bowl era and to work with farmers to enhance the fertility of the land (it later became the Soil Conservation Service when it was transferred to the Department of Agriculture in 1935, and in 1994 it was renamed the Natural Resources Conservation Service to reflect its expanded mission of protecting and restoring wetlands, watersheds, and native plants as well as soils). Not until the late twentieth century did governing bodies all over the world also begin to recognize that working with farmers as partners in soil conservation, rather than as adversaries, led to more effective sustainability efforts and policies that were finely turned to local conditions (Pretty and Shah 1997).

The Federal Surface Mining Control and Reclamation Act of 1977 required that topsoils be stored and replaced following coal strip mining, that damage to the hydrology be limited, and that the surface contours and plant communities be restored to a state similar to those existing prior to mining. The Society for Ecological Restoration (SER) was founded in 1987 "to advance the science and practice of ecological restoration as a tool for recovering biodiversity and ecosystem services" and began publication of the journal *Restoration Ecology* in 1993. By the latter decades of the twentieth century, restoration of the soil itself had become a goal of ecosystem restoration, including the analysis and, in some cases, manipulation of the soil microbial communities as a key to restoring natural habitats to their

pre-disturbance state (Allison 2012). Some scientists, engineers, and practitioners also began to explore the roles of healthy, diverse microbial communities in sequestering carbon in soils as a means of stemming climate change, in addition to improving soil quality and productivity (Goreau 2015).

This chapter will explore the effects of various major land uses on soil microbes and microbial communities, including ecosystem restoration and reclamation activities, as well as the effects that soil microbial communities have on the ecosystem in turn. Although space does not allow a thorough review of these issues, it is hoped that this overview will illustrate the importance of the topic, provide a conceptual framework, and direct readers to key current literature for additional details.

7.2 Importance of Soil Microbial Communities for Sustainability

7.2.1 Soil Microbial Community Structures

Microbial communities perform many ecosystem services in soils, carry out transformations of organic and inorganic compounds that are key to nutrient cycling, alter the microenvironments surrounding them, interact with other organisms in the soil, and influence the physical structure of the soil. Therefore, our understanding of biogeochemical cycling, plant productivity, soil formation, and climate change all depend upon an understanding of this critical microbial community. In addition, certain soil microbial parameters may be useful indicators of soil quality and functional efficiency, including how these parameters may change with land use or ecosystem restoration. Good soil quality indicators should be sensitive to variations in land management, well-correlated with beneficial soil functions, useful for explaining ecosystem processes, and useful to land managers in the field (easy and inexpensive; Doran and Zeiss 2000). The effects of land use and the success of restoration efforts cannot be monitored effectively without taking into account the size, composition, and activity of the soil microbial communities (Harris 2003). However, due to the spatial and temporal heterogeneity of soil ecosystems at many scales, from sub-micrometer to global distances and from microseconds to decades in time, determining the nature of the soil microbial community is a task fraught with many sampling, technical and modeling challenges. For this reason, ecological understanding of terrestrial microbial systems has generally tended to lag behind advances in aquatic microbial ecology.

One of the challenges surrounding the sampling of soils is the fact that there are functionally many different "compartments" in soil ecosystems, having wide-ranging microenvironmental characteristics which are often difficult to detect, separate, and sample: the rhizosphere is heavily influenced by plant roots and their exudates, the detritusphere has recognizable plant and animal remains undergoing

active decomposition, the drilosphere is enriched by earthworm or other invertebrate activities, the aggregatusphere is made up of soil aggregates of various sizes colonized and largely held together by soil microbes and their extracellular products, and the porosphere is composed of the voids between soil particles and aggregates (Giri et al. 2005). Each of these areas has distinct microbial communities, activities, and functions, with huge differences in microbial activity across small spaces resulting in "hot spots" that dominate soil function (Kuzyakov and Blagodatskaya 2015).

7.2.1.1 Microbial Biomass

Biomass represents the mass of living organisms in a particular habitat or ecosystem at a particular time point and is typically one of the easiest ecosystem parameters to measure. It has been estimated that one gram of soil typically contains 10^7–10^{12} microbial cells (Watt et al. 2006). However, due to their small size, researchers can rarely quantify the exact biomass of each microbial species in situ. Due to technical and budgetary constraints, estimates are usually made of the total soil microbial biomass, microbially associated carbon (C_{mic}), or microbial nitrogen (N_{mic}) from a pooled sample of soil that includes some specified depth profile (usually a core of soil extending from the surface to between 3 and 20 cm depth). This can obscure small spatial differences in microbial communities between depths and different soil aggregates (Gupta and Germida 2015). Many environmental factors can affect soil microbial biomass on micro and macro scales (e.g., bacterial growth tends to correlate positively with pH, while fungal growth is negatively correlated; Rousk et al. 2010). Nonetheless, total microbial biomass has often been found to be responsive to ecosystem disturbances, land use changes, seasonality, pollution, and other variables, often making it a good indicator of soil quality and overall microbial community health (Kaschuk et al. 2010; Dequiedt et al. 2011; Holden and Treseder 2013; Xu et al. 2013; Murugan et al. 2014), although exceptions have been reported (Kemmitt et al. 2008).

Interpretation of total microbial biomass data is complicated by the fact that the assay used may or may not allow one to discriminate between viable and dead organisms (Vestal and White 1989) or between metabolically active, potentially active, and dormant living organisms (Blagodatskaya and Kuzyakov 2013). Due to the presence of interfering material, direct microscopic observation often lacks precision as a method of counting microbes in soil, although recent advances in imaging and specific staining techniques have helped elucidate some spatial relationships between species in soils and on roots (Nunan et al. 2003; Cardinale 2014). Culture methods relying on growth on media were found to vastly underestimate numbers of bacteria and fungi due to the unculturability of most species. Other methods of estimating soil microbial biomass initially included biochemical assays such as total protein, ATP (Martens 2001; Contin et al. 2002), or DNA (Martin-Laurent et al. 2001), all of which were problematic due to a lack of specificity to

microbes, variability due to dynamic physiological states (Nannipieri et al. 1978), low turnover times (counting dead biomass; Agnelli et al. 2004), and inefficient extraction or interference from soils. Chloroform fumigation methods [fumigation extraction (Brookes et al. 1985; Vance et al. 1987; Jenkinson et al. 2004; Joergensen et al. 2011), fumigation incubation (Anderson and Domsch 1978; Ross 1987), and substrate-induced respiration (SIR; West and Sparling 1986; Beare et al. 1990)] were developed in the 1980s and 1990s and widely adopted by soil scientists. These methods of determining C_{mic} (microbial carbon) were shown to largely correlate with ATP, microbial activity, and SOM (soil organic matter). Fumigation methods have also been useful in developing a physiological approach to interpreting microbial biomass based on measured respiration per unit of biomass (qCO_2; Anderson and Domsch 2010), but they require assumptions regarding the efficiency of killing, effects on the surviving microbes, penetration into aggregates, appropriate incubation times, and differential effects on various types of microbes (Kaiser et al. 1992; Horwath and Paul 1994; Bailey et al. 2002). At roughly the same time, new biochemical methods of determining microbial biomass based on measurement of phospholipid fatty acids (PLFAs) from cell membranes were developed. Advantages of PLFAs included clean and efficient extraction from soils and quantification in picomole amounts; best of all, PLFAs rapidly degraded upon cell death, making them a good measure of instantaneous viable microbial biomass (Vestal and White 1989; Frostegård and Bååth 1996; de Vries et al. 2013). In addition, PLFAs could also be separated and identified using GCMS (gas chromatography followed by mass spectrometry) to give information about the relative amounts of certain groups of microorganisms that contain specific PLFAs (see "fingerprinting" below). Later, DNA extraction methods for soils improved (Frostegård et al. 1999; Thakuria et al. 2008), allowing for better quantification from soils. However, DNA does not degrade quickly after cell death (Agnelli et al. 2004; Nielsen et al. 2007; Levy-Booth et al. 2007; Pietramellara et al. 2008), limiting its use as a sensitive indicator of changes in microbial community structures as land uses are altered (Duncan et al. 2016).

Most researchers have found that using more than one biomass indicator in soil surveys and experiments is the optimal approach to avoiding bias under different environmental conditions. These biochemical methods of measuring microbial biomass have enabled soil microbial ecology to advance with great strides and are now commonly used by soil researchers worldwide for developing better models of soil ecosystem functions, but they are technically and economically out of reach for most land managers and restoration practitioners. Fortunately, in many cases, the measurement of soil organic matter (SOM) is a cost-effective and useful tool for determining overall soil quality and for tracking long-term changes due to land use or restoration (Karlen et al. 1997; Carter 2002; Franzluebbers 2002; Bending et al. 2004; Dexter 2004; Kibblewhite et al. 2008; Schmidt et al. 2011; Six and Paustian 2014).

7.2.1.2 Microbial Diversity

Diversity is an ecological measure of the complexity of a community of organisms. Assessments of changes in diversity over time, space, or environmental conditions are crucial to understanding ecosystem function, resilience, resistance, and sustainability. With an estimated 10^4–10^6 species in a single gram of soil (Dykhuizen 1998; Torsvik et al. 2002; Bent and Forney 2008), the challenge of quantifying and untangling soil microbial diversity is extreme.

Since 1898 when Heinrich Winterberg first noticed that the number of bacterial colonies on his Petri plates was far less than the number of bacteria he observed under the microscope (Winterberg 1898), microbiologists have known of the "Great Plate Count Anomaly" (Staley and Konopka 1985). Whether the vast majority of bacteria in soils and other environments are truly "unculturable" or whether most are simply uncultivated ultraslow-growing or dormant cells (Jones and Lennon 2010; Buerger et al. 2012), the fact remains that culture-dependent methods of assessing bacterial biomass and diversity vastly underestimate microbial life in most environments (Epstein 2009). Therefore, culture-independent methods are now widely used by microbial ecologists and soil scientists.

Culture-independent biochemical methods commonly used by microbial ecologists fall into two fundamental groups: (a) phylogenetic analyses of nucleic acid sequences [16S rRNA sequencing, targeted amplicon gene sequencing, whole metagenome shotgun (WMS) sequencing] and (b) "community fingerprinting" methods based on either phospholipids (PLFA) or DNA (DGGE, T-RFLP, ARISA). The latter fingerprinting methods rely on proxy indicators of diversity that do not largely align with either actual species or genetic diversity (Bent and Forney 2008). Genetic sequencing methods on the other hand, while also not necessarily aligning perfectly with "species," do allow for an analysis of genetic diversity and phylogenetic relationships (Franzosa et al. 2015). All of these methods have various problems of extraction efficiency, detection limits, biases, and interpretation, but all have been found to be useful in detecting differences and changes in microbial communities under a variety of stresses associated with land management and use, as well as other environmental conditions. Fingerprinting profiles of PLFAs (Guckert et al. 1985; Vestal and White 1989; Zelles et al. 1992; Harris 2003; McKinley et al. 2005; Frostegård et al. 2011; Mathew et al. 2012) and DNA using denaturing gradient gel electrophoresis (DGGE; Agnelli et al. 2004; An et al. 2014), terminal restriction fragment length polymorphism (T-RFLP) (Mummey and Stahl 2003; Gomez et al. 2004; Park et al. 2006; Hartmann and Widmer 2008; Bennett et al. 2008; Schütte et al. 2008; Enwall and Hallin 2009; Chauhan et al. 2011), or automated ribosomal intergenic spacer analysis (ARISA; Kennedy et al. 2006; Yamamoto et al. 2010; Banning et al. 2011; Mathew et al. 2012) are quicker and more cost-effective, but sequencing costs are falling rapidly. So many studies have utilized 16S rRNA sequencing as a measure of diversity that some comparisons and generalizations across biomes and ecosystems have become possible (Chaffron et al. 2010; Shade et al. 2013).

Many researchers use more than one method to gain insights into microbial diversity, and taken together "meta" (overarching) techniques have been found to discriminate between communities in sites experiencing different land uses (McKinley et al. 2005; Coolon et al. 2013; Khodakova et al. 2014), changing environmental parameters over time (Tedersoo et al. 2012; Fernandes et al. 2013; Shade et al. 2013), or plant–microbe interactions (Marschner et al. 2011; Rosenzweig et al. 2013; Knief 2014), as well as taxonomic and functional differences in soils from different biomes (grassland, forest, mangrove, desert, tundra; Xu et al. 2014).

The simplest measure of diversity is the total number of species (species richness), but this fails to take into account the relative numbers of individuals of each species and the possible influences of dominant and rare species; modeling has shown that richness is not generally a reliable estimate of functional microbial diversity (Bent and Forney 2008). Other diversity indices weight species according to their abundance (e.g., the Shannon and Simpson indices) and describe the equitability of the abundance of each species (species evenness). Recent reviews (Nielsen et al. 2011; Bardgett and van der Putten 2014) have explored the roles of biodiversity and community evenness in soils and how these factors influence the microbial community's resistance and resilience in the face of disturbances and environmental changes (Wittebolle et al. 2009). Microbial community diversity can also be described at three levels: α-diversity (diversity within a given community, habitat, or area, usually species richness), β-diversity (the difference in the species present in two different areas, habitats, or ecosystems), and γ-diversity (overall diversity in a wider area, ecosystem, or biome). These comparisons are useful in studies comparing effects of land use or restoration when treatment plots, time series, or environmental gradients can be compared, but there is still much that we do not understand about interactions within the very complex communities of viruses, bacteria, fungi, protists, microfauna, and macrofauna in soils.

Some argue that since fingerprinting methods do not align with species, the use of diversity indices for these analyses becomes problematic from a theoretical standpoint (Frostegård et al. 2011); many researchers do, however, use diversity indices to describe 16S rRNA, metagenomic, or functional diversity in microbial communities. This makes practical sense, since many bacteria (and bacteriophage) can transfer genes across species and between genera through horizontal gene transfers (transformation, transduction, and conjugation), making the community's microbial gene pool a fluid resource (Committee on Metagenomics 2007) and making the taxonomic definition of a bacterial species controversial and transient (Land et al. 2015). Newer single-cell genetic sampling techniques may ultimately address this problem, but these are currently untenable for sampling whole communities or large areas. There is some indication that measures of genome size may also have ecological significance, as copiotrophic taxa adapted to fast growth in rich substrates and nutrient environments tend to have smaller genomes than oligotrophic taxa adapted to chronic starvation (Leff et al. 2015).

Some have criticized the widespread use of 16S rRNA/18S rRNA genes as the operational taxonomic units (OTUs) and as proxies for species abundance due to the fact that copy numbers per cell may vary, different sequence copies may exist within

a single cell, horizontal gene transfer may affect its reliability, and rare species are underrepresented; the use of ubiquitous housekeeping genes have been suggested as a better alternative (Wooley et al. 2010). Normally undetectable rare microbial genomes can be selected by in situ enrichment incubations and discovered by metagenomics (Buerger et al. 2012; Delmont et al. 2015) revealing underestimates of microbial richness in soils. The Genomic Encyclopedia of Bacteria and Archaea (GEBA) project led by the Joint Genome Institute (Wu et al. 2009; Rinke et al. 2013) is attempting to improve whole genomic sequence databases for uncultivated, phylogenetically distant "dark matter" prokaryotic phyla.

Many researchers have focused on bacterial biomass and diversity in soils, but recent advances in viromics (viral metagenomics) have highlighted the ecological importance of viruses in structuring the bacterial communities and in ecosystem function. Although mycorrhizal fungi have been recognized as playing important roles in plant health and productivity, other microeukaryotes (protozoa, algae, yeasts, and other non-mycorrhizal fungi) have also been largely neglected in soil microbial diversity surveys. Recent molecular surveys using 18S rRNA genes suggest that protozoa may be as diverse as bacteria in soil but that their community structure is driven by different sets of environmental parameters, e.g., primarily soil moisture rather than pH (Bates et al. 2013).

7.2.1.3 Community Structure: Connectedness, Resilience, and Function

Phylogenetic diversity alone does not shed much light on the active interactions occurring among the many members of the microbial community (competition, mutualism, parasitism, or predation) which are essential to the functioning of food webs, nutrient cycling, pathogenesis, biofilm formation, and other complex microbial processes essential to ecosystem health. For example, analysis of 16S rRNA genes neither reflects the full functional diversity of the genome nor accounts for horizontal gene transfers; therefore, analysis of the full genomic DNA is more appropriate for gaining insight into functional relationships in the microbial community. Targeted amplification, sequencing, and cloning of genes into vector species (like *E. coli*) to detect their functions are laborious and often biased based on gene dominance, primer selection, and vector suitability but may be useful for finding novel genes of economic importance (de Bruijn 2011). Random shotgun high-throughput (next-generation) sequencing, filtering, and assembly of large metagenomes followed by annotation and data mining to find predicted protein coding regions have been successful in illustrating differences in microbial diversity and metabolic functions between soils with different land uses (e.g., a 100-year agricultural field and a native grassland), but most of the coding regions detected are not similar to any found in the reference databases and so remain a mystery as to their function (Howe et al. 2014). However, microarray methods like the GeoChip (He et al. 2007, 2010; Tu et al. 2014) can directly screen DNA from environmental samples for thousands of targeted known gene families using probes for genes

related to C, N, or P cycling, energy metabolism, antibiotic resistance, metal resistance/reduction, stress responses, bacteriophage, virulence, and more.

Beyond metagenomics, techniques to analyze extracted mRNA (transcriptomics; Jiang et al. 2016), proteins (proteomics; Siggins et al. 2012), or small molecular metabolites (metabolomics; Reuben et al. 2008; Mosier et al. 2013) from mixed communities are on the horizon but are as yet technically challenging due to assay interference factors (particularly in proteomics and metabolomics). Stable isotope labeling has been helpful in isolating compounds from "dirty" samples based on mass/charge ratios (Mosier et al. 2013; Bueschl et al. 2014). By using two or more of these "-omics" approaches, the functional activities of microbial communities can theoretically be aligned with their phylogenetic potential, giving a clearer picture of not only "who" is in the soil along with their genetic potential but "what" they are doing at the moment of sampling and how the members of the community are interacting (Franzosa et al. 2015). However, neither gene nor transcript abundance has been found to reliably correlate with measured biogeochemical processes (Wood et al. 2015a). According to a recent meta-analysis encompassing 10 years of studies, process reactants or products correlated with their corresponding gene abundance in only 38% of cases (Rocca et al. 2015). These associations were highly variable over studies, ecosystems, and genes, with agricultural sites showing stronger correlations than other terrestrial habitats, "narrow" physiological processes performed by few organisms correlating better than "broad" processes performed by many groups, and correlations between gene abundance and flux rates faring better than associations between genes and pool sizes of either reactants or products. In addition, many studies have targeted only bacterial genes and failed to take account of the potential contributions of the Archaea. In an agricultural study comparing conventional cropping with an early successional grassland, no significant differences in measured soil gas flux rates of CO_2, CH_4, or N_2O were detected between treatments even though the composition of the bacterial communities differed significantly according to pyrosequencing of the 16S rRNA genes (Lauber et al. 2013). Therefore, gene or transcript abundances cannot be used to draw conclusions concerning land use effects on biogeochemical processes unless verified by habitat-specific studies of corresponding process rates.

Spatial and temporal dynamics must be taken into account as well, since the regulation of genes and gene products can change rapidly. Some species of soil organisms appear to be evolutionarily adapted to grow as fast as possible in nutrient- and substrate-rich (copiotrophic) environments (r-selection), while others are adapted to long-term slow growth under low-nutrient, starvation (oligotrophic) conditions (K-selection). The proportion of soil microbial biomass that is metabolically active at any one time may not exceed 5% (often 0.1–2%), with the remainder being either potentially active, but able to respond quickly to carbon inputs (10–50%) or dormant (40–50%; Blagodatskaya and Kuzyakov 2013). The large proportions of the bacterial community found to be in a state of dormancy, particularly in nutrient-poor systems, potentially act as a "seed bank" of diversity that survives disturbances to recolonize the ecosystem and thus provide the community with resilience (Jones and Lennon 2010). Reviews of the literature suggest, however,

that many soil microbial communities are sensitive rather than resistant to a variety of disturbances (e.g., fertilization, temperature changes, carbon amendments, and elevated CO_2), often not returning to their undisturbed state for some time (at least a few years). Such a lack of functional redundancies can then lead to subsequent dissimilarities in rates of some soil processes (Allison and Martiny 2008).

The term "interactomics" refers to the meta-analysis of the biochemical interactions occurring within a cell or, more broadly, within an ecosystem (Committee on Metagenomics 2007). Using a network analysis, the number of nodes (species or genetic functions) along with their pair-wise links (interactions) can be calculated from metagenomes or transcriptomes, in addition to analyzing connections between metabolic processes within individual cells (e.g., enzyme-catalyzed reactions, protein–protein interactions, transport and regulatory interactions). At the ecosystem level, network analysis has been used to describe the connectedness, modularity (subgroup organization, possibly reflecting strong trophic interactions), hierarchy (nestedness, possibly reflecting more mutualistic interactions), and small-world behavior (shorter paths between nodes reflecting tighter communication or feeding loops; Montoya and Sole 2000) of the community (Deng et al. 2012). These network properties have been shown to be important for the robustness and stability of complex systems of macroorganisms, but research on microbial molecular networks is in its early stages. Network analysis may shed light on ecological organism interactions, both those that are negative/agonistic (competition, predation, parasitism, inhibition) and others that are positive in effect (mutualism, symbiosis, commensalism, co-metabolism, syntrophy, and structural cooperation to form biofilms or mats). Many, if not all, of these interactions rely on either direct contact (specific cell surface interactions involving structures like pili and cell surface receptors) or on molecular "sensing" and communication mechanisms (signaling pathways, sensor kinases, and quorum sensing), for which genes may be identified (Zhuang et al. 2013). For example, metagenomic analyses are now being used to explore the phylogenetic diversity and distribution of quorum sensing genes for the synthesis, regulation, and inactivation of *N*-acyl-L-homoserine lactone (AHL) signal molecules in soils and other habitats (Ferluga et al. 2008; Kimura 2014), and a database of signaling peptides has been created (Wynendaele et al. 2013). Global meta-analysis of 16S rRNA sequences across a wide range of environments indicates that the genomes of coexisting microbes are more similar than expected by chance; there are specific and recurring associations and partnerships among these microbial lineages, as well as stability in their habitat preference, indicating that the connectedness of certain microbial species may have previously been underestimated (Chaffron et al. 2010).

Many more studies of microbial ecosystems at various scales are needed to build in-depth baseline and comparative data sets to understand how different community architectural patterns affect community stability and resilience in the face of environmental change and to allow us to predict community responses to those changes (Nesme et al. 2016). To date, much of the research on soils has involved small-scale studies on particular habitats or treatment effects (e.g., various agricultural methods, forestry strategies, or particular local environmental gradients), which, although

important, have often utilized disparate sampling, recovery, and analytic and statistical methods such that the results may be difficult to compare or assimilate into wider meta-analyses. The primary goal of the TerraGenome project is to generate a complete metagenome for a reference soil that can be used for methodological development and as a comparative reference (Cole et al. 2010), and the Earth Microbiome Project (EMP) is coordinating efforts to "construct a microbial biomap of planet Earth" (Gilbert et al. 2014). These and other large, coordinated explorations of soil microbial communities and their associations will be vital to peeling back the veil of mystery surrounding this essential component of the biosphere.

7.2.2 *Ecological Functions and Ecosystem Services Performed by Soil Microbial Communities*

"Ecosystem services" are the benefits that ecosystems provide to people, supporting their sustainable well-being, health, livelihoods, and survival (Costanza et al. 2014). These services can be grouped (Millenium Ecosystem Assessment 2005) into "provision services" that provide tangible goods (food, fiber, fuel, clean water, or other products), those that support life (soil formation, nutrient cycling, flood control, pollination), those that regulate ecosystem processes (climate regulation, disease control, detoxification), and those that provide "cultural services" of recreational, aesthetic, or cultural value. The monetary value of ecosystem services can be estimated based upon valuations of the benefits of a wide variety of ecosystem services to individuals, human communities, and sustainability (Costanza et al. 1997; de Groot et al. 2012). Global estimates currently reach $145 trillion per year (Costanza et al. 2014), a sum that, while most likely an underestimate, is much larger than the total global gross domestic product (GDP). However, the global sum of ecosystems services is declining, and so maintenance of ecosystem health and biodiversity are critical issues (Costanza et al. 2014). This "natural capital" is largely composed of ecosystem components that are not economic commodities traded in human markets and so are commonly overlooked when human development leads to ecosystem disturbance or destruction, hence the need for ecological risk assessments (Faber and Wensem 2012). The concept of ecosystem services began to gain wider appreciation with the publication of a recent series of major international reports: the Millennium Ecosystem Assessment (Millenium Ecosystem Assessment 2005); a series of reports on The Economics of Ecosystems and Biodiversity (TEEB; Kumar 2011) funded by the UN Environment Programme, the European Union (EU), and the governments of Germany, the UK, the Netherlands, Sweden, Norway, and Japan; and the 2010 report of the UN Interagency Panel on Biodiversity and Ecosystem Services (IPBES; (Díaz et al. 2015). Recently, a Common International Classification of Ecosystem Services (CICES) was developed (Haines-Young and Potschin 2013) for use in environmental accounting by the EU and UN Statistical

Division (United Nations 2014). Ecosystem health can also be gauged by the status of indicator species, guilds, or thermodynamic assessments of work capacity and efficiency (the eco-exergy index) of the ecosystem as a whole (Jørgensen et al. 2010).

Soils and the functions provided by their biodiversity provide many critical ecosystem services, particularly for agriculture (Power 2010; Brady et al. 2015), grazing, and forestry (Wang and Fu 2013), as well as less obvious services such as water purification, biogeochemical cycling, and provision of habitats for soil decomposers (Dominati et al. 2010; Pascual et al. 2015). Soils are, however, one of the least understood components of the biosphere. Soil microbes contribute significantly to provisioning (e.g., producing antibiotics and soil organic matter) and regulating types of support services (water purification and storage, soil stabilization, litter decomposition, carbon sequestration, methane cycling, nutrient cycling, energy storage, pathogen regulation, and crop support via rhizosphere interactions; Robinson et al. 2013), but to date no comprehensive valuation of services linked specifically to soil microbes has been made (Comerford et al. 2013; Robinson et al. 2014). However, recent empirical evidence suggests that maintaining soil microbial diversity is key to sustaining the multifunctionality of soils in a wide range of ecosystems (Allison and Martiny 2008; Delgado-Baquerizo et al. 2016). Four main ecosystem functions collectively and interactively comprise the basis for the ecosystem services provided by soil: (1) maintenance of soil structure, (2) transformations of carbon, (3) cycling of nutrients, and (4) biological regulation of soil populations in food webs, including pathogens and pests (Groffman and Bohlen 1999; Kibblewhite et al. 2008).

7.2.2.1 Soil Structure

The physical structure of the soil is determined not only by the abiotic components of the soil (relative proportions of sand, silt, and clay) but crucially also by the amount and type of soil organic matter (SOM). The SOM affects soil structure and aggregation, with aggregation of soil particles playing important roles that enhance porosity, aeration, water holding capacity, and the ability of fungal hyphae and plant roots to penetrate soils. Thus, aggregate size, quantity, and stability (turnover rate) are important soil qualities affecting ecosystem function. A significant portion of SOM is either comprised of microbial biomass or derived from microbial necromass (Miltner et al. 2012). Bacterial and fungal cells, cell fragments, and cell products play a crucial role in microaggregate formation. Stable microaggregates can then be connected and bound together by plant roots, fungal hyphae, and polysaccharides of plant and microbial origin in a more transient manner (Tisdall and Oades 1982; Nunan et al. 2003; Feeney et al. 2006; Miltner et al. 2012; Six and Paustian 2014).

Soil aggregates of various sizes lead to spatial heterogeneity and microenvironmental gradients that can enhance microbial biomass and diversity (Mummey and

Stahl 2003, 2004; Gupta and Germida 2015), and, in turn, microbial and microfaunal activities related to soil particulate organic matter turnover affect aggregate formation and breakdown in a dynamic balance (Six et al. 2000; Mummey et al. 2006). Production of the glycoprotein glomalin by arbuscular mycorrhizal fungi (AMF) plays a particularly important role in the formation of water-stable aggregates in many soils (Lovelock et al. 2004; Six and Paustian 2014) as well as in soil carbon sequestration (Comis 2002; Lovelock et al. 2004). Aggregates can also shield soil organic matter from decomposition by microbes (Lehmann et al. 2007; Schmidt et al. 2011; Dungait et al. 2012; Plaza et al. 2013), thus further contributing to carbon sequestration and soil quality (Schmidt et al. 2011). Human activities that physically disturb soils (e.g., tilling, mining, and development) have a negative impact on soil aggregates and soil structure, often leading to changes in soil community structure, including the ratio of bacteria to fungi and the presence versus absence of soil fauna (Six et al. 2000; Piccolo 2012; Plaza et al. 2013; Ponge et al. 2013; Devine et al. 2014) (Fig. 7.1).

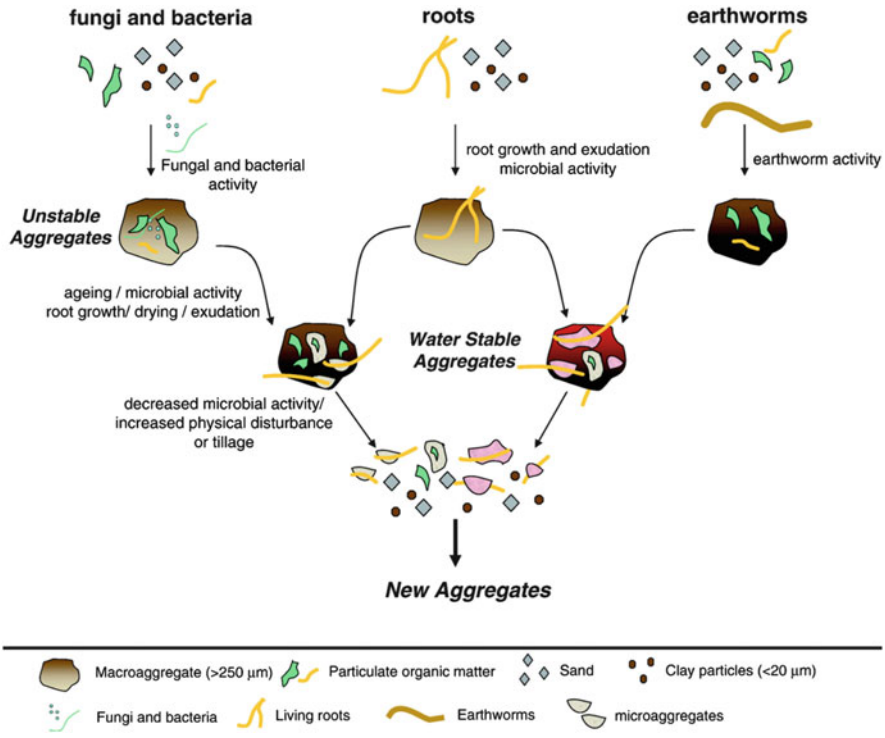


Fig. 7.1 Biological mechanisms of soil aggregate formation and turnover in temperate soils [Barrios 2007; modified from Six et al. (2002)]

7.2.2.2 Biogeochemical Cycling

Perhaps the most fundamental ecosystem service provided by soil communities is biogeochemical cycling—the "recycling" of the elements that make up living organisms (C, N, P, S, and all of the less prominent elements that make up various biochemicals in cells). All of these elements must eventually be released from the cells, waste products, or metabolites of organisms (upon death, if not before) and chemically converted by decomposers either directly into new biomass or into chemical forms that are available for uptake by other living organisms. These elements may pass through many chemical transformations and environmental reservoirs before once again becoming part of a living organism, and turnover times may be relatively short (e.g., for C in CO_2) or very long (e.g., for C in coal) depending upon where and under what conditions a particular atom ends up. In addition to playing significant roles in all biochemical cycling processes, prokaryotic microorganisms are the sole processors of critical segments of all biogeochemical cycles (e.g., nitrogen fixation, nitrification, denitrification, methanogenesis, methylotrophy, sulfate reduction, sulfur oxidation, breakdown of certain recalcitrant polymers, etc.). In all biogeochemical cycles, environmental conditions like soil pH, nutrient availability, moisture (Cook and Orchard 2008), oxygen content (Kuzyakov and Blagodatskaya 2015), and litter quality (Perez et al. 2013; Fanin et al. 2014; Fanin and Bertrand 2016) may restrict or accelerate rates and fluxes in these cycles (Graham et al. 2014), and microbes can either mineralize, mobilize, or immobilize nutrients under differing conditions.

Although all biogeochemical cycles are essential to life on Earth, the carbon cycle not only processes the greatest quantity of matter but is now of urgent concern due to the roles of CO_2 and CH_4 in the greenhouse gas effect and climate change (Stocker et al. 2013). Photosynthesis and decomposition are the two largest components of the carbon cycle, and the soil reservoir contains more carbon (>2400 Pg) than the atmosphere (5900 Pg) and terrestrial vegetation (350–550 Pg) combined (Ciais and Sabine 2013). Both organic and inorganic forms of carbon reside in soils, but the inorganic carbonates are only slowly cycled, while soil organic matter turnover is relatively active (Kandeler et al. 2005). The SOM pool contains up to 2000 Pg of C in the top 1 m of depth (Smith et al. 2015), with greater amounts in northern permafrost areas due to temperature-limited decomposition rates (Tarnocai et al. 2009). It is important to understand that SOM concentrations vary considerably by latitude, climate, and land use (Dilly 2005), with stabilization and sequestration largely governed by the type and amount of organic carbon inputs from terrestrial plants (Gougoulias et al. 2014) and the microbial usage efficiency relative to these inputs (Smith et al. 2015). Plant-mycorrhizal associations and ectomycorrhizal turnover also appear to have major effects on SOM formation and decomposition (Johnson et al. 2005; Godbold et al. 2006; Averill et al. 2014; Finzi et al. 2015).

Some components of dead biomass and plant litter are relatively labile (e.g., simple sugars and amino acids) and readily used by almost all saprophytic organisms, while other components are more recalcitrant (e.g., cellulose, lignin, and

chitin) and can only be degraded by a few specialists (limited species of fungi and bacteria) under certain conditions (Malik et al. 2013). Plant litter decomposition involves physical fragmentation by small invertebrates, colonization and subsequent chemical degradation by bacteria and fungi, and leaching of soluble organic and inorganic molecules. Local inputs of plant litter, root exudates, faunal waste products or decay, and other rich sources of organic matter lead to temporary, intense increases in microbial biomass and activity which greatly accelerate C turnover and induce a "priming effect" as microbes mobilize N and P from ancient recalcitrant SOM to support their carbon-fueled growth (Heuck et al. 2015). Within these "hot spots" the active fraction of the microbial community increases from 2 to 20 times that of the bulk soil during "hot moments" as the labile C is quickly mineralized or converted into microbial biomass and SOM (Kuzyakov and Blagodatskaya 2015). Under favorable environmental conditions, these accelerated flux rates (up to two orders of magnitude greater than those in the bulk soil) tend to balance out the C inputs, preventing large increases in C stocks.

Mineralization of SOM under aerobic conditions results in an efflux of CO_2 from soils which, together with rhizosphere respiration and inorganic C weathering, represents a transfer flux an order of magnitude larger (~60 Pg C) than the anthropogenic atmospheric CO_2 emissions from the burning of fossil fuels and changes in land use (~1.1 Pg C $year^{-1}$; Ciais and Sabine 2013). However, global data on greenhouse gas emissions from soils may contain a high degree of uncertainty due to variations in methodology, underrepresentation of urban soils, and a bias toward the temperate climate zones (Oertel et al. 2016). Many studies have shown that soil respiration and N mineralization rates have a strong linear relationship with labile soil organic matter, and some researchers have used measurements of these metabolic rates as proxies for labile SOM (Laik et al. 2009; Wang et al. 2012). After a severe drought, rainfall can stimulate large pulses of CO_2 release from soils as a result of the resuscitation of the microbial community, although the origin of the carbon thus released has not yet been determined (Placella et al. 2012). Under anaerobic conditions in flooded or low porosity soils SOM is reduced to CH_4, a greenhouse gas more potent than CO_2. Thus, changes in environmental conditions that affect these flux rates of CO_2 and CH_4 could have significant effects (and feedback effects) related to climate change. Since the SOM content of the soil greatly influences overall soil quality, understanding how to manage land use to maximize carbon sequestration in the form of SOM can lead to benefits not only from decreasing atmospheric carbon stocks but also improving soil health for food production and biodiversity maintenance (Fig. 7.2).

Soil microbes also actively cycle nitrogen (Philippot and Germon 2005) and phosphorus, the two main limiting nutrients for terrestrial primary producers. Starting in the 1950s, the intensification of agriculture to increase food production has relied primarily on N and P fertilizers derived either from mined minerals or from industrial synthesis. Today, the anthropogenic addition of reactive N (ammonia or nitrate) is double that derived from natural nitrogen fixation by microorganisms (Erisman et al. 2008). Unfortunately, only about 17% of this anthropogenic fertilizer ends up in products consumed by humans, while the rest enters other pathways of the

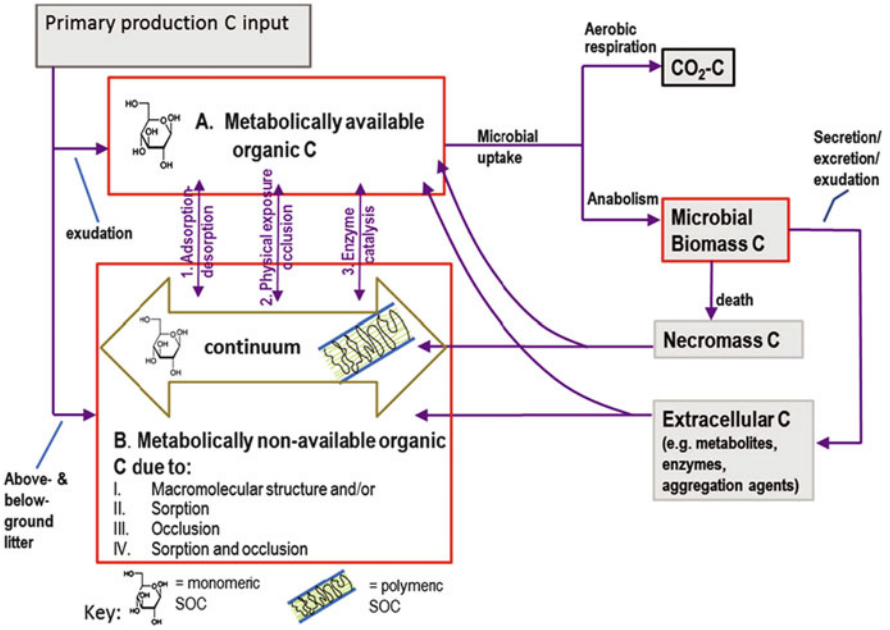


Fig. 7.2 Fate of primary production carbon inputs to soil. Plant-derived organic carbon is processed by soil microorganisms to CO_2, microbial biomass, and extracellular substances. Microbial necromass and metabolites are the precursors for stable soil organic carbon (SOC; red boxes), which may be protected in soil aggregates. Dissolved and exposed organic carbon (A) is available for microbial uptake to produce CO_2 and new biomass. Macromolecular, sorbed, or occluded SOC is metabolically unavailable (B) but may become available via enzymatic depolymerization, desorption, or exposure (I–III, respectively) given adequate water, electron acceptors, heat, pH, and nutrients for microbial activity (Gougoulias et al. 2014)

nitrogen cycle to return to the atmosphere as unavailable N_2 or to cause unintended pollution like nitrate leachates in groundwater, nitric acid in acid rain, nitrous oxide in atmospheric greenhouse gases, ammonia or nitrate runoff causing eutrophication of freshwaters and coastal areas, and various negative effects on the ozone layer (Erisman et al. 2008). This is in stark contrast with natural nitrogen cycling, in which various forms of N cycle tightly between plants, SOM, and soil microbes with minimal losses from this terrestrial system. Biological nitrogen fixation by nodulating bacterial symbionts of legumes (e.g., *Rhizobium*, *Actinomycetes*), non-legume symbionts, endophytic bacteria (e.g., *Azospirillum*, *Azotobacter*), and free-living bacterial nitrogen fixers (e.g., *Azotobacter*, *Klebsiella*, *Rhodospirillum*) can provide 60% or more of the N required by some plants, thus greatly reducing the need for fertilization. Soil N transformations can influence rates of C sequestration, greenhouse gas emissions, and primary production, and thus in situ estimates of N fluxes (Khanna and Raison 2013) and models of N availability (Paul et al. 2002) are important to understanding many soil ecosystem functions (Figs. 7.3 and 7.4).

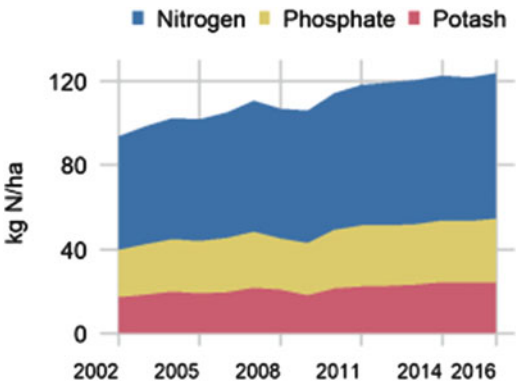


Fig. 7.3 Global nitrogen, phosphate, and potash fertilizer use from 2002 to 2015 (FAO 2015b)

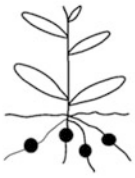
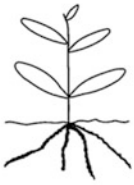
System of N_2 fixation ($N_2 \rightarrow NH_3$) and microorganisms involved	 Symbiosis (e.g., *Rhizobium, Actinomycetes*)	 Associations (e.g., *Azospirillum, Azotobacter*)	 Free living (e.g., *Azotobacter, Klebsiella, Rhodospirillum*)	
Energy source (organic carbon)	Sucrose and its metabolites (from the host plant	Root exudates from the host plant	Heterotroph: Plant residues	Autotroph: Photosynthesis
Estimates of amounts fixed (kg N $ha^{-1}yr^{-1}$)	Legumes: 50-400 Nodulated non-lugumes: 20-300	10-50	1-2	10-80

Fig. 7.4 Type, energy source, and fixation capabilities of biological N^2 fixation systems in soils [Barrios 2007, modified from Marshner (1995)]

Phosphorus can be the most limiting nutrient in soil, primarily due to the fact that many forms are insoluble and therefore unavailable to plants and many saprophytes. The ultimate source of P is parent rock material from which minerals are released very slowly, sometimes with the aid of phosphate-dissolving microbes (Guggenberger 2005), and cycles tightly in the plant–soil system between organic and inorganic forms. Low phosphorus levels in agricultural soils is a serious issue in parts of Africa, South America, and Russia, but anthropogenic fertilization with phosphates mined from either guano or rocks has led to soil P surpluses in North America, Europe, and Asia (West et al. 2014) along with significant water quality

impacts (Bennett et al. 2001). Endomycorrhizal AM (arbuscular mycorrhizal) fungi in particular can greatly aid plants in obtaining P (as well as N, S, and micronutrients) by greatly increasing the volume of soil explored and the surface area for uptake. Plant–AMF symbioses are the most common plant associations, with up to 80% of vascular plants having AM fungi within their root cells (Schübler et al. 2001). However, not all species of AMF are equally efficient in acquiring P (Pandey et al. 2005; Barrios 2007; Schnepf et al. 2008), different plant species may not receive equal benefits, and plants can regulate their mycorrhizae depending on their nutrient status (Hammer et al. 2011). Microbes can also mineralize phosphorus from organic sources (e.g., nucleotides and other phosphorylated compounds), and there is evidence that, even in P-poor soils, the microbial community is limited more often by available C rather than P (Heuck et al. 2015). With many gaps in our knowledge of microbial P cycling (Sindhu et al. 2014), there is a potential to improve the nutrient status of phosphorus-limited soils with further research.

Understanding the rates, limiting conditions, and mechanisms of these transformations in soils is critical to understanding vitally essential processes both locally (e.g., in agricultural productivity or soil reclamation following mining) and globally (e.g., as they affect climate change, ocean chemistry, and acid rain). Research in soil biogeochemical cycling takes advantage of a wide range of methods (Blagodatskaya and Kuzyakov 2013; Sinsabaugh et al. 2015), including microcosm and in situ tracer experiments using either radioactive or stable isotopes (Tiunov 2007; Dungait et al. 2011; Templer et al. 2012; Verastegui et al. 2014), BIOLOG substrate utilization assays (Moynahan et al. 2002; Nair and Ngouajio 2012; Xu et al. 2015), enzyme activity analysis (Romaní et al. 2006; An et al. 2013; Kruse et al. 2015; Tischer et al. 2015; Loeppmann et al. 2016; Zuber and Villamil 2016), and mass balance experiments (Kemmers et al. 2013), as well as the recent advances in transcriptomics and proteomics. Regardless of the methods utilized, rates of activity should be related to the size of the microbial biomass present. For example, the "microbial metabolic quotient," or qCO_2, is the ratio of microbial respiration to biomass, indicating the catabolic demand or efficiency of carbon use by the community. Higher values reflect faster microbial turnover rates and less efficient use of carbon (Dilly 2005), and the qCO_2 has been used successfully to monitor trends in soil disturbance and development (Saviozzi et al. 2001; Marinari et al. 2006; Melero et al. 2006; Anderson and Domsch 2010). There is still much that we do not understand about these processes and the organisms carrying them out, but we do know enough to be able to enhance soil quality and biodiversity through improved nutrient and land management (Smith et al. 2015), as will be discussed below.

7.2.2.3 Soil Food Webs

Food webs model nutrient cycling and energy flow through communities. The abundance of each member of the food web can be controlled either by top-down pressures (consumers and parasites) or bottom-up restrictions (resources like substrates and nutrients). In the soil food web, the soil microbiota (bacteria, archaea, fungi, protozoa,

and algae) are functionally connected to microfauna, macrofauna, and plants in complex ways that are still only partially understood. The heterogeneity of the soil ecosystem at scales from nanometers to hectares provides for an extreme number of habitats and niches that allow for high biodiversity at all trophic levels (Tiedje et al. 2001). Members of soil food webs can be categorized by size into the virome (<1 μm viruses), microbiome (1–100 μm bacteria, Archaea, algae, and fungi), microfauna (5–120 μm protozoa and nematodes), mesofauna (80 μm–2 mm collembolan, acari, etc.), and macrofauna (500 μm–50 mm earthworms, termites, etc.) fractions, all of which tend to decrease in abundance with depth (Ekelund et al. 2001). In general, the smaller the organism, the less we know about its overall diversity and function (Wall et al. 2001), but understudied groups like soil protozoa may be good indicators of soil quality and pollution (Ekelund et al. 2001). The soil food web can also be understood based on key functional groups that carry out essential processes (Swift et al. 2004): decomposers (degraders of cellulose, lignin, chitin, and other SOM), microsymbionts (e.g., N-fixers, mycorrhizae, rhizosphere symbionts, faunal gut symbionts), elemental transformers (nutrient cyclers like nitrifiers, denitrifiers, sulfate reducers, chemolithotrophs, etc.), soil engineers (earthworms, termites, and other fauna that structure the soil), pests and diseases (grubs, parasitic nematodes, pathogenic fungi, and bacteria), and microregulators (grazers, predators, and parasites). Obviously, there is considerable overlap between these groups, and many organisms fulfill multiple roles either simultaneously or at different times.

In the detrital food web, soil macrofauna enhance decomposition by fragmenting detritus, increasing the surface area for microbial colonization, partially digesting the litter, and enhancing colonization during passage through the gut, as well as aerating, mixing, and bioturbating the soil structure (Aira et al. 2008; Gómez-Brandón et al. 2012). Some macrofaunal species actually reduce efflux of CO_2 from litter decomposition through complex physical and chemical interactions with the soil and microbial community (Chang et al. 2016). As bacteria and fungi colonize detritus, protozoa, and nematodes graze on these microbes and are in turn consumed by higher trophic level carnivores. Faunal waste products consequently provide yet more resources for the microbes in the decomposer web (a feedback loop not shown in many food webs and which has not been adequately quantified).

Apart from the detritus food web, soil macrofauna can also feed directly on plant roots and mycorrhizae, releasing additional resources from living plants. Undoubtedly all soil macrofauna depend on their own mutualistic microbiomes, but the microbes associated with the bodies of soil macrofauna have not been thoroughly studied except for the cases of the highly specialized wood-digesting flora of the termite gut (Noda et al. 2007), the more generalist earthworm system (Sampedro and Whalen 2007), and in the nematode *Caenorhabditis elegans* (Cabreiro and Gems 2013). Ecosystem stability is often linked to the abundance, diversity, and interaction strengths of these various functional groups of macrofauna (Ruiter et al. 1995), but how much functional redundancy and resiliency exists at each trophic level is largely unknown. It appears, however, that ecosystem function will most likely be affected by loss of faunal groups having either low species diversity or those

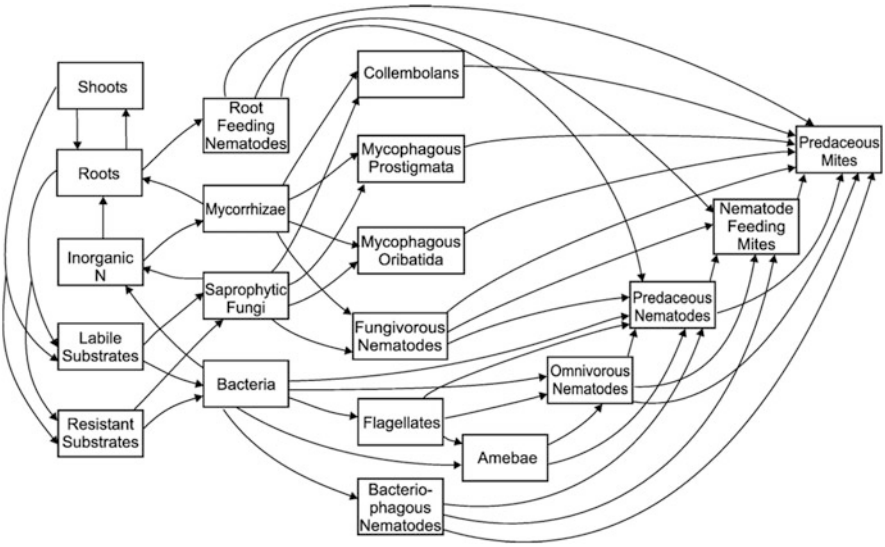


Fig. 7.5 Graphic representation of a detrital food web in a shortgrass prairie [Barrios 2007, modified from Hunt et al. (1987)]. Note that faunal waste products are not represented

occupying trophic levels near the base of the detrital food web, thus affecting nutrient availability and flux (Chapin et al. 1997; Laakso and Setälä 1999) (Fig. 7.5).

7.2.2.4 Plant–Microbe Interactions

Plant communities have a large role in structuring soil microbial communities. Plant species richness can increase archaeal diversity, while plant species evenness tends to increase bacterial diversity (Lamb et al. 2011). Across a latitudinal gradient from rainforests to boreal forests, soil microbial diversity has been observed to correlate well with herbaceous plant diversity, but not tree diversity (Wang et al. 2016) in a manner similar to that found previously in grasslands (Prober et al. 2015). On a smaller scale, plant species patchiness influences the patchiness and spatial heterogeneity of soil microbial communities (Franklin and Mills 2009; Ushio et al. 2010; Ben-David et al. 2011). Land use changes most directly affect the plant communities in an ecosystem, either changing the plants present or eliminating them entirely. Not only do plant communities capture the energy that sustains the vast majority of primary production in terrestrial ecosystems and drives the carbon cycle, they also directly interact with the soil microbial community below ground. Recent isotope tracer studies and community manipulation experiments have shown that aboveground food webs and belowground food webs are more intimately and specifically connected than previously recognized. The direct interface between the two is primarily the rhizosphere zone where plant roots modify the structural and chemical environment of the soil (Strickland et al. 2012; Murphy et al. 2015), but plant litter

and fecal deposits from aboveground macro-herbivores are additional routes by which resources from plants can enter the detrital community. The specific plant species (Ladygina and Hedlund 2010; Becklin et al. 2012; Vries et al. 2012; Rosenzweig et al. 2013; Turner et al. 2013), and even cultivar genotypes, growing in an ecosystem structure the surrounding microbial community, but the soil microbial community also affects the health, growth rates, and disease resistance of the plants.

The "rhizosphere effect" holistically describes the processes taking place at the soil–root interface, including root rhizodeposits, gradient diffusions, genetic exchanges, root and microbial signaling molecules, microbial activities, and nutrient transformations (Haldar and Sengupta 2015). As noted earlier, the rhizosphere is a microbial "hot spot" since plants allocate one-third to one-half of their assimilated carbon belowground, and 15–25% of that carbon is exuded directly into the rhizosphere (Kuzyakov 2002). Due to this surplus of organic carbon in the rhizosphere N and P are limiting there, in contrast to the bulk root-free soil in which carbon tends to be limiting (Wardle 1992), but rhizosphere bacterial genes have been identified that may enhance phosphate solubilization (Chhabra et al. 2013). In most rhizospheres there is a tightly knit relationship between the plant roots, mycorrhizal fungal hyphae, and bacterial cells in which the bacteria can colonize root and fungal surfaces as well as exist as endosymbionts within the plant or the mycorrhizae (Bonfante and Anca 2009), and the relative dominance and activities of the bacterial and fungal communities appear to vary according to environmental conditions like water and nutrients (Rolli et al. 2015).

Rhizodeposits from plant roots include small molecules lost passively (ions, monosaccharides, amino acids, and organic acids), polymers that are actively transported (carbohydrates, protein, and lipids), insoluble mucilage (composed of polysaccharides and polygalacturonic acid), secondary metabolites (antimicrobials, nematicides, and flavonoids), and dead root cells. Plant productivity (Ladygina and Hedlund 2010), genetics, age (Chaparro et al. 2014), health, root architecture (Rosenzweig et al. 2013), soil environmental factors (Bonito et al. 2014), plant neighbors, aboveground herbivores, and the surrounding soil microbial community can affect the amount and type of root exudates released by a plant (Wardle et al. 2004; Huang et al. 2014) and hence the associated microbial community structure. Plant root exudates help mediate plant–plant, plant–faunal, and plant–microbe interactions, but the roles of protozoa and microfauna in the rhizosphere are far from clear (Bonkowski 2004). Some of these root exudates attract and select for a rhizosphere microbial community that is beneficial to the plant, and the abundance of substrates also boosts microbial biomass in the rhizosphere and increases the proportion of microbial cells that are active rather than dormant (Lennon and Jones 2011; Zhuang et al. 2013). The rhizosphere microbial community may be less diverse than in the surrounding bulk soil but it generally includes taxa of bacteria and fungi selected by the plant to aid in its defense against plant pathogens via competition and antagonism (biological control agents), regulate concentrations of plant growth-regulating molecules, and enhance the plant's acquisition of both water (Rolli et al. 2015) and nutrients (plant growth-promoting organisms; Philippot et al.

2013; Huang et al. 2014; Knief 2014; Haldar and Sengupta 2015). In turn, the soil microbial communities available to the plant can affect plant succession and community structure by favoring some plants over others and even affect the plant's interactions with aboveground fauna like herbivores (Khaitov et al. 2015) and pollinators (Becklin et al. 2011). Arbuscular mycorrhizal fungi appear to have a mutualistic relationship with many plants under P-limited conditions, but when available P is in excess, the relationship between mycorrhizal fungi and many plants shifts toward antagonism, limiting plant productivity and altering plant species composition (Gaowen Yang et al. 2014). Thus, the rhizosphere represents both a mutualistic relationship between the plant and its microbial community, analogous to the human gut microbiome's relationship, and a competitive arena in which plants, microbes, and fauna have coevolved survival strategies. If perturbations, transplantation, or other land use changes alter or eliminate the rhizosphere community, it can have negative effects on both the soil food web and the aboveground community.

7.3 Impacts of Land Use on Soil Microbial Communities

Against the backdrop of the basic science and ecological theories described above, we now turn to the effects of human land uses on soil microbial communities, along with their feedback effects. Significant areas of certain biomes have been converted to agriculture or development, and projections for continued loss are significant. Land use changes or disturbances have both immediate effects on the soil ecosystem and longer-term effects once the system has stabilized in a new equilibrium; this chapter will focus on the latter. Each form of land use has its own implications for ecosystem goods and services, as well as for long-term sustainability. A select set of examples of land use effects on soil microbial communities will be explored here to highlight these challenges to sustainability from an increasing human population (Figs. 7.6 and 7.7).

7.3.1 Agriculture and Grazing

Food production is arguably the most fundamental ecosystem service provided by soils. As the primary medium for terrestrial plant growth, soil is an essential resource for agriculture and domestic animal grazing. Modern intensive agricultural methods are increasingly seen as unsustainable in the long run and detrimental to many ecosystem services, like climate change mitigation, aside from direct food or fiber production. Sustainable agriculture aims to balance the trade-offs between crop production and other ecosystem services, like building soil organic matter and maintaining robust biogeochemical cycles, through the use of either low-impact or reduced input and integrated pest management systems (Smith et al. 2015). The

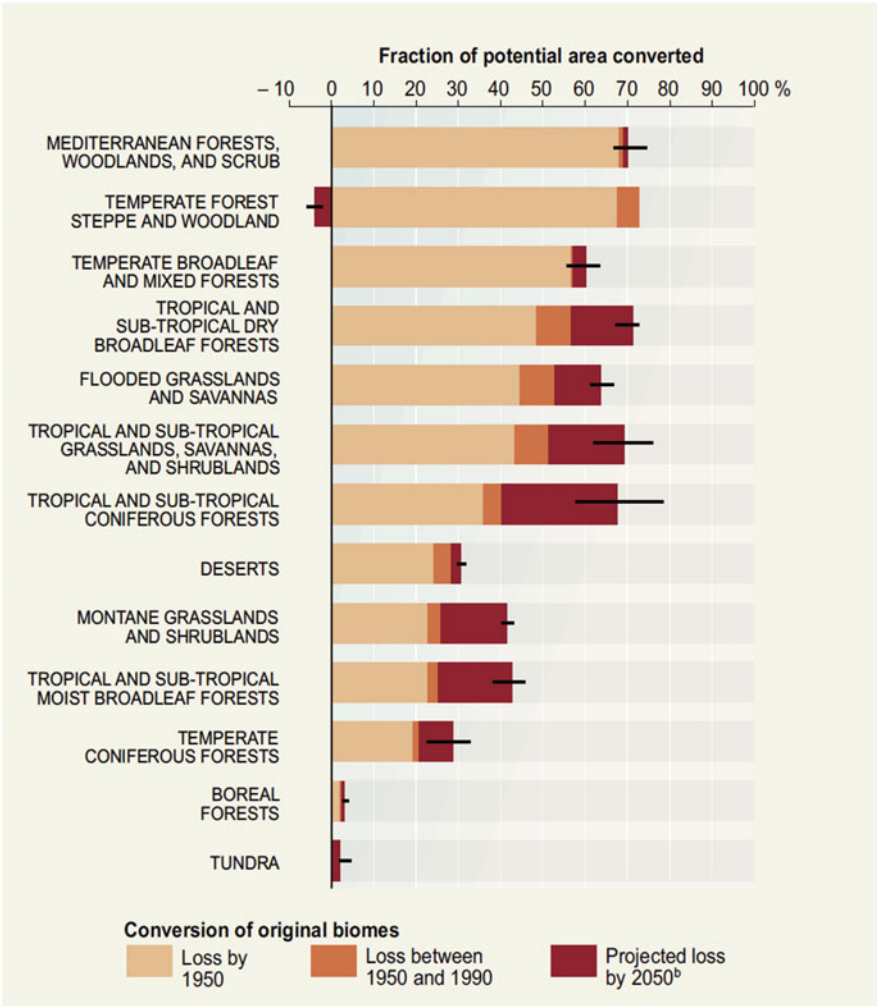


Fig. 7.6 The amount of potential biome area prior to significant human impact that is estimated to have been converted to cultivation or development by 1950 (medium certainty), converted between 1950 and 1990 (medium certainty), and will be converted (low certainty) between 1990 and 2050. Mangroves are not included because the area was too small to be accurately assessed (Millenium Ecosystem Assessment 2005)

effects of various agricultural methods on soils and their microbial communities have been studied extensively for decades, and here we will review only the most recent results of field experiments and observations. About 80% of agricultural lands are in Asia, sub-Saharan Africa, and Latin America (FAO 2015b), but the majority of agricultural studies have been conducted in North America and Europe.

Conventional, intensive agriculture systems in industrialized countries generally use a combination of annual or more frequent tilling, high levels of N + P

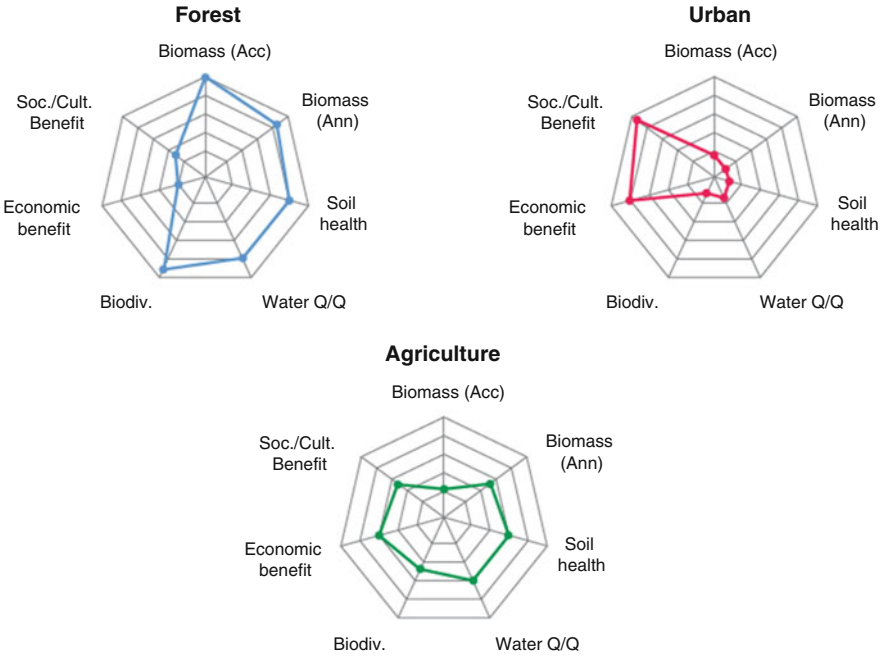


Fig. 7.7 Example of the effect of land use on indicative factors for ecosystem goods and services (FAO 2015a)

fertilization, herbicide and pesticide applications, and frequent irrigation to generate maximal monoculture crop yields. In essence, as soils lose their fertility and SOM over time, these artificial inputs replace many of the services previously provided by the soil structure, SOM, and naturally occurring microbial communities. However, these agricultural methods rely on nonrenewable energy sources (typically fossil fuels), increase greenhouse gas emissions from soils (CO_2, CH_4, and N_2O), and greatly reduce the potential for carbon sequestration in the form of SOM as a form of climate change mitigation. Globally, agriculture accounts for 20–35% of greenhouse gas emissions (West et al. 2014). In addition to these climate change ramifications, intensive agriculture also decreases soil ecosystem diversity and regulatory functions, alters soil structure, increases erosion, decreases soil water holding and purification capacities, increases nutrient runoff and pollution, and can lead to salinization and compaction, thus impairing overall soil health (Kibblewhite et al. 2008; Morugán-Coronado et al. 2014). The magnitude of these effects may vary from site to site based on soil and climatic properties (Alele et al. 2014). Conversion of tropical or temperate forests or pastures to long-term agriculture can lead to an increase in α-diversity in some soil bacterial communities but decreases in β-diversity as a result of a loss of variability due to a homogenization of the soil during tilling and cropping (Jesus et al. 2009; Rodrigues et al. 2013; Montecchia et al. 2015). Conversion of tropical forest to pasture can also stimulate soil microbial

biomass and respiration due to shifts in nutrient status and pH, mobilizing carbon from the soil to the atmosphere (Potthast et al. 2012).

Effects of different agricultural methods on plants, soil, and soil microbial communities are perhaps one of the most studied areas of soil science. The two most widely examined practices are the physical disturbance of tillage and the chemical disturbance of mineral fertilization, as these practices have the largest impacts on soil in many regions.

7.3.1.1 Tillage vs. No-Till Cropping

When native grasslands or forests are initially converted to agricultural production, tillage (plowing or disking) has an immediate effect on the soil, leading to a large spike in carbon mineralization, a reduction in methane consumption (Levine et al. 2011; Zuber and Villamil 2016), and increases in nitrate leaching (Schindler et al. 2007) due to aeration, mixing effects on substrates, priming effects, and breakage of aggregates (Vargas Gil et al. 2009). Disruption of larger aggregates and mixing of depth profiles also redistributes and homogenizes much of the spatial organization, heterogeneity, and niche diversification of the soil ecosystem, leading to loss of microbial biomass and diversity (Wortmann et al. 2008), and tillage intensity can have profound effects on microbial biomass, composition, and nutrient cycling (Cookson et al. 2008; Benhua Sun et al. 2011). Larger organisms, in particular, suffer losses of abundance and often richness of K-selected species, including fungi (Jangid et al. 2008; Vargas Gil et al. 2009; Navarrete et al. 2010), microarthropods, nematodes, and macrofauna (Ponge et al. 2013), largely due to physical disruption, loss of pore structure for habitat, and food web effects. Fungal (Wu et al. 2008) and bacterial community diversity may also be affected (Shange et al. 2012). Temporary increases in bacterial biomass as a result of sudden releases of carbon and penetration of oxygen during initial tillage can lead to short-term increases in grazer nematode populations in some cases (Wardle et al. 1995). One-time tillage of no-till crop fields significantly reduced the abundance of all microbial groups, however, and all populations recovered within 3 years except for the AM fungi (Wortmann et al. 2008).

Long-term tillage leads to higher bulk density, lower SOM, and lower C–N ratios, causing lower water holding capacity, pore size, aggregation (Six et al. 2000), and nutrient dynamics (Azooz et al. 1996; Trojan and Linden 1998; Wander et al. 1998; Steenwerth et al. 2002; Balota et al. 2003; Babujia et al. 2010), as compared with either never-tilled land or no-till cultivation. Conventional tillage tends to decrease the biomass and activity of the microbial community (including bacteria, fungi, AMF, and actinobacteria) in the surface layers of the soil, as well as to increase the dominance of aerobic organisms (Kandeler et al. 1999; Staley 1999; Steenwerth et al. 2002; Balota et al. 2003; Feng et al. 2003; Mathew et al. 2012). However, some studies have failed to detect any effects of different tillage methods on the microbial community, with significant effects found only for any form of cropping compared with uncultivated land (Buckley and Schmidt 2001; Wakelin et al. 2008). These

differences may be the result of the use of relatively insensitive DNA methods in the latter studies, seasonal differences in which plant effects are stronger during the growing season but tillage effects more prominent in winter (Feng et al. 2003), or overriding effects of depth profiling of nutrients in some systems (Helgason et al. 2010). A meta-analysis of Brazilian studies found that microbial biomass carbon (C_{mic}) was a more sensitive indicator of no-till effects at an earlier stage following cessation of tillage than total soil organic carbon, with C_{mic} increasing by as much as 118% (and averaging 58%) within 10–15 years before stabilizing (Kaschuk et al. 2010). In a long-term study site in Georgia, USA, more bacterial taxa (by 16S rRNA gene sequencing) were found in no-till plots compared with conventional tillage, but the overall diversity was not significantly different (Upchurch et al. 2008).

In addition to microbial biomass, microbial activity has also been found to be higher in no-till systems, as assessed by enzyme activities in global studies (Zuber and Villamil 2016) and a BIOLOG substrate utilization study in China (Guo et al. 2016). Recent pyrosequencing analysis of long-term, 50-year no-till versus continuous till experimental plots in Ohio revealed that the tilled soils had fewer dominant species of bacteria, and the no-till soil had more rare species (Sengupta and Dick 2015), while 16S rRNA sequencing of soils in 27-year-old no-till and conventional till fields in Brazil revealed that tillage led to reduced microbial diversity, fewer anaerobes, and an absence of methanogens (Dorr de Quadros et al. 2012).

Reduced tillage (Zhang et al. 2011; Ziadi et al. 2014; Ghimire et al. 2014) or cessation of tillage (Drijber et al. 2000), rather than changes in vegetation or soil characteristics, has been found to have an immediate effect on the soil microbial community, with increases in total microbial biomass, fungi, filamentous actinobacteria, and protozoa. On the Argentine Pampas, no-till management favored oligotrophic, K-selected groups of microbes based on metagenomic analysis of relative gene abundances, compared with conventional tillage, both of which were fertilized (Carbonetto et al. 2014). Over the long term, the effects of plant diversity and inputs following cessation of tillage may have a greater impact on the soil than the absence of tillage alone, with accumulation of SOM and improved soil structure slowly progressing toward the native state in old fields and restored prairie chronosequences (Allison et al. 2005; McKinley et al. 2005; Rosenzweig et al. 2013).

Overall, the vast majority of studies have shown that conservation tillage or no-till farming not only benefits the soil microbial food web but also significantly increases soil organic matter, often with attendant increases in N and P availability (e.g., Ziadi et al. 2014). The incorporation of carbon into aggregates is an important mechanism for long-term carbon sequestration (Six et al. 2000), and protection of root-derived carbon within soil aggregates can partially explain the fact that root C contributes a greater proportion of retained SOM than do shoots (Gale et al. 2000; Wander and Yang 2000; Puget and Drinkwater 2001; Kong et al. 2011). In particular, the occlusion of microaggregates within macroaggregates can explain nearly all of the difference in SOM between conventional and no-till agricultural systems (Denef et al. 2004).

The specific effects of tillage on fungi are controversial. Annual cropping compared with perennial cropping tends to limit the development of AM fungi in plant root systems due to high root turnover (Drijber et al. 2000; de Vries et al. 2013). Some researchers have found significantly more fungi and a higher fungi–bacteria (F/B) ratio in untilled land compared with tilled when measuring mean free hyphal lengths, but others found increased abundance of both fungi and bacteria but no difference in the F/B when using methods that measured viable biomass (PLFA); disturbance may not only break fungal hyphae but may also increase their decomposition rates, preventing accumulation of dead hyphae that sequester carbon and help to structure the soil (Feng et al. 2003; Helgason et al. 2010; Wu et al. 2008).

7.3.1.2 Mineral Nutrient vs. Organic Fertilization

Mineral nutrient fertilization is another widespread agricultural practice that has a significant negative impact on soil biota in both test plots and in active agricultural settings. Many studies, recently reviewed (Diacono and Montemurro 2010), have compared differing amounts of mineral fertilizers (N, P, K, and S) individually and in various combinations in contrast to either organic fertilizers or controls lacking fertilizer. The results from these comparison studies almost always show that long-term mineral fertilization leads to decreased microbial biomass, diversity, and activity, along with poorer soil quality as gauged by SOM, total N, and other correlating variables, although there may be short-term stimulation of microbial activity upon initiation of nutrient addition. It is often difficult to discriminate the direct effects of fertilization from indirect effects that are the result of changes in plant productivity, pH, or other factors that tend to occur along with long-term changes in agricultural management. However, in general, there appears to be a consistent advantage to the use of either organic farm wastes or industrial wastes as fertilizers compared with mineral fertilizers, even when the NPK ratios are balanced. In particular, composted stabilized manures typically give the largest increases in microbial biomass (up to 100% more), enzyme activities (up to 30% more), SOM, and aggregate stability, and the lowest DOC and N leaching rates compared with mineral fertilizers, with important implications for carbon sequestration (Birkhofer et al. 2008; Kaschuk et al. 2010). Some studies have also found that manure tends to decrease the qCO_2 compared with mineral fertilizers (Kaschuk et al. 2010; Heinze et al. 2010; Reeve et al. 2010), indicating that the microbial community is utilizing carbon more efficiently and the microbial biomass turnover rate is slower (Dilly 2005). Using isotope tracing methods, it has been determined that bacterial communities tend to preferentially incorporate recent plant material vs. older SOM (Kramer and Gleixner 2006) into their biomass, and carbon sequestration can be up to 14 times faster in organic vs. mineral fertilizations (Kong and Six 2010). These trends appear to hold in both temperate and tropical regions; a meta-analysis of Brazilian studies found that conversion to organic from conventional fertilization led to increased soil C_{mic}, SOC, and respiration but decreased qCO_2 rates for apple, coffee, sugarcane, and acerola crops (Kaschuk et al. 2010).

Microbial diversity and abundances of various taxa are also significantly affected by conversion of conventional fertilization to organic inputs: community structure (PLFA, DGGE, or T-RFLP) is often significantly different, with microbial diversity being significantly greater with organic inputs (Marschner 2003; Esperschütz et al. 2007; Jangid et al. 2008; Ding et al. 2013; Berthrong et al. 2013; An et al. 2014). Sequencing of 16S rRNA genes typically reveals that mineral fertilization favors copiotrophic taxa, including *Bacteroidetes*, *Actinobacteria*, and *γ-Proteobacteria* (Fierer et al. 2012; Ding et al. 2013; Wood et al. 2015b), while organic amendments or no fertilization favor more oligotrophic taxa, including *Acidobacteria*, *Deltaproteobacteria*, *Betaproteobacteria*, *Clostridia*, and *Bacteroidetes* (Jangid et al. 2008; Chaudhry et al. 2012; Ding et al. 2013; Sul et al. 2013; Wood et al. 2015b). Substrate utilization patterns (BIOLOG) are also more diverse with organic fertilization (Chaudhry et al. 2012; An et al. 2014). Grasslands and organic crops in the Netherlands had significantly more taxa of AM fungi and more variability between sites than conventionally fertilized crops (Verbruggen et al. 2010), while P fertilization reduced AMF hyphae and phylotype richness while increasing numbers of spores in Canadian maize fields (Ziadi et al. 2014).

7.3.1.3 Burning and Pesticide Use

Slash-and-burn farming and crop burning to recycle nutrients are common practices, particularly in tropical regions. Land is burned to clear it for planting or after winter fallow cover cropping. In grassland areas, fire is a natural part of the environmental regime, and many organisms in those ecosystems are adapted to it; however, for cropping or grazing purposes, the same areas are often burned more frequently than occurs naturally. The intensity of a fire, and hence the heat delivered to the surface soils, is largely dependent upon the fuel load; when fields are burned annually, the amount of residual biomass is generally low enough to limit the effects of heat. In Brazilian savannas burning has been shown to stimulate C_{mic} temporarily, while rates of C mineralization and immobilization were unaffected and annual inorganic N cycling slowed (Nardoto and Bustamante 2003). In another Brazilian grassland case, C_{mic} and SOM were unaffected by annual burning, but the qCO_2 rate increased, a possible microbial adaptation to disturbance (Baretta et al. 2005). In Ghana, burning winter fallow vegetation led to increases in bacterial spore-forming genera (Sul et al. 2013), but elsewhere in the tropics the burning of sugarcane fields decreased C_{mic} by about 20% (Cerri et al. 1991; Mendonza et al. 2000), and the clearing of tropical forests followed by burning the remaining wood decreased C_{mic} by about 80%, leading to reduced productivity and field abandonment within a few years (Pfenning et al. 1992; Kaschuk et al. 2010).

The use of pesticides, mostly synthetic organic compounds that are energy-intensive to manufacture and apply (herbicides, insecticides, and fungicides) is also common in conventional agriculture in industrialized countries. Most studies on the effects of pesticides on soil microbiota have been culture-based lab or microcosm experiments (Spyrou et al. 2009; Kumar et al. 2012; Yousaf et al.

2013). Field studies examining pesticide effects on soil microbes are often confounded by other intensive agricultural practices like tillage and fertilization (Alletto et al. 2010; Nguyen et al. 2016) and may not agree with lab toxicology experiments (Karpouzas et al. 2014). Although the results have been highly variable, depending upon the type of pesticide, rate of application, and the soil characteristics, in general it appears that the most significant effects are on the higher trophic levels of the soil food web (macrofauna, microfauna, and fungi; Iqbal et al. 2001b; Kibblewhite et al. 2008), which may then indirectly affect the microbial community. Pesticide application can lead to a temporary boost in bacterial biomass and activity due to pesticide degradation activity, but negative short-term and long-term effects have also been seen on microbial diversity and activity (Bishnu et al. 2008; Spyrou et al. 2009; Floch et al. 2011; Srinivasulu and Rangaswamy 2013), particularly among the AM fungi (Sainz et al. 2006; Jan et al. 2014) and nitrifying bacteria (Iqbal et al. 2001a; Feld et al. 2015). Pesticide degradation has also been shown to be spatially highly variable (Huang et al. 2009; Dechesne et al. 2014), highlighting the roles of AM fungi and "hot spots" like wormholes and burrows in the transport and degradation of pesticides (Badawi et al. 2013) (Fig. 7.8). In addition, both the effects of pesticides on microbial activities and the rates of pesticide degradation are influenced by interactions with mineral fertilization, compost addition (Muñoz-Leoz et al. 2012), and tillage regimes (Alletto et al. 2010). These findings, taken together, have profound implications on the fates and destinations of these chemicals. In the long run, legacy effects of historic pesticide use prior to regulatory restrictions can reveal

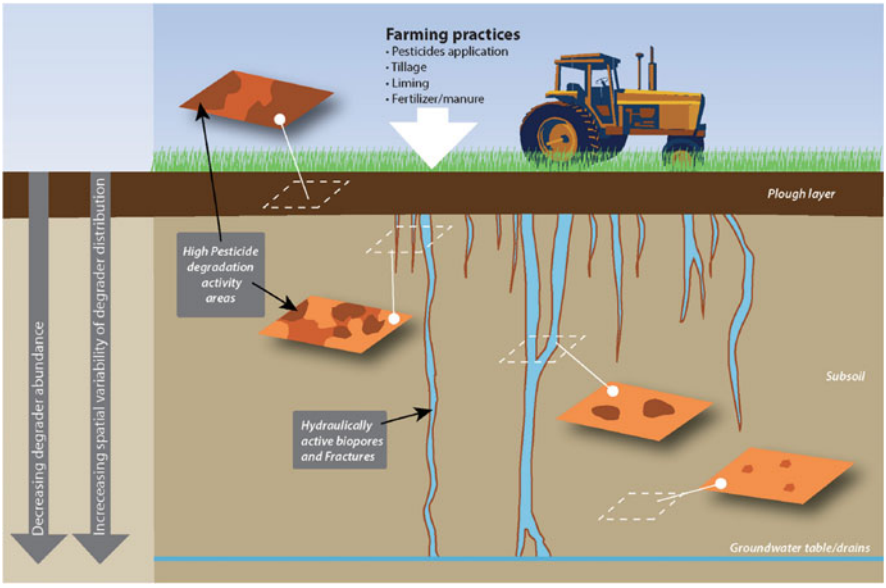


Fig. 7.8 Spatial distribution of 2-methyl-4-chlorophenoxyacetic acid (MCPA) degradation (Dechesne et al. 2014)

themselves when those soils are disturbed by later land uses (Renshaw et al. 2006). Lately, there has been a movement toward using "natural," botanically derived pesticides in an effort to make agriculture more sustainable; the measured effects on soil microbial communities have been mixed, but for the most part, the botanical pesticides do not impact the microbiota as negatively as either synthetic organic compounds or fumigants (Spyrou et al. 2009; Ipsilantis et al. 2012).

7.3.2 Other Land Uses

About 12% of the Earth's land area is currently devoted to agriculture, and agriculture is the major driver of deforestation, particularly in the tropics. Even so, over 30% of the land area remains in boreal, temperate, and tropical forests (FAO 2015b) which store up to 45% of global terrestrial carbon (Bonan 2008). This includes areas in which forests are managed and harvested, activities that can stress many components of the ecosystem, including soils. Other human activities that lead to major soil disturbances include mining and the development of urban and suburban cities and industrial complexes.

7.3.2.1 Forestry

In addition to the conversion of forested lands to agriculture or development, disturbances that can affect forest soil microbial communities include wood harvesting, fire, blowdowns due to major storms, and widespread infestations or diseases affecting trees. Harvesting can affect soils in various ways, depending on the extent and mechanism of tree cutting. Clear-cutting all of the trees in a given area has the largest impact on soils as well as the ecosystem as a whole, while selective or partial harvesting is usually less disruptive. Forestry harvesting effects on soils can include compaction by heavy machinery, short-term increases in nutrients due to plant waste left behind by loggers, and long-term decreases in nutrients due to loss of litter and root exudate production (Johnson and Curtis 2001; Zhou et al. 2013) as well as soil erosion. Loss of vegetation cover also leads to soil warming and consequent loss of moisture in many cases.

Harvest management practices both directly and indirectly affect soil biogeochemical cycling not only through removal of plant biomass from the ecosystem but also by additions of organic C and N from harvest residues and inorganic N fertilizers. Carbon and nitrogen cycling are coupled in these systems, with plant litter quality and flux affecting N mineralization and immobilization by the decomposers, and thus long-term N availability (Parolari and Porporato 2016). In addition, plants and decomposers may compete for nutrients when N is limiting. Hardwood forests are more susceptible to N loss following harvesting than are coniferous forests because they have greater rates of N mineralization and nitrification and lower litter

C–N ratios (Vitousek et al. 1982). In the first year after clear-cutting in a Northern Michigan hardwood forest, a resulting doubling of net N mineralization rates led to increased NH_4^+ availability, a doubling of nitrification rates and 25% more NO_3^- leaching compared with intact forest, but no increases in either denitrification rates or microbial biomass, suggesting rapid microbial N turnover after harvest (Holmes and Zak 1999). Postharvest N availability is mainly controlled by soil microbial community N retention and may be manipulated to improve nitrogen use efficiency (NUE) in harvested ecosystems. For example, experimental plots of southern pines where organic residue was left on site immobilized 20% more N 2 years postharvest than did plots with residual removal and herbicide application (Vitousek and Matson 1985), while 8 years of N fertilization reduced microbial biomass and net N mineralization in northern hardwood forests but increased microbial respiration quotients (qCO_2) and growth efficiency (Fisk and Fahey 2001). Comparisons between continuously harvested and clear-cut rotational models show that, given the same yield, clear-cut systems exhibit greater NUE and mineralize more N than do continuously harvested systems. In addition, large fluctuations in N leaching rates over the clear-cut rotation cycle can lead to pollution impacting local streams, while leaching rates are slower and more stable in continuous harvest systems (Parolari and Porporato 2016).

Classic ecosystem theories predicted that the total amount of CO_2 released by soil microbes would increase following forest disturbances due to increases in soil temperature and labile carbon (Chapin et al. 2002). However, in situ measurements of soil respiration, microbial biomass, and soil C following forest harvesting have been mixed. Partial harvesting allows for retention of C in standing live tree biomass and increased leaf and wood litter inputs when compared with clear-cutting, and it has a lower impact on decomposition dynamics of both wood and leaf litter. Over a period of 9 years in a boreal aspen forest, neither partial nor clear-cut harvesting had an effect on soil C pools, indicating that the forest soil C was resistant to disturbance, but maintaining high and continuous snag and large log inputs in partially harvested systems allowed for slowly decomposing wood to become integrated into the soil over time, thus enhancing C sequestration (Strukelj et al. 2015). On average, whole-tree harvests reduce A horizon C and N by 6%, while leaving more limb and bark residues on site (saw log harvest) leads to an 18% increase (Johnson and Curtis 2001). Other meta-analyses have found little effect of forest cutting on overall soil C stocks (forest floor combined with mineral soils at depth) in temperate (Nave et al. 2010) or global studies (Zhou et al. 2013), but a meta-analysis of 432 temperate studies found that forest cutting can reduce carbon storage on the forest floor alone by as much as 20% in coniferous and mixed stands and by 36% in hardwood stands (Nave et al. 2010). In addition, the conversion of forests to other land uses significantly decreases soil C globally (Table 7.1), with the exception being changes from forest into pasture. Again, the types of trees matter when considering the effects of tree plantations: replacing native forest or pasture with broadleaf plantations did not significantly alter soil C stocks, while pine plantations reduced C stocks by 12–15% (Guo and Gifford 2002).

Table 7.1 Percentage change in soil C due to land use alteration, with number of observations from a meta-analysis of 74 publications by Guo and Gifford (2002)

Ecosystem conversion	Soil carbon change (%)	Number of observations
Pasture to crop	−59	97
Forest to crop	−42	37
Forest to plantation	−13	30
Pasture to plantation	−10	83
Forest to pasture	+8	170
Crop to plantation	+18	29
Crop to pasture	+19	76
Crop to secondary forest	+53	9

Although natural wildfires are ecologically important disturbances in both forests and grasslands, the frequency and extent of fires in northern forests are expected to increase 3.5–5.5 times by the end of the twenty-first century due to changes in climate (Balshi et al. 2009). About 5.1×10^8 ha of forests burn globally each year, and human activities are directly or indirectly responsible for a large portion of these (Caldararo 2002). Slash burning and prescribed fires are widespread management techniques used in plantation forests to clear harvest wastes, release nutrients, suppress competitive plants, and prepare sites for replanting (Johnson et al. 2009; Boerner et al. 2009). Economic pressure to salvage timber from either burned or insect-killed forests in Canada's boreal forests is increasing (Schmiegelow et al. 2006), but this repeated disturbance of salvage logging following fires or infestations has been found to result in greater long-term impacts on the forest floor than did either harvesting or wildfire alone. Changes in soil carbon and nitrogen pools and cation exchange capacity following salvage logging persisted for 10 years and exhibited different recovery patterns than for either fire or harvesting (Kishchuk et al. 2015). Forest floor depth is consistently lower under salvage logging, and depleted soil calcium (Ca), magnesium (Mg), and phosphorus (P) may not return to pre-disturbance levels within the planned rotation time (Brais et al. 2000).

Fire affects soil microbial respiration and N mineralization rates by altering soil moisture, nutrient availability, and microbial activity (DeLuca and Zouhar 2000; Hamman et al. 2008), and the effects of fire on soil generally decrease with increasing soil depth and with time after the fire, with vegetation type, fire type, fuel type, and fuel consumption amount also affecting the outcome (Wan et al. 2001). Prescribed fire often decreases soil acidity since the burning of organic matter releases alkaline substances and destroys organic acids; this alkalinity in turn can increase the retention and availability of cations like K^+, Ca^+, and Mg^+ (Scheuner et al. 2004). A meta-analysis of 76 studies (Wang et al. 2012) found that, on average, fire decreased soil organic C in mineral soil layers by 20%, a finding that could have negative implications for C sequestration, but a meta-analysis of a network of North American sites found no SOC changes in burned forests compared with control sites (Boerner et al. 2009). Fire also tended to decrease microbial biomass C, microbial

respiration, and N mineralization but increased the soil total N, microbial biomass N, dissolved organic C, and total N (probably due to nutrient releases from litter and wood), with wildfires often having greater effects than prescribed fires (Wang et al. 2012). However, forest type had an effect; wildfires in broadleaved forests and Mediterranean zones increased both soil organic C and total N, while there were negative responses in coniferous forests and temperate zones, with wildfire significantly decreasing N mineralization in coniferous forests. Another meta-analysis of 185 data sets from 87 studies published from 1955 to 1999 (Wan et al. 2001) found that soil NH_4^+ pools increased twofold immediately after fire and gradually declined to pre-fire levels within a year, while the increases in soil NO_3^- were smaller (24%) immediately after fire and reached a threefold maximum within 0.5–1 year after fire before declining. In severely burned soils, revegetation and plant secondary succession can aid in the recovery of the microbial community biomass, activity, and diversity (Knelman et al. 2015).

7.3.2.2 Mining

Although mining is a temporary land use change, extraction of minerals from the earth through surface mining is one of the most destructive human activities that impacts habitats and their soils, often having long-term consequences (Lewis et al. 2012). Mining operations cause degradation of land through: (1) removal of vegetation and topsoil, (2) excavation and dumping of overburden or tailings, (3) changes in the landscape topography, (4) disruption of surface and subsurface hydrology, (5) loss of soil fertility, and (6) serious environmental pollution (Keskin and Makineci 2009). Surface mining excavation and dumping of overburden destroys large areas of habitat, and tailings from underground mining can have an equally devastating effect (Frouz 2014). Overburden (the subsoil and rock removed to expose the ore-containing seams) and mine tailings (the crushed rock that remains after the useful ore has been removed) have very low cation exchange capacities, minimal microbial populations and activity, almost zero organic matter content, and can contain high concentrations of heavy metals (Pepper et al. 2012; Poncelet et al. 2014). Weathering of disturbed overburden can lead to oxidation of chemical constituents and lower the pH of the surrounding area (Poncelet et al. 2014). Tailings and overburden are often deposited to a depth of up to 30 m or more in deserts or other uninhabited areas, and, if not remediated, the scant vegetative growth that results leads to erosion, dust storms, and toxic runoff. Runoff can chemically alter the environment through acidification and pollution by heavy metals, with the effects on the soils and microbial communities being widely variable depending upon the specific ores, minerals, soil, and water chemistry present. Establishment of plant communities is highly dependent on the reestablishment of the microbial communities in these harsh conditions (Markowicz et al. 2015).

Soil contamination with heavy metals (primarily Hg, Pb, Cd, Cu, Ni, As, and Zn) is a locally serious problem related to ore mining and smelting, but discarded

manufactured products, coal ash, agriculture, and transportation contribute more metal inputs into soils globally than smelting (Dudka and Adriano 1997). Coal, oil sands, and natural gas extraction also cause environmental disturbance and contamination with hydrocarbons, drilling chemicals, fracturing fluids and metals, while quarrying and sand mining result in soil removal and landscape alterations. It is beyond the scope of this chapter to delve into all of the effects that these forms of land use have upon soil microbial communities but, as a prelude to discussing ecosystem restoration in subsequent sections, a sample of mining effects on soil microbial communities will be reviewed. Ecosystem reconstruction of affected mining areas is mandated in some, but not all, countries, and as yet no systematic meta-analysis of its efficacy is available.

When topsoil layers are removed and stockpiled prior to surface mining, the microbes inhabiting the original surface layers are buried under compacted subsoils (Sheoran et al. 2010). A flush of activity, including respiration, occurs in the overturned new upper layer as the bacteria are exposed to atmospheric oxygen. Some topsoils are stored for 10 or more years before being used to reclaim the mined lands (Mummey et al. 2002). After 2 years of storage, there is little change in the bacterial numbers at the new surface, but less than one-half the initial topsoil populations buried at depths below 50 cm survive (Williamson and Johnson 1991). At depth in the stockpile, anaerobic bacteria dominate (Harris et al. 1989) thus inhibiting nitrification and leading to an accumulation of ammonia. Stockpiled topsoils typically experience loss of microbial biomass, diversity, enzyme activity, SOC, and soil nutrients and increased bulk density (Baldrian et al. 2008; Lewis et al. 2010, 2012; de Souza et al. 2013; Cruz-Ruíz et al. 2016). Once the soil from the stockpile is reinstated on the disturbed overburden in a 20–30 cm deep layer (Baldrian et al. 2008), aerobic microbial populations reestablish, and subsequent nitrification of the accumulated ammonia can lead to nitrate generation and leaching into local waters with potentially serious environmental consequences for drinking water (Johnson and Williamson 1994). Viability of mycorrhizae in stored soils may decrease to less than 1/10 those of the undisturbed soil (Rives et al. 1980; Gould and Liberta 1981), with soil–water potential being a significant factor affecting mycorrhizal viability: in dry soil of less than −2 MPa, mycorrhizal propagules can survive longer, but in moist soil greater than −2 MPa, survival can be limited by storage time (Miller et al. 1985). However, mycorrhizae can become reestablished once stockpiled topsoil has been spread due to windblown spores and release from dormancy (Jasper 2007). Topsoil is not always reapplied to the overburden after mining has ceased, and in coal mining areas, a significant amount of the organic carbon available for microbial incorporation into biomass is in the form of lignite (Rumpel and Kögel-Knabner 2004).

Heavy metal and other chemical pollutants are toxic to many microorganisms (particularly endospore-formers and oligotrophs), suppressing both soil biomass and activity (Šmejkalová et al. 2003), but these contaminants also provide selection pressures that spur the development of resistance and metabolic capabilities to either detoxify or degrade the contaminants. Therefore, mine tailings and contaminated soils are prime locations for discovering microbes (Wolfaardt et al. 2008; Rastogi

et al. 2010) that can be used in bioremediation of contaminated soils and concentration of useful minerals through such processes as biosorption, bioaccumulation, and biotransformation, although high concentrations of toxic heavy metals can be a factor that limits such processes (Fashola et al. 2016).

7.3.2.3 Urban and Industrial Development

Urban land use is increasing and is predicted to reach at least 1.4% of global land cover by 2030 (Seto et al. 2012). As rapid urbanization continues, the potential of urban soils to serve as carbon (C) sinks and the important role of soil microbes in urban soil C fluxes has been more widely recognized (Chen et al. 2013). Whereas soil and ecosystem changes as a result of transitions between agricultural and native land use have been well studied, conversions to urban land uses remain less well understood. Urban land use changes may be irreversible, including increases in variability and patchiness compared with other land use types (Pouyat et al. 2007), and non-native species commonly grown in the urban areas can alter nitrogen cycling, primary productivity, and other biogeochemical processes (Ehrenfeld 2003).

Urban land development practices (vegetation removal, A horizon topsoil removal, storage and reapplication, surface grading, subsoil compaction, and building construction) generally result in degraded urban soils with low vegetative cover, high bulk density, low infiltration rates, and altered C cycles (Kaye et al. 2006; Woltemade 2010). Unlike mining sites, topsoil stockpiled at urban construction sites that is later reapplied on site is typically stockpiled for less than 1 year (Chen et al. 2013). Like mining sites, however, urban soils are often contaminated with heavy metals from air pollution, industrial activities, and technological waste (Kelly et al. 1996; Jim 1998; Madrid et al. 2002; Chen et al. 2005; Lee et al. 2006; Wei and Yang 2010). Post-development human structures and activities, including impervious surfaces (ranging from 10 to 80% cover in various cities), engineered water and wastewater flows, artificial landscaping, fertilizer and other nutrient inputs, and herbicides and pesticide use, can affect both soil quality and biogeochemical cycling (Kaye et al. 2006). In humid environments, the growing season in cities is about a week longer than in nearby rural areas due to the "heat island effect" (White et al. 2002), which can increase annual nutrient and carbon uptake by plants.

Soil management practices typical of urban land development (topsoil removal, short-term stockpiling, and reapplication) have been observed to result in significant losses of soil C in surface soils for over 4 years when compared to undisturbed sites, and this holds true with regard to both the mineral-bound C pool and the less stable labile C pools in surface soils (Chen et al. 2013). A study estimating C dynamics of urbanized lands in the Southern United States from 1945 to 2007 (Zhang et al. 2012) suggested that soil C rapidly decreases at the beginning of land conversion to urban uses and then gradually increases. The greatest increases in urban soil C in the years following development have been observed in highly managed soils; for example, SOM in golf course fairways increased from 1.76% 1 year after turfgrass planting to

4.2% after 31 years (Qian and Follett 2002). Comparisons of carbon storage in six US cities and nearby native soils found that urban areas have the potential to either sequester or lose SOC depending largely on the climate (Pouyat et al. 2006); cities in the Northeast with high concentrations of C in native soils (Boston and Syracuse) experienced a 1.6-fold decrease in SOC pools following urbanization, while cities located in warmer and drier climates (Chicago and Oakland) increased slightly (6% and 4%, respectively). While variation in SOC was higher within cities than among cities for a given soil type when all urban land uses were included (Pouyat et al. 2002), residential yards in Chicago, Moscow, and Baltimore exhibited relatively little variation in soil C (12.9–18.5 kg m^{-2}) with the presence of trees possibly adding C to deeper levels of soil (Pouyat et al. 2002; Huyler et al. 2014). Relative to native soils, some urban soils have been shown to have higher fluxes of both CO_2 and N_2O greenhouse gases (Koerner and Klopatek 2002; Kaye et al. 2005), but in some cases the mechanisms controlling these processes in urban areas are not well understood as the usual moisture and nutrient controls on microbial processes appear to be uncoupled (Pataki et al. 2006; Koerner and Klopatek 2010).

Recent reviews of the literature found that both negative and positive effects on soil microbial biomass and activity had been recorded following slight compaction as well as strong compaction, but above an effective bulk density of 1.7 g cm^{-3}, only negative effects were found (Beylich et al. 2010; Nawaz et al. 2013). The North American Long-Term Soil Productivity study conducted in North Carolina, Louisiana, and California found that neither microbial biomass nor activity were affected by severe compaction from multiple passes of heavy machinery in loam forest mineral soils (Busse et al. 2006), but other studies in forest and agricultural soils have found significant decreases in microbial biomass and enzymatic activity following compaction (Tan and Chang 2007; Tan et al. 2008; Pupin et al. 2009; Nawaz et al. 2013). Although research into the effects of compaction on soil respiration rates has been mixed, soil compaction can cause an increase in denitrification and emissions of N_2O up to 500% (Nawaz et al. 2013). The residence time of N_2O in the soil, however, is dependent on soil conditions, especially moisture. The anaerobic conditions resulting from compaction can also result in increased methanogenesis, exacerbating greenhouse gas emissions (Nawaz et al. 2013). The variation in microbial biomass responses to compaction could be due to differences in measurement techniques, SOM, land use, the severity and duration of compaction, site history, and management practices (Beylich et al. 2010; Nawaz et al. 2013). It has been proposed that urban soils could be sinks for atmospheric C sequestration (Lorenz and Lal 2009), and subsoil has been recognized recently as an important C sink that contains more stable forms of C (Salomé et al. 2010; Helfrich et al. 2010; Sanaullah et al. 2011; Lorenz et al. 2011; Rumpel et al. 2012). The addition of calcium-rich construction and demolition wastes derived from cement to the soil and subsoil profile during urban excavation and development could potentially capture carbon from the atmosphere in the form of carbonates (Washbourne et al. 2012). Further research and large-scale demonstration projects are needed to confirm this hypothesis.

7.4 Ecosystem Restoration Strategies and the Roles of Soil Microbes

Restoration is broadly defined as the repair of degraded and/or fragmented ecosystems to improve their health, integrity, and sustainability (Aronson et al. 1993; Dilly et al. 2010; Allison 2012). In a strict sense, the Society for Ecological Restoration (SER) defines ecological restoration as "the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed" (Aronson et al. 1993; Society for Ecological Restoration International Science & Policy Working Group 2004). An idealistic form of restoration to match the original, native ecosystem is rarely possible due to lack of historical knowledge regarding native species and their dynamics, but in practice restoration often aims to work with any indigenous species still present and manage the land in a way to suppress invasive species and reassemble as much of the local native biodiversity as possible (Allison 2012).

The goals of ecological restoration can range from ameliorating highly degraded abiotic conditions (e.g., toxic pollutants or lack of topsoil on old mining sites), to reinstatement or enhancement of key ecosystem functions (e.g., productivity, carbon sequestration, biogeochemical cycling, erosion control, water flows, and quality), to the reestablishment of a local biotic community (e.g., rare species, native species, high diversity, or eradication of invasive species) (Eviner and Hawkes 2008). Landscapes having no remaining native species (e.g., areas that have been farmed, mined or sustained industrial disturbance) require more radical ecosystem reconstruction, including amending or transplanting soil, plants, and animals (Dilly et al. 2010). Ecosystem rehabilitation, compared with restoration, focuses on reinstating ecosystem functions, productivity, and services rather than particular species, but as with restoration it aims for ecosystem resilience and sustainability through reestablishing the original hydrology, energy flows, and nutrient cycling (Aronson et al. 1993; Ehrenfeld 2000; Shackelford et al. 2013). There are as of yet no standard methods for evaluating the effects of either restoration or rehabilitation (Wortley et al. 2013), but a meta-analysis of 89 restoration assessments around the globe found that, on average, biological diversity and ecosystem function were improved by 44% and 25%, respectively, and were correlated with each other (Benayas et al. 2009). In recent years the potential of ecosystem restorations to ameliorate climate change through carbon sequestration has also been emphasized, but climate change will likely also affect the long-term survival of former native species in some areas (Harris et al. 2006; Seabrook et al. 2011).

Plant–soil interactions are the key drivers necessary for realizing of any of these restoration goals. Soil conditions will constrain plant survival, growth, and productivity, as well as community composition (Pywell et al. 2003). Plant composition can also impact soil structure, function, and microbial communities (Wardle 2002; Eviner and Chapin III 2003; Wardle et al. 2004; Lange et al. 2014). Therefore, specific plants can be used as tools to alter soils (Whisenant 1999; Eviner 2004; Eisenhauer et al. 2010), but some plants can become impediments to restoration (e.g., non-native invasive species that alter soil conditions for their own benefit while

inhibiting native species) (Suding et al. 2004a; Ehrenfeld et al. 2005; Levine et al. 2006; Scharfy et al. 2010).

Although much of ecosystem restoration practice, research, and evaluation has focused primarily on plant species (Wortley et al. 2013), in this section we will briefly review various ecosystem restoration strategies with particular attention to soils and their microorganisms, along with their interactions with plants. Sites that are extremely degraded due to soil loss, compaction, or contamination generally require more soil remediation either prior to or during the restoration process. In such cases, the soil conditions may prevent many plants from growing, and other ecosystem services may be impaired due to inadequate pore space, pH balance, water holding capacity, SOM, nutrient content, and microbial activity. Depending upon the severity and history of the soil degradation, the local environmental context and the restoration goals, soil remediation strategies can range from simply modifying a single physical, chemical, or biological factor to a more integrated, multifactorial restoration management plan (Callaham et al. 2008; Heneghan et al. 2008).

7.4.1 Single-Factor Soil Restoration Methods

For economic, logistical, and practical reasons, most ecosystem restoration projects that include soil remediation have relied on modification of a single soil factor (Callaham et al. 2008). In highly degraded sites soil structure can often be improved by physical manipulations, including tillage (e.g., disking, ripping, subsoiling; Scullion and Mohammed 1991; McNabb 1994; Ashby 1997), incorporation of polyacrylamide beads (Vacher et al. 2003), activated charcoal (Glaser et al. 2002) or biochar (Lehmann et al. 2011; Beesley et al. 2011) into the soil, or topdressing (e.g., with manure; Johnson et al. 2006). Transplantation of topsoil or other organic amendments can introduce plant seeds, mycorrhizal symbionts, pathogen, and other soil microbes, as well as altering soil microenvironments and water relations; thus possible unintended effects should be considered (Heneghan et al. 2008).

Chemical amendments (organic or inorganic) are commonly used to improve nutrient status (C, N, P, or K), pH, cation exchange potential, or sources of electron donors and acceptors (Lu et al. 1997; Marrs 2002; Saquing et al. 2016), or to detoxify soils contaminated with heavy metals, PAHs, or PCBs (Cao et al. 2011; Beesley et al. 2011; Gomez-Eyles et al. 2013). Former agricultural lands with high residual levels of inorganic nitrogen from long-term fertilization may require amendments to reduce soil fertility (e.g., carbon additions in the form of biochar, compost, or straw) and permit growth of native plant species adapted to nitrogen-limited systems (Wilson and Gerry 1995; Blumenthal et al. 2003; Suding et al. 2004b). In addition to their effects on plant growth, all of these treatments may also affect bacterial and mycorrhizal biomass (Luo et al. 2013; Zhang et al. 2014), biogeochemical cycling rates (Zimmerman et al. 2011; Lehmann et al. 2011; Wang et al. 2013), and biochemical cell–cell signaling between bacteria, fungi, and plants (Warnock et al. 2007; Masiello et al. 2013). Most studies have, for example, found

that biochar amendments positively influence overall microbial biomass (Luo et al. 2013; Zhang et al. 2014), but the effects on mycorrhizal biomass and symbiosis tend to vary depending on the nutrient status of the soils (Warnock et al. 2007; Solaiman et al. 2010; Hammer et al. 2011; LeCroy et al. 2013). Long-term nitrogen enrichment in a tallgrass prairie was found to significantly decrease the soil bacterial richness and diversity, while burning and P enrichment did not (Coolon et al. 2013); therefore, reducing the N content could potentially enhance the microbial community structure. High levels of nutrients have also been found to increase the proportion of soil microbial genes related to a copiotrophic, *r*-selected lifestyle (Fierer et al. 2012), but geographical and climatic conditions may also influence microbial communities in native tallgrass prairie remnants and restorations (Fierer et al. 2013).

Biological manipulations are common during ecosystem restoration—seeding grasses and forbs (herbaceous flowering plants other than grasses) or planting trees is almost always part of a restoration plan unless either a good reservoir or seed bank of desirable plants is extant. Biological manipulations of the soil community are less common, however. It is relatively rare that soil fauna are manipulated; the introduction of earthworms to improve soil porosity, SOM, and aggregate structure has met with mixed success (Butt 2008; Zhang et al. 2015), and more research is required to understand the potential benefits of inoculating other terrestrial macroinvertebrates such as either millipedes and isopods (Snyder and Hendrix 2008) or microinvertebrates like nematodes (Heneghan et al. 2008). Although bacteria and other microbes often come along with organic amendments like composts and topsoil (Middleton and Bever 2012; Sun et al. 2014), intentional inoculation with specific bacterial species is usually limited to cases in which either N-depleted soils require nitrogen-fixing symbionts or specific toxins require bioremediation (Herrera et al. 1993). Recent metagenomic analyses of soil microbial communities are beginning to allow researchers to determine more specifically what the native soil communities were, and to provide targets for restoration soil community function and diversity (Fierer and Jackson 2006; Bates et al. 2013; Fierer et al. 2013).

Numerous restoration researchers have, however, added mycorrhizae to soils, either through additions of spores, direct inoculation of plants, or addition of mycorrhizae-containing soil from undisturbed locations. The choice to apply mycorrhizae depends upon the site conditions (Ehrenfeld and Toth 1997; Jeffries et al. 2003). Although mycorrhizae have good potential for aiding in phytoremediation of heavy metals (Gaur and Adholeya 2004), some mycorrhizae may not grow at sites heavily contaminated with heavy metals or at sites containing very low nutrients (Wardle 2002; Entry et al. 2002). On the other hand, mycorrhizae may be inhibited by high levels of nutrients such as nitrogen from fertilizers (Egerton-Warburton et al. 2007). Plants depend more on mycorrhizae when P availability is low, but mycorrhizae may offer a less predictable benefit of increased plant survivorship during drought (Richter and Stutz 2002; Allen et al. 2003; Walker et al. 2004; Augé 2004).

The indications for use of mycorrhizae also vary according to how dependent the dominant and rare plant species of the community are on these associations (Bever et al. 2001; Bever 2002). For example, if the dominant species depend on mycorrhizae, restoring ecosystem function may depend upon a healthy stock of fungi in the

soil (Richter and Stutz 2002), while reintroduction of rare species may require direct inoculation with specific mycorrhizal species for their establishment (van der Heijden et al. 1998). Likewise, inoculation may be necessary to reclaim extremely degraded sites and maximize productivity of a limited species pool under such circumstances (Frost et al. 2001; Mergulhão et al. 2010). Since restoring native mycorrhizae to degraded soils can be difficult (Cardoso and Kuyper 2006), the use of commercially prepared mycorrhizal inocula has been proposed for use in restorations. In addition to concerns about possible negative effects of non-native inocula, recent field trials have shown that transplanted and resident soil fungi often outperform commercial AMF mycorrhizal preparations in terms of plant colonization and/or growth (Paluch et al. 2013; Emam 2016), and no single commercial treatment will enhance all of the desirable native plants (Perkins and Hatfield 2016). This may, in part, be due to the importance of the whole soil community, including mycorrhizal "helper" bacteria (Bending 2007; Kurth et al. 2013). Additional research is needed to determine the best ways to promote and reinoculate these important microbial communities.

7.4.2 Integrated Restoration Management

The single-factor restoration manipulations described above result in cascading effects within the ecosystem, changing multiple physical, chemical, and biological factors as they modify the environment. An integrated restoration management plan attempts to anticipate and intentionally take advantage of these various changes, in addition to using multiple manipulations to achieve a result that closely resembles the native habitat. At the most basic level, many of the earliest restoration projects aimed at establishing vegetation of any sort, but complex plant species assemblages are now the aim of most ecosystem restorationists (Temperton et al. 2004). Improving a restoration's resistance to invasion by either weedy or non-native plant species following disturbance is another common problem that can best be addressed through an integrated management approach. Restorations of native grasslands on former agricultural land, for example, are commonly plagued by encroachment of woody species in the absence of fire and invasion of non-native weedy pioneer plants due to soil disturbance and residual fertilizers (Burke and Grime 1996; Davis et al. 2000; Krueger-Mangold et al. 2006). In addition to a regime of mowing, grazing, and burning to control woody growth, excess soil N and P nutrients can be exported and sequestered through resource removal (mowing and removal of plant biomass; (Maron and Jefferies 2001; Antonsen and Olsson 2005), burning, immobilization of nutrients in microbial biomass through the addition of carbon to the soil (Morghan and Seastedt 1999; Blumenthal et al. 2003; Averett et al. 2004; Kulmatiski 2011), and reduction in P availability by addition of gypsum (Suding et al. 2004b). These types of integrated strategies have been effective in North American prairie (Wilson and Gerry 1995; Baer et al. 2003; Suding et al. 2004b; Averett et al. 2004; Vinton

and Goergen 2006), coastal sage scrub (Cleland et al. 2013) and Australian tussock grassland restorations (Prober et al. 2005), among others.

Both positive and negative feedbacks between plants and soil microbes and conditions may also influence the outcome of an invasion. For example, the invasive C_3 grass smooth brome (*Bromus inermis*) prefers more N than the native prairie species in North America, and its N-enriched litter is decomposed more rapidly than is that of the native C_4 switchgrass (*Panicum virgatum*); therefore, smooth brome not only has a competitive edge in soils that contain elevated N from fertilization or atmospheric deposition, but it also sustains itself through a positive feedback loop of rapid N cycling (Vinton and Goergen 2006). Frequent burning to simultaneously reduce the N content of the soil and suppress invasive plants has been found to be more effective in this case than addition of C in the form of simple sugars. Activated carbon, however, has been shown to aid in suppression of diffuse knapweed (*Centaurea diffusa*) and cheatgrass (*Bromus tectorum*), possibly due to absorption of allelopathic substances produced by these invasive plants in tallgrass prairies (Kulmatiski and Beard 2006). Choosing the best combination of treatments can be complicated and should be tailored to the specific needs of the region, habitat, or soil type; therefore, pilot studies may be required to determine the optimum outcome for large-scale restorations (Griffith et al. 2001).

Thus, evaluation, monitoring, and, when appropriate, manipulation of soil quality have an important place not only in land use research but also in ecosystem restoration research and practice (Singer and Ewing 1999; Carter 2002; Karlen et al. 2003; Heneghan et al. 2008). Inexpensive soil test kits for nutrient and pH evaluation as well as low-tech SOM determinations make it possible for restoration and land management practitioners to evaluate not only the easily visible plant community but also some key indicators of soil quality. Real-time assessments of the resistance (maintenance of function) of the soil ecosystem to disturbance and the resilience of the soil in recovering from degradation can also be made using these indicators, although detecting nuanced changes in soil microbial communities requires a higher level of technical expertise and instrumentation. Not only does knowledge of ecosystem and soil ecology benefit the practice of ecosystem restoration, but well-documented restoration methods and outcomes can also help to inform ecological theory (Palmer et al. 2006).

7.5 Conclusions and Climate Change Implications

By now it is clear that the interactions of soil microbial communities with their surroundings, both biotic and abiotic, are complicated even in the absence of such human interventions as land use changes and ecosystem restorations. Although a large body of research on the local and regional scales has been developed, how these principles relate to the global scale of climate change is less well understood (Singh et al. 2011). There is an urgent need for more research to determine the possible negative and positive feedbacks between soil microbes and climate,

particularly as they relate to fluxes of the greenhouse gases CO_2, CH_4, and N_2O (Bardgett et al. 2008; Ayres et al. 2010; Singh et al. 2010). Microbes are central to regulating amounts of these gases in the atmosphere through both production (addition) and transformation (removal), and globally, soil organic matter contains three times more carbon than do either the atmosphere or all terrestrial vegetation (Schmidt et al. 2011). The possibility of positive feedbacks between elevated temperatures and terrestrial SOM decomposition rates, for example, has serious implications for the rate and extent of climate change (Davidson and Janssens 2006; Heimann and Reichstein 2008). Understanding these mechanisms and their drivers is essential to building more accurate climate models and predictions (Wall et al. 2008; Singh et al. 2010; Schmidt et al. 2011; Wallenstein and Hall 2012), as recent progress shows (Castro et al. 2010; Wieder et al. 2013). In addition, estimating the potential for climate mitigation through conservation, ecosystem restoration, and soil manipulations will likely have to be a critical component of our overall climate adaptation and amelioration strategy (Guo and Gifford 2002; Lal 2004a, b, 2014; Lal et al. 2012; Pachauri and Meyer 2014). The climate is already changing due to human-induced fossil fuel combustion and other activities, and soil microbial communities are likely changing as a result (Frey et al. 2008, 2013; Briones et al. 2009). Even if the atmosphere and temperatures were stabilized immediately, the changes already induced in soil C stocks and microbial communities would persist, and recovery would lag until new equilibria could be established, but we have little understanding of these dynamics. We now have the tools we need to get better insights into the microbial world and its biogeochemical cycling through metagenomics, stable isotope methods, and meta-analyses of large data sets. The question is, will the necessary research and pilot studies be funded in time to make a difference?

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Compliance with Ethical Standards

Conflict of Interest Vicky L. McKinley declares that she has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Chapter 8
Microbial Communities in Salt Marsh Systems and Their Responses to Anthropogenic Pollutants

Jonna M. Coombs

Abstract Salt marshes are vegetated terrestrial systems that develop along coastlines in temperate to arctic environments, in areas where surface or groundwater mixes with flooding from coastal tides. These environments perform essential ecosystem services such as storm buffering, carbon-trapping, and the protection of estuarine waters through the removal of land-based nutrients. The sediments in these environments are chemically complex, with gradients of salinity, redox, and pH that give rise to some of the most abundant and diverse microbial communities yet characterized. However, the significant loss of marsh surface area over the past 150 years has raised concerns about the stability of these microbial communities and their ability to deliver ecosystem services in the future. Many reasons have been proposed for the loss in marsh surface area, including anthropogenic pollutants, changes in predator and herbivore ecology, and global sea level rise. This chapter examines the structure of baseline microbial communities and their role in salt marsh biogeochemical cycling—and how anthropogenic land-based pollutants may negatively affect the ecosystem services provided by these microbial communities.

8.1 Introduction

Estuaries are coastal ecosystems that develop in partially enclosed land areas where freshwater and salt water meet. In these environments, mud flats that have been colonized by salt-tolerant vegetation give rise to elevated platforms known as salt marshes. The elevation of these platforms is dictated by the sea level and the rate of sediment deposition (Morris et al. 2002) as well as rates of primary productivity that result in increases in belowground plant biomass (Kirwan and Gutenspergen 2012). Salt marshes typically have very low plant diversity, and within estuary sediments natural gradients of salinity, soluble sulfide, pH, and redox potential exert selective pressure that results in the formation of distinct zones of salt marsh vegetation (Bertness 1991) (Table 8.1). Studies have shown that soil drainage and salinity

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Table 8.1 Significant physical, chemical, and biological factors influencing salt marsh sediments

Low marsh	High marsh
Low elevation	High elevation
Frequently flooded	Infrequently flooded
Tidal flooding generally results in lower salinities	Lack of tidal exchange combined with evapotranspiration generally results in higher salinities, except where local topography and hydrogeology provides a significant influx of freshwater
Stress-tolerant vegetation	Competition-tolerant vegetation
Representative plant species include: – *Spartina alternaflora* – *Spartina maritima* – *Bolboschoenus robustus*	Representative plant species include: – *Spartina patens* – *Halimione portulacoides* – *Juncus roemarianus* – *Phragmites australis*

appear to be the primary drivers of plant zonation in marshes worldwide (i.e., Bertness 1991; Pennings et al. 2005; Brownstein et al. 2013; Tabot and Adams 2013), with local freshwater hydrogeology also playing a role (Wilson et al. 2015). Life in many of these environments is limited by suboptimal levels of vital requirements such as bioavailable nitrogen and phosphorous. Plant species often compensate for these limitations by obtaining essential nutrients through symbiotic relationships with sediment rhizosphere microbes.

Salt marshes exist in temperate, boreal, and arctic ecosystems and involve a land area of approximately 3.8×10^5 km^2 (Maltby 1988). Worldwide, it is estimated that 25–50% of the land area of vegetated coastal ecosystems, i.e. salt marshes as well as mangroves and seagrass beds, has vanished in the last 50–100 years (Pendleton et al. 2012), with 1–2% of salt marsh land area continuing to be lost each year (Duarte et al. 2008b). This is of significant ecological concern, since the annual primary productivity of salt marsh ecosystems can range from 343 to 3200 grams of carbon per square meter per year (g C m^{-2} year^{-1}), ranking salt marshes among the most productive ecosystems in the world (Schubauer and Hopkinson 1984; Morris et al. 2013). Algae on the surface of the marsh sediments may contribute another 80–190 g C m^{-2} year^{-1} (Pomeroy et al. 1981), and chemoautotrophic sulfur-oxidizing bacteria may contribute an additional 275–500 g C m^{-2} year^{-1} (Howarth 1984). In organic-rich peat marshes such as those found in the northeastern United States, much of this produced carbon becomes sequestered in coastal sediments, removing it from the atmospheric carbon pool, and this carbon has informally come to be known as "blue carbon" (Nellemann et al. 2009). It has been estimated that 20–30% of the world's soil carbon is stored in salt marshes and other wetland systems (Mitsch and Gosselink 2007). In addition to carbon sequestration, salt marshes also provide essential ecosystem services such as nutrient cycling, habitat for breeding and early development of commercial finfish and shellfish, and residential protection from storms (Barbier et al. 2011). These ecosystem services have been valued at an annual rate of $14,397 per hectare (ha^{-1} year^{-1}), with 66% of these services attributed to nutrient removal and transformation (Gedan et al. 2009). Several factors have been proposed to

contribute to marsh losses, including groundwater withdrawal, dam construction, human land use developments, sea level rise, eutrophication, pollution and ecological shifts caused by species invasions, and the loss of keystone predator populations (Kirwan and Megonigal 2013; Tong et al. 2013; Bertness et al. 2014). All of these factors can act as plant stressors, affecting plant root biomass and sediment-trapping efficiency and therefore leading to loss of land elevation and marsh ecosystem collapse (reviewed in Kirwan and Megonigal 2013). Therefore, it has been proposed that circumstances influencing the growth of plants are likely to be key determinants for whether marsh areas demonstrate either resiliency or loss. Since plant productivity and sediment microbial activity are inexorably linked, it is necessary to develop a greater understanding of the impact of anthropogenic pollution on the physiology and ecology of salt marsh sediment microbes.

The purpose of this review is to examine microbial community structure and processes in salt marsh sediments and the positive and negative feedbacks that these can have on salt marsh resiliency.

8.2 Microbially Mediated Biogeochemical Processes in Salt Marsh Sediments

Many of the ecosystem services provided by salt marsh environments are a direct result of the activities of microbes in biogeochemical nutrient cycling. An in-depth discussion of each of these processes is beyond the scope of this review. However, a brief overview of these processes in salt marshes is provided as background prior to discussing anthropogenic effects on microbial activity.

8.2.1 Salt Marsh Nitrogen Cycling

Nitrogen is an essential macronutrient for living organisms and can be found in the environment in multiple oxidation states, ranging from N^{5+} to N^{3-}. The global biogeochemical nitrogen cycle involves several microbially mediated transformations that shift the oxidative state of nitrogen. Assimilatory processes in the cycle such as assimilation and assimilatory nitrate reduction to ammonia are important pathways for the biosynthesis, incorporating nitrogen into essential biological macromolecules such as protein and DNA, while mineralization removes nitrogen from these compounds. Dissimilatory processes, including dissimilatory nitrate reduction (also known as denitrification), dissimilatory nitrate reduction to ammonia (DNRA), anaerobic ammonia oxidation (anammox), and nitrification, all allow nitrogen to act as an electron donor or acceptor for the purposes of cellular energy generation (Maia and Moura 2014). Dissimilatory nitrate reduction can also provide reducing energy

that allows cells to maintain redox balance (Morozkina and Zvyagilskaya 2007). A model of the nitrogen cycle is shown in Fig. 8.1.

Nitrogen can naturally enter salt marsh systems through a variety of mechanisms, such as the deposition of terrestrial or marine organic matter (i.e., marine wrack or bird feces), dissolved organic nitrogen originating from freshwater sources (i.e., groundwater and surface waters), atmospheric deposition, and biological nitrogen fixation. Nitrogen cycling in salt marshes is strongly tied to other biogeochemical cycles, particularly the carbon and sulfur cycles. In salt marshes that have not been impacted by anthropogenic nutrient loading, nitrogen is primarily found in sediment pore water in the form of ammonia (DeLaune et al. 1983; Cartaxana et al. 1999), while total soil nitrogen is mainly in the form of organic nitrogen (Buresh et al. 1980; Cartaxana et al. 1999).

Microbial processes that contribute to the formation of ammonia (NH_4^+) in salt marsh sediments include nitrogen fixation ($N_2 \rightarrow NH_4^+$), assimilatory reduction of nitrate to ammonium (also known as assimilatory ammonification) ($NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$), dissimilatory reduction of nitrate to ammonium (DNRA) ($NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$), and mineralization of organic nitrogen ($-NH_2 \rightarrow NH_4^+$) (Fig. 8.1). Since the nitrogenase complex that transforms atmospheric nitrogen (N_2 gas) into NH_4^+ during nitrogen fixation is sensitive to oxygen exposure, nitrogen fixation typically occurs either in naturally anaerobic environments or is compartmentalized within living cells to protect nitrogenase from oxygen, (i.e., in cyanobacterial heterocysts). In natural salt marsh environments of coastal North America and Europe, nitrogen is often a limiting nutrient

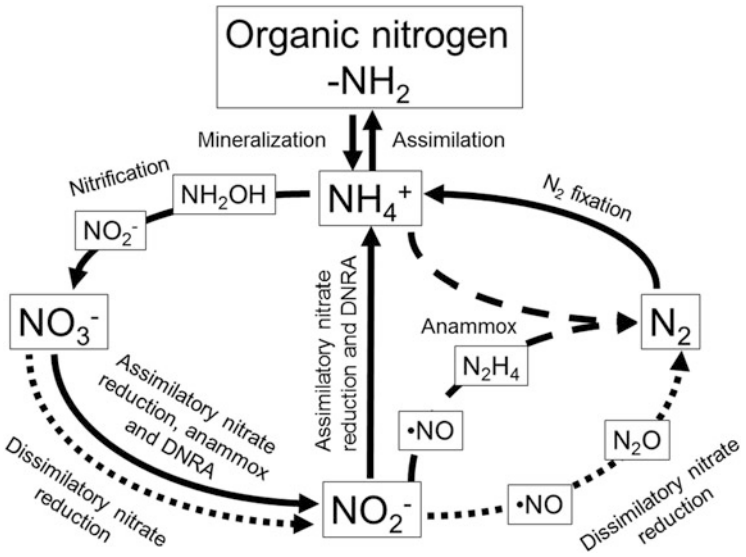

Fig. 8.1 Common microbially mediated pathways of the nitrogen cycle. Dotted lines represent the pathway for denitrification via dissimilatory nitrate reduction. Dashed lines represent the two parts of the anammox pathway. Adapted from Maia and Moura (2014) and Morozkina and Zvyagilskaya (2007)

(Valiela and Teal 1974; Kiehl et al. 1997; Rozema et al. 2000), and nitrogen fixation is generally carried out by diazotrophs that either may be plant root-associated or freely living in soil and sediment. Diazotrophs are supported in part by root exudates produced by salt marsh plants, and stimulation of photosynthesis in the smooth cordgrass *Spartina alterniflora* through the addition of CO_2 has been shown to increase bacterial nitrogen fixation rates 5–6× (Whiting et al. 1986). Mineralization of nitrogen to ammonia occurs during the breakdown of organic matter and is carried out by a wide variety of heterotrophic salt marsh bacteria in both oxic and anoxic microenvironments. Mineralization rates are highest at the surface of soil and sediment and decrease with depth due to declining organic N quality (Hopkinson and Giblin 2008). Rates for organic nitrogen mineralization to ammonia within salt marshes are site-dependent and can range from 15 to 84 g N m^{-2} $year^{-1}$ (Anderson et al. 1997; Hopkinson and Giblin 2008).

The conversion of ammonia to other forms of nitrogen is accomplished through the processes of assimilation, nitrification, and anaerobic ammonia oxidation (anammox). Nitrogen assimilation involves the incorporation of NH_4^+ into amino acids and other nitrogen-containing compounds. Nitrification takes place when NH_4^+ is converted to NO_2^- and then to NO_3^-, with each of these steps requiring two distinct groups of aerobic chemoheterotrophic bacteria. In the first step, ammonia is used as an energy source, with O_2 acting as the electron acceptor and some of the energy being used to fix CO_2. The ammonia-oxidizing bacteria that perform this reaction mainly belong to the *Gamma-* and *Betaproteobacteria*. The betaproteobacterial genera *Nitrosomonas* and *Nitrosospira* have been shown to be active in estuary sediments, with *Nitrosomonas* dominating in freshwater and *Nitrosospira* increasing in prevalence with a transition to more brackish water (Bernhard et al. 2005; Freitag et al. 2006; Ward et al. 2007). The existence of ammonia-oxidizing archaea that are able to catalyze the conversion of ammonia to nitrite has also been documented (Treusch et al. 2005; Könneke et al. 2005), and these organisms have been found to play an important role in nitrogen cycling in salt marsh sediments (Bernhard and Bollmann 2010; Bernhard et al. 2015). The second step of nitrification, the conversion of nitrite to nitrate, can be carried out by many bacterial genera, with *Nitrobacter* serving as the best-characterized representative of this functional group. Some Archaea are also able to carry out this reaction (Könneke et al. 2005; Hatzenpichler et al. 2008). Nitrification rates are controlled by O_2 and NH_4^+ availability, but salinity also appears to be important and sulfide levels significantly impact nitrification (Seitzinger et al. 1991; Joye and Hollibaugh 1995). Lastly, NO_2^- can be used to oxidize ammonia and convert it directly into N_2 gas without the formation of nitrate via the anammox reaction. Currently only four candidate taxonomic groups within the *Planctomycetes* are known to carry out this reaction, with the genus *Scalindula* (Schmid et al. 2007; Dale et al. 2009) predominating at high salinities.

One of the most important ecosystem services provided by salt marshes is the conversion of nitrate to other forms of nitrogen. This can occur through assimilatory reduction of nitrate to ammonia and also through dissimilatory processes such as dissimilatory nitrate reduction (denitrification), anammox (utilizing NO_2^- as an intermediate), and dissimilatory nitrate reduction to ammonia (DNRA) (Fig. 8.1). Dissimilatory nitrate reduction (denitrification) reduces nitrate to nitrite to nitric oxide (NO) to

nitrous oxide (N_2O) and ultimately to N_2 in anaerobic environments. Salt marshes support some of the highest rates of denitrification measured (Hopkinson and Giblin 2008). Some of the NO and N_2O produced during these processes may escape into the atmosphere, acting as greenhouse gases. Genes encoding catalysts involved in this pathway are widespread in bacteria, particularly in anaerobic or facultatively anaerobic heterotrophs where nitrogen reduction is tied to organic carbon utilization, although removal of nitrate can also occur via utilization of H_2, H_2S, or Fe (II) as electron donors. In salt marsh environments, removal of nitrate from groundwater is more efficient than removal of nitrate from surface waters (either from runoff or tidal influx) due to more effective delivery of the nitrate dissolved in groundwater to vegetated areas at the center of marshes (Teal and Howes 2000). The process of DNRA for nitrate removal is favored over dissimilatory nitrate reduction under strongly reducing conditions in anoxic sediments (An and Gardiner 2002). This process may be coupled with fermentative pathways or the utilization of H_2, H_2S, or Fe (II) as an electron donor (Burgin and Hamilton 2007). Sulfide, which inhibits coupled nitrification/denitrification, has been shown to be a controlling factor for DNRA (An and Gardiner 2002).

8.2.2 Salt Marsh Sediment Carbon Cycling

The mechanism by which carbon enters salt marsh systems depends on the characteristics of the marsh sediments. In mineral marshes, where mineral sediment inputs determine accretion rates, such as in the southeastern United States and in northwestern Europe, there tends to be a high input of allochthonous carbon generated from sources such as sediment-bound organic material entering from freshwater sources and marine phytoplankton entering from marine environments, while in peaty, sediment-starved marshes such as those found in the northeastern United States, carbon tends to be generated autochthonously due to on-site primary production (Middleburg et al. 1997). Mineralization of autochthonous or allochthonous organic carbon can occur aerobically or anaerobically, through the activities of sediment bacteria. Heterotrophic microbes that can utilize aerobic respiration for the breakdown of organic materials have been identified on the surface of standing plant biomass and on submerged leaf litter, with both biomass and heterotrophic activity greater on standing material (Kuehn et al. 2000). Consistent with this observation, Lowe and Dichristina (2000) found high densities of aerobic heterotrophs in salt marsh creek bank sediments, where bioturbation by benthic macrofauna helped oxidize the sediments, while culturable aerobic heterotrophs were absent from the more reduced mid-marsh areas, which were dominated instead by high levels of cultivatable anaerobic heterotrophs such as Fe (III)-reducing bacteria (FeRB) and sulfate-reducing bacteria (SRB). Microbes can contribute considerably to the pool of organic matter that is available for respiration in salt marsh systems. Tremblay and Benner (2006) examined highly decomposed submerged detritus and found that 20–40% of the associated carbon and 60–75% of the associated nitrogen were of bacterial origin. Marsh plant biomass, which is also a source of salt marsh organic

matter, is composed of a large part of lignocellulose. This cross-linked complex of cellulose, hemicellulose, and lignin has been shown to be recalcitrant to degradation; therefore, bacterial utilization of this source of autochthonously produced organic can proceed at very slow rates (Van Dyk and Pletschke 2012; Deng and Wang 2016). Salt marsh detritivores positively affect microbial respiration rates in a species-dependent and plant leaf litter-dependent fashion, directly affecting soil carbon and nitrogen ratios (Zimmer et al. 2004). In particular, phenolics and tannins produced by some plant species appear to be completely degraded, while phenolics and tannins of other plant species appear completely recalcitrant to degradation (Zimmer et al. 2004). Culture-dependent as well as culture-independent studies have identified microbial populations in marsh sediments with the capacity to utilize plant-produced lignocellulose (Darjany et al. 2014; Deng and Wang 2016).

Acetogenesis involves the fixation of atmospheric CO_2 to produce acetate via the Wood-Ljungdahl pathway. Acetogenesis has long been known to occur in salt marsh sediments, and studies have utilized primers for the gene encoding formyltetrahydrofolate synthetase (FTHFS), a key enzyme in the acetyl-CoA pathway for the conversion of C1 compounds to acetate, to detect acetogens in salt marsh sediments (Leaphart et al. 2003). Previously it was thought that methanogens and sulfate-reducing bacteria were energetically favored over acetogens in marine environments; however, recently Lever (2011) has made the case that metabolic diversity and the reduced energetic cost of biosynthesis may enable acetogens to coexist with other taxa. The products produced by acetogens, acetate, H_2, and CO_2, can be used as substrates for other organisms such as sulfate-reducing bacteria and methanogens.

Methanogenesis generally is defined as a reduction of carbon dioxide into methane in the presence of hydrogen. Methanogenesis rates are often low in marsh sediments, producing fluxes of methane that vary from 0.10 to 40 g C m^{-2} $year^{-1}$ (King and Wiebe 1978; Adams et al. 2012), with higher rates in vegetated vs. unvegetated sediments and the highest rates occurring in the most waterlogged soils with the highest salinities. Most methanogens are hydrogenotrophic, fixing carbon dioxide and using hydrogen, formate, or acetate as an electron source (Zeleke et al. 2013). However, members of the genus *Methanosarcina* are metabolically versatile and have the ability to use acetate as well as methylated compounds (methanol, monomethylamine, dimethylamine, and trimethylamine). Methanogens use the acetate and the methylated compounds as either a source for methane or electron donors for the process of methanogenesis (Liu and Whitman 2008). Methane fluxes are inversely correlated with sulfate concentrations, and at high sulfate concentrations, community structure shifts to favor methylotrophic organisms (Smith et al. 2008)

8.2.3 Salt Marsh Sediment Sulfur Cycling

Sulfate reduction rates mediated by sulfate-reducing bacteria (SRB) are high in salt marsh sediments, ranging from 6 to 75 mol S m^{-2} $year^{-1}$ (Giblin and Wieder 1992), and correlate with salt marsh plant primary productivity (Hines et al. 1999). Since

sulfate is generally not limiting in salt marsh environments, sulfate reduction in these environments depends on the availability of organic carbon. Sulfate reduction has been estimated to account for 67–80% of all marsh sediment respiration (Howarth and Teal 1979; Howarth and Giblin 1983) and may comprise up to 90% of the total depth-integrated rate of organic carbon oxidation (Howarth and Hobbie 1982). Molecular studies indicate that a majority of the deltaproteobacterial SRB found in marsh sediments are related to organisms known to be complete oxidizers of organic compounds, and these sediment microbes are likely to be nutritionally versatile (Klepac-Ceraj et al. 2004). Sulfate-reducing microbes are generally strict anaerobes, although some may be aerotolerant. These organisms can reduce sulfate to sulfide using several substrates such as hydrogen, formate, acetate, butyrate, propionate, and ethanol as electron sources. They are found in several phylogenetic groups, including the *Deltaproteobacteria* and the *Firmicutes*, as well as phylum *Nitrospirae*, phylum *Thermodesulfobacteria*, and in some archaeal genera (Zeleke et al. 2013)

The sulfide produced by sulfate reduction in salt marshes is a suitable substrate for subsequent sulfur oxidation. A variety of chemoautotrophic or facultative chemoautotrophic bacteria can capture the energy of sulfur oxidations using oxygen or nitrate as an oxidant and using the energy to fix carbon dioxide and produce labile organic compounds (Thomas et al. 2014). Two main pathways for sulfur oxidation exist—the SOX pathway, where reduced sulfur compounds are completely oxidized to sulfate (Kelly et al. 1997; Friedrich et al. 2001), and the branched thiosulfate oxidation pathway, where sulfur intermediates are sequentially oxidized to sulfite and sulfate through the activity of several enzymes including reverse-acting dissimilatory sulfite reductase (rDSR) (Pott and Dahl 1998; Kappler and Dahl 2001). Expression of marker genes for both pathways has been detected in *Spartina alterniflora*-dominated salt marshes in the northeastern United States, with the gammaproteobacterial orders *Chromatiales* and *Thiotrichales* dominating (Thomas et al. 2014).

8.2.4 Salt Marsh Sediment Iron and Manganese Cycling

In salt marsh sediments, iron (III) is often abiotically reduced by hydrogen sulfide (Luther et al. 1992); therefore, the activities of iron reducers have generally been believed to be low in salt marsh sediments. Like iron reduction, reduction of manganese (IV) was originally believed to occur at very low rates, if at all, in the mainly anaerobic environment of salt marsh sediments (Keith-Roach et al. 2002). In addition, the solubility of both Fe and Mn increases as they are converted to their +2 state, resulting in the dissolution of Fe/Mn precipitates (Kerner 1993; Lovley 1997). However, recent work has shown that microbially mediated reduction of both metals may occur in sediments, either directly through anaerobic respiration or indirectly through microbial alteration of the redox potential. Lowe and Dichristina (2000) used cultivation and molecular techniques to show the presence of iron-reducing bacteria, particularly in the 0–2 cm layer of sediment where amorphous Fe (III)

oxyhydrides were available. Roots of short-form *S. alterniflora* (King and Garey 1999) and tall-form *S. alterniflora* (Kostka et al. 2002) have both been shown to support iron reduction. This activity has been attributed to specialist iron-reducing bacteria, likely supported by active oxygenation of the sediments by *Spartina* and bioturbation by invertebrates which serve to increase the availability of oxygen and therefore Fe (III) in deeper sediments (Kostka et al. 2002). Koretsky et al. (2005) performed a follow-up study at the same site and found that iron oxidizers, manganese oxidizers, and sulfate reducers coexisted in the top 10 cm of the bioturbated *S. alterniflora*-vegetated sediments without a clear vertical separation. A study performed in the United Kingdom showed that a marsh vegetated with the halophytes *Halimione portulacoides*, *Puccinellia maritima*, *Suaeda maritima*, and *Salicornia* spp. also supported anaerobic respiration of Mn (IV) (Keith-Roach et al. 2002). Sediment oxygen measurements obtained during that same study indicated sediments of this marsh remained mildly oxygenated throughout the year. Likewise, Lin and Taillefert (2014) demonstrated the reduction of manganese oxides coupled to oxidation of ammonia in salt marsh sediments, indicating the occurrence of Mn (IV)-catalyzed anaerobic nitrification. Together, these studies indicate that iron and manganese reduction may play a much greater role in salt marsh sediment nutrient cycling at some sites than previously thought.

8.3 Baseline Microbial Community Structure

The rhizosphere of salt marsh environments and the layers of sediment beneath the rhizosphere are home to complex communities of microorganisms. In general, these communities are expected to change with depth, consistent with redox chemistry changes that determine favorable electron acceptors for microbial respiration. The slow rate of oxygen diffusion into waterlogged sediments can result in anoxia within a few millimeters of the sediment surface (Piceno et al. 1999), although during the normal tidal cycle pore water may drain into adjacent tidal creeks or be lost due to evapotranspiration resulting in an increase in increasing oxygen flux into sediment pore spaces (Howarth 1993; Agosta 1985). It should be noted that in the sediment root zones, the impacts of plants and macrofauna have been shown to produce heterogeneous microenvironments rather than distinct chemical layers in sediments. Many rhizosphere sediments are actively ventilated by salt marsh vegetation such as *Spartina* spp., which have specialized tissues known as aerenchyma which transport oxygen produced via photosynthesis through the plant to be released into the sediments (Teal and Kanwisher 1961). This oxygen has the chemical effect of oxidizing toxic hydrogen sulfide which is produced in the anoxic sediments by sulfate-reducing bacteria (Sundby et al. 1998). In addition, sediment burrowing activity (bioturbation) carried out by macrofauna such as polychaetes, bivalves, and crustaceans provides a mechanism for sediment irrigation and gas exchange (reviewed in Kristensen and Kostka 2005). Bioturbation combined with the active ventilation of the sediments by plants stimulates the growth of aerobic bacteria at

sediment depths that would be completely anoxic in the absence of vegetation and allows the development of complex communities of organisms utilizing a variety of electron acceptors. In these chemically complex sediment microenvironments, heterotrophic aerobic bacteria, oxygen-tolerant species, and strict anaerobes can exist in close proximity.

There are conflicting views about what drives the structure of microbial communities in marsh sediments. Many studies indicate that community structure is closely associated with environmental variables, with sites similar in environmental conditions having similar bacterial communities (Horner-Devine et al. 2004; Hewson et al. 2007). However, an examination of salt marsh pore water chemistry demonstrated no correlate between chemical gradients and microbial community structure in the top 10 cm of sediment (Koretsky et al. 2005). Dominant species of marsh vegetation have been shown to have an effect on sediment microbial communities in some studies (Blum et al. 2004) but not all (Horner-Devine et al. 2004). High levels of labile organic compounds are suspected of reducing competition between microbes and contributing to microbial diversity (Koretsky et al. 2005). A study by Franklin et al. (2002) examined microbes in salt marsh creek bank sediments at differing elevations and found that bacterial abundance was correlated in space with local environmental factors; however, these researchers did not find a clear relationship to community structure. The authors proposed that this difference was due to environmental heterogeneity.

Because redox and the availability of nutrients are affected by sediment depth, this chapter discusses baseline community structure in the context of three main sections: the surface layer (depth of 0–2 cm), the rhizosphere (depth of 2 cm to approximately 25 cm), and below the rhizosphere (depth of below 25 cm). There is some overlap of functional taxa among the sediment layers described in the following subsections. For the purposes of simplicity, each subsection describes the dominant microbial taxa and functional assemblages (see also Figs. 8.2 and 8.3).

8.3.1 Community Structure of the Marsh Surface (The Uppermost 2 cm of the Sediment)

The surface layers of marsh sediment receive organic compounds from leaf litter of the dominant marsh vegetation. Microbial community structure in this layer (defined as 0–2 cm depth), as well as the deeper rhizosphere zones, tends to be seasonal, with specific taxa dominating at different times of the year, consistent with changes in aboveground biomass and seasonally varying environmental conditions such as temperature (Burke et al. 2002b; Koretsky et al. 2005; Bañeras et al. 2012). There is limited fungal decomposition activity at the sediment surface due to intolerance of fungi to anaerobic environments caused by periodic flooding, although fungi are commonly isolated from non-immersed portions of standing *Spartina* spp. (Calado and Barata 2012; Calaldo et al. 2015). Decomposition of plant biomass in

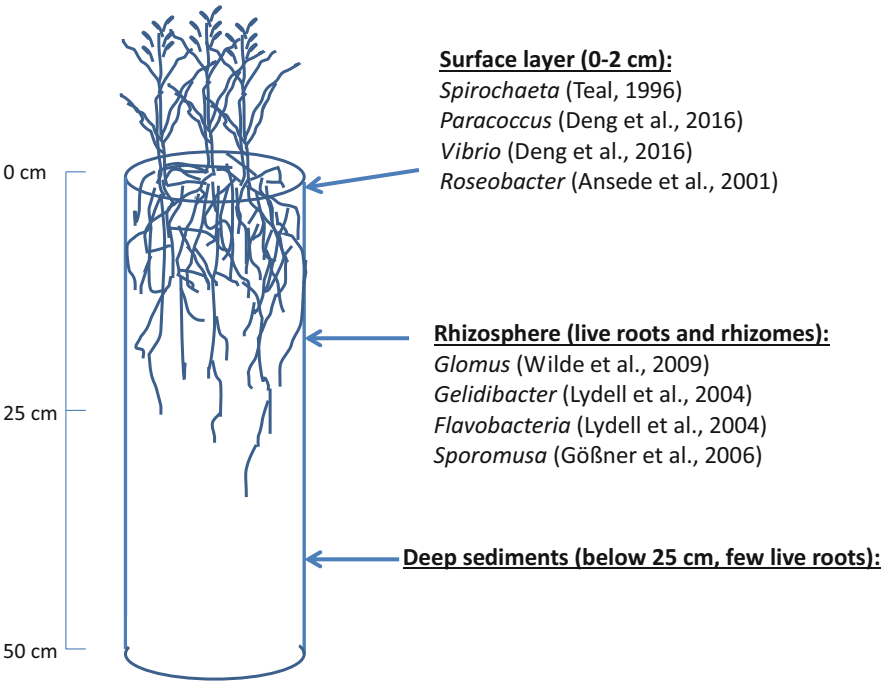

Fig. 8.2 A representation of a salt marsh sediment core, with some select cultivated or microscopically observed genera detected at specific depths in marsh sediments

the sediments is driven primarily by microbial respiration, with rates that appear to be influenced by both the plant species producing the detritus and prior processing of the litter by specific detritivore species (Zimmer et al. 2004; Treplin et al. 2013).

A significant portion of salt marsh plant detritus is composed of lignocellulose, and Darjany et al. (2014) examined 1-cm-deep sediment cores from a California salt marsh using stable isotope probing to identify lignocellulose-responsive microbes. Bacterial groups that were most responsive to additions of carbon-13-labeled lignocellulose included the complex organic substrate utilizing *Desulfosarcina*, as well as gram-negative *Kangiella* and anaerobic *Spirochaeta*. Spirochetes have been detected in other salt marsh studies (i.e., Teal et al. 1996), including a study by Weber and Greenberg (1981) that found higher numbers of spirochetes were present in the top 1–2 cm of sediments as compared to 3–5 cm in a Massachusetts salt marsh. Darjany et al. (2014) also detected significant numbers of *Sedimentibacter* (of the *Flavobacteriacea*) in both control sediments and those sediments which had received lignocellulose addition treatments, but nucleotide sequence analysis for presence of these organisms revealed the *Sedimentibacter* to be twofold more abundant in the treated samples, indicating enrichment of the organism in response to the addition of lignocellulose (Darjany et al. 2014). Another study using pure-culture techniques identified nine types of lignocellulolytic salt marsh bacteria

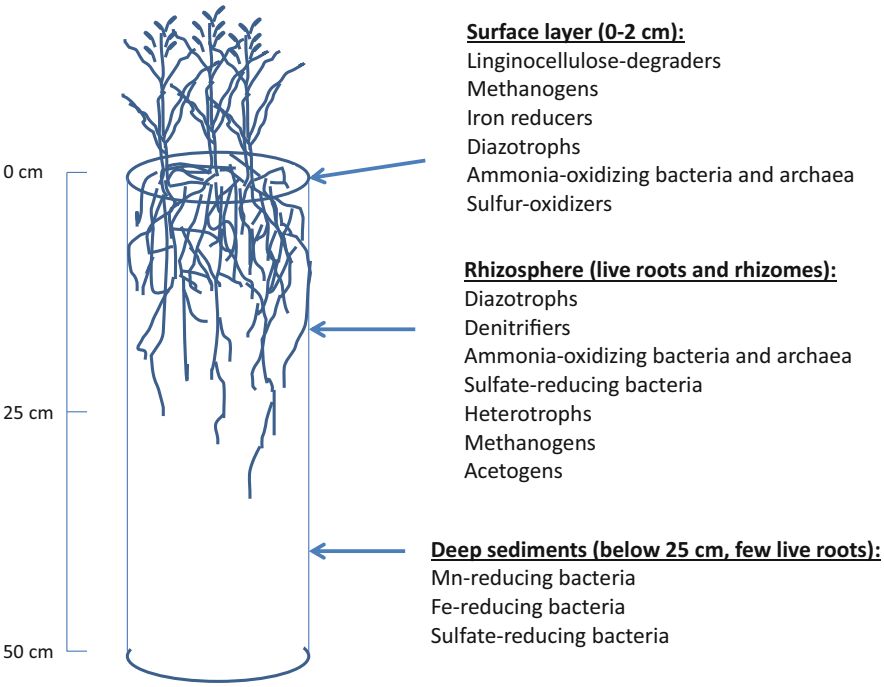

Fig. 8.3 A representation of a salt marsh sediment core, with some microbial functional groups detected using either pure-culture or molecular techniques at specific depths in marsh sediment

growing on the surface of fallen salt marsh detritus, eight of which belonged to either the *Alpha-* (*Labrenzia* sp. and *Paracoccus* sp.) or *Gammaproteobacteria* (*Vibrio* spp., *Gallaecimonas* sp., *Hahella* sp., and two isolates belonging to the families *Vibrionaceae* and *Alteromonadaceae*) with the ninth belonging to the *Actinobacteria* (Deng and Wang 2016). The greatest rates of lignocellulose degradation in subsequent physiologic testing occurred when consortia of three different organisms were co-cultured in lignocellulose medium (Deng and Wang 2016).

Other studies have used pure-culture techniques to examine the prevalence of specific microbial groups at the surface of salt marsh sediments. Jones and Paynter (1980) examined culturable methanogens in marsh sediments, detecting the highest numbers at the 0–2 cm depth compared to 5–36 cm depth in sediments vegetated with tall-form *Spartina alterniflora* at a marsh in Georgia; however, samples from a South Carolina marsh showed no difference in culturable methanogen abundance at depths from 0 to 22 cm (Jones and Paynter 1980). Lowe and Dichristina (2000) found high numbers of culturable iron reducers in the top 2 cm of sediment in the same Georgia salt marsh, associated with a high concentration of amorphous Fe (III) oxides. This same study also examined culturable SRB in surface sediment samples and found that they were at or below the detection limit. Dicker and Smith (1980) examined culturable diazotrophs in the top 1 cm of sediment from a salt marsh in the

northeastern United States, detecting putative *Azotobacter*, *Desulfovibrio*, and *Clostridium* spp., with cyst-forming cells identified as *Azotobacter* being the most prevalent diazotroph based on plate count methodology.

Archaea have also been detected in the surface layer of salt marsh sediments. Oliveira et al. (2012) used fluorescence in situ hybridization (FISH) to examine sediments vegetated by *Halimione portulacoides* and *Spartina maritima*, finding that 25% of the prokaryotic population was comprised of archaeal cells. Another study comparing sediments at three sites vegetated by short-form *S. alterniflora*, tall-form *S. alterniflora*, or *S. patens* detected both *Euryarchaeota* and *Crenarchaeota* 16S rRNA sequences in all three sites, with *Crenarchaeota* sequences—particularly sequences similar to the aerobic ammonia-oxidizing "Candidatus Nitrosopumilus maritimus"—predominating. Rarefaction analysis indicated that there was additional archaeal diversity that was not captured by the sampling techniques used in the study (Nelson et al. 2009).

Bowen et al. (2012) used pyrosequencing of the 16S rRNA gene to examine 1-cm-deep sediment cores from a stand of tall-form *Spartina alterniflora* from a marsh in the northeastern United States and found surprising diversity in the microbial community which was distinct from the community structure of samples taken from a nearby pond. Those researchers found representatives of 41 different bacterial phyla in the sediments of the tall-form *Spartina alterniflora* stand. The dominant group detected in the study was *Proteobacteria* (~61%), followed by *Bacteroidetes* (~9.4%), *Acidobacteria* (~7%), *Planctomycetes* (4.6%), *Verrucomicrobia* (~4.4%), *Chloroflexi* (3.2%), and *Gemmatimonadetes* (~2.9%). These findings relating to some of the dominant bacterial groups are consistent with pure-culture work that has detected *Alpha-* and *Gammaproteobacteria* (Ansede et al. 2001) in salt marsh surface samples and the fact that *Firmicutes* and *Bacteroidetes* have been detected in mixed surface and rhizosphere samples (approximately 0–7 cm; Bharathkumar et al. 2008; Lydell et al. 2004).

The pyrosequencing study performed by Bowen et al. (2012) demonstrated that *Proteobacteria* comprised a majority of the sequences (approximately 61%) obtained from the tall-form *S. alterniflora* surface sediments examined, with 39 of the 47 recognized orders of *Proteobacteria* detected. The bacterial orders most abundant in these surface sediment samples were *Rhodobacteriales* (12%), *Myxococcales* (13%), and *Xanthomonadales* (14%) with an additional 10% that were described as unidentified *Deltaproteobacteria*. The remaining sequences detected in that study were from rare taxa present at less than 1% of the total abundance. Rarefaction analysis of the pyrosequencing data indicated that the sediment sequencing coverage was not complete and that there was remaining diversity in the sediment that had not been sampled. Using 3% taxonomic clustering, sediment samples contained twice as many observed operational taxonomic units (OTUs) as the nearby salt marsh pond water column (Bowen et al. 2012).

Activity rates for microbial pathways at the sediment surface have been assayed in a number of studies. A majority of the nitrogen fixation occurring in the top 2 cm of sediment within a *Spartina maritima*-dominated marsh site in the United Kingdom was found to be associated with phototrophic bacteria such as cyanobacteria,

with rates of nitrogen fixation being affected in locations where shading by marsh plants was heavy (Aziz and Nedwell 1986). Gandy and Yoch (1988) measured nitrogen fixation in a *Spartina alterniflora*-dominated marsh in South Carolina and found that in the top 5 cm of sediment, 70% of the activity was inhibited by molybdate and therefore could be attributed to sulfate-reducing bacteria. Evidence from extracellular enzyme assays of nitrification and nitrate reductase shows the greatest levels of activity in the 0–2 cm sediment layers (Costa et al. 2007), indicating that nitrogen-cycling organisms may be most active at the sediment surface. Activity of β-glucosidase, cellulase, and alkaline phosphatase measured in the sediments of three *Spartina maritima*-dominated marshes in Spain was likewise highest in this 0–2 cm range. Urbanized and industrial areas in this study had the highest levels of microbial activity, associated with the relatively higher levels of available organic carbon in the sediments at these sites (Costa et al. 2007).

Functional gene analysis of salt marsh surface sediments has been performed for select microbial populations. Some genes that are commonly targeted for functional analysis encode proteins involved in nitrogen and sulfur cycling. These include *nirS* or *nirK* (encoding functionally equivalent but structurally distinct nitrate reductases for the process of denitrification), *amoA* and *amoB* (encoding two of the three subunits of the ammonia monooxygenase enzyme used to convert ammonia to nitrite), soxB (encoding part of a multi-enzyme complex which generates sulfate from reduced sulfur compounds), and *rdsrAB* (encoding reverse-acting dissimilatory sulfite reductase, which enables cells to oxidize intracellularly stored sulfur). Bernhard et al. (2015) examined functional genes of denitrifiers (*nirS*) as well as the *amoA* gene of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB), finding significant differences between samples from the top 2 cm of sediment in a New England salt marsh as compared to samples collected at the 6–8 cm depth using TRFLP (terminal restriction fragment length polymorphism) analysis. Thomas et al. (2014) examined sulfur oxidation (*soxB* and *rdsrAB*) in a different New England marsh and found highest levels of functional gene transcripts in the upper 5 cm of the sediment.

8.3.2 Community Structure of Rhizosphere Sediment (Here Defined as Depth 2 cm to 25 cm)

The microbial community structure of the rhizosphere is by far the best studied microbial environment of salt marsh sediments. Microbial activity in *Spartina*-dominated marshes has been found to be directly correlated with plant growth, achieving the highest levels during the plant growth season (e.g., Piceno et al. 1999), when most of the labile organic compounds produced by the plants are entering the rhizosphere. In fact, several studies have shown seasonality of specific rhizosphere taxonomic groups (Table 8.2). Culturable aerobic bacteria from 0 to 10 cm depth in a *Spartina*-dominated marsh in the southeastern United States were

Table 8.2 Response of salt marsh microbial communities to seasonal effects

Taxonomic or functional group	Seasonality detected	Method of analysis	Location of marsh	Reference
Aerobic bacteria	Yes	Culture	Sapelo Island, GA	Koretsky et al. (2005)
Proteobacteria	*Alphaproteobacteria*, yes; *Gammaproteobacteria*, weak; Sulfate-reducing bacteria, weak; *Betaproteobacteria*, no	DNA hybridization	Piermont Marsh, NY	Burke et al. (2002b)
Sulfate-reducing bacteria	Bulk sediment, no; root-associated, yes	RNA hybridization	Chapmans Marsh, NH	Hines et al. (1999)
Manganese-reducing bacteria	Yes, higher abundance in summer	Culture	Sapelo Island, GA	Koretsky et al. (2005)
Iron-reducing bacteria	Yes, higher abundance in winter	Culture	Sapelo Island, GA	Koretsky et al. (2005)
Diazotrophs	Yes	DGGE with *nifH*	North Inlet estuary, SC	Gamble et al. (2010)
Diazotrophs	Yes	DGGE with *nifH*	North Inlet estuary, SC	Davis et al. (2011)
Diazotrophs	No	RFLP	Piermont Marsh, NY	Burke et al. (2002b)

shown to be present at higher detectable CFUs in the summer, with a decrease in the winter (Koretsky et al. 2005). Burke et al. (2002b) detected seasonal variation in the *Spartina patens* and *Phragmites* rhizosphere community using in situ hybridization probes. At the 1.5–3.5 cm depth, *Alphaproteobacteria* showed a high degree of seasonality, while *Gammaproteobacteria* and SRB were weakly seasonal and *Betaproteobacteria* showed no seasonality. Hines et al. (1999) detected little seasonality of SRB from bulk sediment; however, *Spartina alterniflora* root-associated SRB abundance was highly seasonal. Culturable manganese-reducing and iron-reducing bacteria have also demonstrated seasonal patterns, with manganese reducers present at higher levels in the summer than in the winter and iron reducers showing the opposite trend with higher levels in the winter (Koretsky et al. 2005). Salt marsh diazotrophic bacterial populations and activity have been shown to demonstrate strong seasonality in the southeastern United States (Gamble et al. 2010; Davis et al. 2011), although studies performed in the northeastern United States do not show the same seasonal trend for nitrogen-fixing microbes (Burke et al. 2002b), indicating that climate conditions may play a role.

Root-associated (rhizoplane and endophytic) microbial communities appear to differ from each other and also differ from non-root-associated rhizosphere communities. Su et al. (2016) examined endophytic, rhizoplane, and rhizosphere and non-rhizosphere bacteria of greenhouse-grown *Spartina alterniflora* from the Jiulong Estuary in China using 16S Illumina sequencing. Each of the four sample types in this study was dominated by four major phyla: *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Chloroflexi.* However, principal coordinate analysis revealed strong separation of endophytic, rhizoplane, and rhizosphere organisms, and sediment sequences showed higher overall diversity. These findings are similar to an earlier metagenomic study using field-collected samples from the same estuary (Hong et al. 2015). Both studies detected higher levels of *Gammaproteobacteria* associated with the rhizoplane than with other environments, higher levels of *Spirochetes* and *Bacteroidetes* associated with the endophyte samples, and the highest levels of *Deltaproteobacteria* and *Chloroflexi* in the sediments (Hong et al. 2015; Su et al. 2016). Additionally, Hong et al. (2015) found *Cyanobacteria* were significantly enriched in the endophyte community compared to other environments, which was not detected in the greenhouse-grown *Spartina* (Su et al. 2016).

Dominant vegetation in salt marshes appears to have an effect on microbial abundance and diversity in rhizosphere sediments. In one study, direct counts detected double the number of microbial cells in *Spartina*-vegetated sediments than for *Phragmites*-colonized sediments (Burke et al. 2002b). A comparison of PLFA (phospholipid fatty acid) profiles from *Salicornia brachiate*, *Aeluropus lagopoides*, and *Suaeda maritima* in India revealed differences in rhizosphere community composition, with molar % PLFA signatures for gram-positive, gram-negative, and actinomycetes significantly higher in *Suaeda* and *Aeluropus* rhizosphere samples compared to *Salicornia* (Chaudhury et al. 2015). *Salicornia* soils also contained lower concentrations of microbial genomic DNA and had lower abundances of genes for rubisco and nitrogenase as determined by qPCR (Chaudhury et al. 2015). PLFA analysis of microbes associated with greenhouse-grown *Spartina* and *Phragmites* roots and rhizomes revealed distinct differences in fatty acid profiles in the two plant species, and these microbial signatures were also affected by variations in flooding treatment (Ravit et al. 2007). Differences in microbial community structure were detected in a clone library comparison of *Spartina alterniflora*, *Phragmites australis*, and *Scirpus mariqueter* rhizosphere samples, including the presence of *Nitrospira* and *Spirochetes* associated with *Scirpus* and *Phragmites* only and *Chloroflexi* associated with *Scirpus* and *Spartina* only (Wang et al. 2007). In that study, diversity and evenness were highest with *Scirpus* soils and lowest in *Phragmites* soils (Wang et al. 2007). A comparison of Illumina sequencing OTUs detected higher diversity in *Spartina alterniflora* rhizoplane and endophytic communities compared to nearby stands of mangrove (Hong et al. 2015).

High-throughput sequencing studies indicate that *Proteobacteria* appear to dominate the salt marsh rhizosphere, followed by *Bacteroidetes* and *Chloroflexi*, with additional groups such as *Acidobacteria*, *Planctomycetes*, *Firmicutes*, and *Chlorobi* more prevalent in some study sites than in others (Su et al. 2016; Hong et al. 2015;

Reitl et al. 2016). This finding is supported by analysis of other locations with other methods, for example, *Alphaproteobacteria* comprised the largest taxonomic group in both *Spartina*-dominated and *Phragmites*-dominated environments as detected by taxon-specific DNA–DNA hybridization, followed by *Gammaproteobacteria* and SRB, with *Betaproteobacteria* and *Cytophaga-Flavobacteria* present at the lowest levels of all the groups tested (Burke et al. 2002b). Clone library analysis has detected *Flavobacteria*, *Planctomycetes*, *Verrucomicrobia*, *Spirochetes*, and *Actinobacteria* in *Spartina*-dominated rhizosphere samples in China (Wang et al. 2007; Shuang et al. 2009). Often, rarefaction analysis of salt marsh sediment molecular data indicates incomplete sampling of diversity (Shuang et al. 2009).

8.3.2.1 Rhizosphere Functional Assemblages: Diazotrophs

Nitrogen fixation is a key process in nitrogen-limited salt marshes, and diazotrophs are one of the best studied marsh sediment microbial groups. This functional group of microbes is taxonomically diverse, including sulfate-reducing bacteria that have been shown to contribute a majority of the fixed nitrogen in the 0–5 cm depth sediments of short-form *Spartina alterniflora* and fermentative anaerobes that are most active (~90% activity) at 5–10 cm (Gandy and Yoch 1988). Culturable diazotrophic microbes exist at high numbers in microaerophilic (~10^5 cells g^{-1}) and anaerobic (~10^6 cells g^{-1}) rhizosphere sediments (McClung et al. 1983; Patriquin and McClung 1978). Diazotrophic activity is directly supported by exudates from *Spartina* roots (Gandy and Yoch 1988; Whiting et al. 1986). Pure-culture studies have identified many different nitrogen-fixing taxonomic groups present in the rhizosphere and rhizoplane of *Spartina* spp., including microaerophilic and facultative anaerobes of the *Gammaproteobacteria* (*Enterobacteriaceae* as well as presumptive *Pseudomonadaceae* and *Vibrionaceae*), *Deltaproteobacteria* (presumptive *Desulfovibrio* spp.), and low G + C gram positives such as *Arthrobacter* spp. and *Bacillus* spp. (Bagwell et al. 1998; Andrades-Moreno et al. 2014; Bagwell and Lovell 2000; Bergholz et al. 2001).

Molecular techniques using the *nifH* gene, which encodes one of the three subunits of the enzyme nitrogenase, have been used to examine diazotrophic microbes in salt marsh sediments. Salt marsh microbe gene sequences for *nifH* tend to cluster within two of the four major phylogenetic groups for *nifH*, of which cluster 1 which appears to be widespread in the *Proteobacteria* and cluster 3 which is common in anaerobic environments include sequences from some known SRB, *Spirochetes*, and archaea (Zehr et al. 2003). Some of the same factors described above that affect the abundance of rhizosphere microbes also hold true for diazotrophs specifically, including seasonality patterns for some environments (Davis et al. 2011; Welsh et al. 2010; Gamble et al. 2010) but not all (Burke et al. 2002b), and an influence of dominant vegetation (Davis et al. 2011; Bagwell et al. 2001; Lovell and Davis 2012; Chaudhury et al. 2015). Marsh elevation and its impact on pore water drainage have also been shown to affect the diversity of sediment diazotrophs (Bagwell et al. 2001; Davis et al. 2011).

Analyses of *nifH* gene sequences indicate that *Proteobacteria* (gamma, beta, and delta, with smaller numbers of *Alphaproteobacteria*) appear to dominate in the rhizospheres of many marsh plants (Lovell et al. 2008; Davis et al. 2011). Gene signatures of diazotrophic *Gammaproteobacteria* are detected frequently, such as pseudomonads, *Vibrionaceae*, *Sulfitobacter*, and *Azoarcus* (Gamble et al. 2010; Lovell et al. 2008; Davis et al. 2011). Sequences similar to *Herbaspirillum* and *Halorhodospira* have also been detected (Gamble et al. 2010; Davis et al. 2011). Anaerobic diazotrophs, particularly *Deltaproteobacteria*, are detected often (Gamble et al. 2010; Lovell et al. 2008; Davis et al. 2011), as well as sequences similar to the genus *Chlorobium* (Davis et al. 2011). Many of the diazotroph gene sequences detected in nature do not form strong phylogenetic clusters with sequences from cultured diazotrophs, indicating that novel diazotrophic organisms are present within the sediment rhizosphere environment (Lovell et al. 2008; Gamble et al. 2010).

8.3.2.2 Rhizosphere Functional Assemblages: Denitrifiers and Ammonia Oxidizers

Denitrifier activity in salt marsh sediments has been found to be dependent on plant species, sampling time, and denitrifier community structure as examined through RFLP (Bañeras et al. 2012). The most common gene targets used to examine the taxonomically diverse denitrifying bacteria are *nirS* (encoding cytochrome cd1-type nitrate reductase) and *nirK* (encoding copper-containing nitrate reductase), and the nitrous oxide reductase encoding gene *nosZ* is also used in some studies. Previous work with sequenced genomes has indicated that the genomes of denitrifying bacteria typically contain either *nirK* or *nirS* but not both (Jones et al. 2008), and an analysis of gene database information collected from environmental studies indicates that denitrifier community composition appears to be impacted by salinity gradients, with *nirS* appearing to dominate in organisms from saline environments (Jones and Hallin 2010). This is supported by gene abundance data collected by Zhang et al. (2013), which showed *nirS* to be dominant over *nirK* abundance in four different estuary habitats, with the ratio of *nirS/nirK* higher in deeper (5.1–20 cm depth) sediments (Zhang et al. 2013). Bañeras et al. (2012) used all three gene targets to examine denitrifier populations associated with *Paspalum distichum*, *Ruppia* spp., and *Phragmites australis* in nine salt marshes in Spain. Bacterial community structure was found to be significantly influenced by plant species, *Ruppia* and *Phragmites*, selected for specific denitrifier communities (particularly *nirK* denitrifiers), while *Paspalum* did not (Bañeras et al. 2012). The communities also had different species distributions, with *nirK* communities often dominated by a single genotype, while *nosZ* and *nirS* communities displayed more community evenness (Bañeras et al. 2012).

Disturbance has been shown to affect both salt marsh sediment denitrifying communities and ammonia-oxidizing communities. An analysis of *Spartina* invasion compared to native mangrove and *Cyperus*-dominated areas in China indicated that *Spartina* invasion influenced both *nirS* and *nirK* sediment denitrifiers, impacting

the abundance of both genes, and the diversity of *nirS* (Zhang et al. 2013). The differences were more pronounced in shallower sediments (0–5 cm) than deeper sediments (5.2–20 cm). An opposite trend between abundance and depth was found in marshes restored after tidal restriction compared to undisturbed marshes using TRFLP (terminal restriction fragment length polymorphism) analysis (Bernhard et al. 2015). In this case, differences between the two comparison sites were more pronounced in deeper sediments at restored sites, where *nirS* abundance was greater. The authors proposed that differences in pore water chemistry, particularly higher concentrations of salinity and ammonium, may have contributed to the differences in community structure (Bernhard et al. 2015).

Similar studies have been performed focusing on ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA). *Spartina* invasion was found to be associated with an increase in AOB and a decrease in AOA in the Jiulong Estuary in China (Zhang et al. 2011). The decrease in AOA was attributed to observed increases in sediment pH and total sulfur concentration, which have been shown to negatively impact AOA. Interestingly, the *Spartina* invasion in this study was associated with an increase in diversity of AOA, though not of AOB. The abundance of both groups was greater in upper, oxidized sediments than in deeper, anaerobic sediments (Zhang et al. 2011). Bernhard et al. (2015) examined TRFLP patterns for deeper sediments of anthropogenically disturbed reconstructed marshes and found that the community structure for AOA and AOB was significantly different than those of undisturbed marshes at 6–10 cm depths.

8.3.2.3 Rhizosphere Functional Assemblages: Sulfate Reducers

Sulfate-reducing bacteria are essential to biogeochemical cycling in the salt marsh rhizosphere. It has been estimated for one salt marsh site that 60–90% of the respiration in marsh sediments (Howarth and Teal 1979; Howarth and Giblin 1983) and over 90% of the organic matter degradation (Howarth and Teal 1979) occur due to the activity of SRB. Sulfate reduction is best characterized in marshes dominated by *Spartina alterniflora*, where it occurs mainly between 2 and 20 cm of depth, coinciding with the active *Spartina* rhizosphere (Howarth and Teal 1979). Sulfate is constantly supplied to the sediments via tidal waters and is not limiting in the salt marsh environment (Howarth and Teal 1979). Sulfate reduction rates in tall-form *Spartina alterniflora* have been shown to be seasonal, increasing fivefold from June to August, correlated with the production of belowground rhizome material (Hines et al. 1989). This observation is supported by molecular work showing that a specific population of sulfate reducers similar to known *Desulfococcus* spp. increased in abundance during *S. alterniflora* vegetative plant growth (Rooney-Varga et al. 1997). Short-form *Spartina* does not follow this same pattern of rhizome production, and peak sulfate reduction and abundance of measurable SRB are instead correlated with the degradation of plant material at the end of the growing season in the August through October northern hemisphere autumn (Howarth and Teal 1979; Edgcomb et al. 1999).

Several studies have used molecular techniques to examine SRB abundance and diversity in salt marsh sediments. Use of SRB-specific 16S hybridization for examining samples that represented depths from 0 to 50 cm has indicated that the highest numbers of SRB 16S RNA transcripts are present in the upper 10 cm of sediments and do not appear to change seasonally in spite of measurable seasonal changes in sulfate reduction rates (Koretsky et al. 2005). Klepac-Ceraj et al. (2004) examined salt marsh sediments utilizing 16S primers specific for deltaproteobacterial SRB. *Desulfobacteraceae* comprised ~80% of sequences (primarily *Desulfosarcina* and *Desulfobacterium*), and *Desulfobulbaceae* comprised ~14% of sequences (such as *Desulfobulbus* and *Desulforhopalus*), with *Desulfovibrionaceae* detected at much lower levels. Bahr et al. (2005) examined these same sediment samples, characterizing SRB using *dsrAB*-specific PCR primers. This study was in agreement with Klepac-Ceraj et al. (2004), finding that nearly 80% of the clones obtained were similar in sequence to either *Desulfosarcina* or *Desulfobacterium*. These taxa are known to be complete oxidizers with high substrate versatility, and previous hybridization studies have indicated that *Desulfobacteraceae* are a dominant group of SRB in marsh environments (Hines et al. 1999). Incomplete oxidizers belonging to the *Desulfobulbaceae* were also detected in the Bahr study, comprising 7% of the clones obtained (Bahr et al. 2005). As with other functional groups, dominant vegetation may impact SRB abundance. In one study, qPCR detected higher copy numbers of *dsrB* in *S. alterniflora* stands than in *Phragmites* stands, with a novel phylogenetic cluster consisting of approximately 11.3% of sequences demonstrating reduced abundance in areas where *Phragmites* is giving way to *Spartina* invasion (Zeleke et al. 2013). Sediments from this salt marsh sampled in the Yangtze River Estuary showed the same dominance of *Desulfobacteraceae* and *Desulfobulbaceae* over other SRB (Zeleke et al. 2013) that have also been observed in North American salt marshes.

8.3.2.4 Rhizosphere Functional Assemblages: Arbuscular Mycorrhizae and Heterotrophic Bacteria

Although fungi are not generally found in the waterlogged sediments of salt marshes, arbuscular mycorrhizal fungi (AMF) have been detected in association with the roots of certain species of marsh plants (Wilde et al. 2009). *Spartina patens*, *Puccinellia* spp., and *Aster tripolium* have been shown to be effectively colonized by AMF, particularly *Glomus* spp., while short-form *Spartina alterniflora*, *Phragmites australis*, and *Juncus* typically do not have associated mycorrhizae (Burke et al. 2002a; Wilde et al. 2009). The root structure of marsh plants appears to play a role in the presence of AMF, as plant species with large root masses and efficient uptake of soil nutrients such as *Spartina patens* are more likely to be colonized than plant species with thick rhizomes and lower rates of nutrient uptake such as *Phragmites* spp. *Spartina*-colonizing AMF have been shown to exhibit seasonality, with increases and decreases correlating with plant productivity and senescence, respectively (Burke et al. 2002b; Welsh et al. 2010). The presence of AMF may impact the

abundance of root-colonizing bacteria. Burke et al. (2003) found a negative association of fungi with root-associated *Alphaproteobacteria* and a positive association of AMF with *Gammaproteobacteria*.

Culturable aerobes are present in the rhizosphere, with the highest abundance assessed as colony-forming units (CFUs) in the upper 5–10 cm of sediment (Koretsky et al. 2005). In a study examining the top 5–7 cm of salt marsh sediment from the Virginia Coast Reserve, pigmented aerobic heterotrophs were detected which contained 16S sequences belonging to the classes *Flavobacteria* and *Sphingobacteria* within the phylum *Bacteroidetes* (Lydell et al. 2004). The most common isolate in this study was a novel species within the genus *Gelidibacter* (Lydell et al. 2004). High-throughput sequencing has detected taxonomic groups with aerobic cultivated representatives that are known to play key roles in the decomposition of dissolved organic matter, such as *Alteromonadaceae*, *Pseudoalteromonadacea*, *Vibrionaceae*, and *Methylophilaceae* (Hong et al. 2015).

8.3.2.5 Rhizosphere Functional Assemblages: Methanogens and Acetogens

Pure-culture studies indicate that dominant vegetation has an effect on methanogenic communities. In a stand of short-form *Spartina* in the southeastern United States, the highest levels of culturable methanogens were found at the 5–7 cm depth, decreasing in abundance in deeper sediments. In contrast, tall-form *Spartina* in the same marsh hosted the highest abundances of methanogens in the 0–2 cm sediment layer (Jones and Paynter 1980). The results are consistent with ^{14}C tracer studies indicating different levels of methane flux for tall-form and short-form *Spartina*-colonized sediments (King and Wiebe 1980). Chen et al. (2015) compared greenhouse gas emission from native *Cyperus malaccensis* salt marsh sediments to *Spartina alterniflora*-invaded salt marsh sediments, finding that methane flux for the *S. alterniflora* sediments was nearly twice that of *C. malaccensis* marshes, indicating that vegetation invasion impacted methanogen community structure. *Spartina alterniflora* invasion of *Phragmites* stands was also found to have an effect on methanogen populations based on higher qPCR abundances of the *mcrA* gene (encoding methyl coenzyme M reductase, which catalyzes the anaerobic oxidation of methane) in the *Spartina*-dominated sediments (Zeleke et al. 2013). In this marsh in the Yangtze River Estuary, the hydrogenotrophic methanogens *Methanomicrobiales* and *Methanococcales* dominated (Zeleke et al. 2013). The diversity of methanogen sequences as assessed by pyrosequencing of the *mcrA* gene was higher in *Phragmites* than in *Spartina*-dominated sediments, with *Methanomicrobiales* and *Methanosaeta* increasing with *Spartina* invasion, while *Methanococcales* populations were reduced. The abundance of *Methanosarcina* (metabolically diverse methanogens) did not change with invasion. Overall, marsh alteration due to *Spartina* invasion caused a measurable increase in the abundance and changes in the community structure of methanogens in salt marsh sediments (Zeleke et al. 2013).

The presence of acetogens in salt marsh sediments has been examined using both microbial cultivation and molecular techniques. Selective media have been used to cultivate H_2 and vanillate-utilizing acetogens from root homogenates of *Juncus roemerianus*, and MPN (most probable number) analysis indicated an abundance of 2×10^2 and 1×10^3 acetogens g^{-1}, respectively (Gößner et al. 2006). One acetogenic isolate was found to exhibit significant trophic interaction when grown in co-culture with a strain of *Clostridium intestinale* also isolated from the *Juncus* root homogenate; the acetogen was characterized as a new species of *Sporomusa* (Gößner et al. 2006). Leaphart and colleagues (2001, 2003) used molecular techniques to examine FTHFS (formyltetrahydrofolate synthetase) gene sequences in sediment vegetated with either *Spartina*, *Juncus*, or *Salicornia* roots, finding sequences that grouped with known pure-culture acetogens (*Sporomusa*, *Clostridium*, and *Acetobacterium*) only in association with the low-marsh *Spartina* (Leaphart et al. 2003). A second phylogenetic cluster contained the greatest diversity of FTHFS sequences and grouped with known SRB including *Desulfoarculus*, *Desulfomicrobium*, and *Desulfovibrio desulfuricans*. The smallest of the three phylogenetic clusters encompassed sequences from a variety of organism types, including *Desulfovibrio salexigens*. Together, these three studies indicate that acetogens are a significant and diverse component of the microbial community in the salt marsh rhizosphere.

8.3.3 *Community Structure of Sediment Below the Salt Marsh Rhizosphere*

Little is currently known about the community composition of salt marsh sediments below the rhizosphere. Belowground biomass of marsh plants such as *Spartina alterniflora* and *Spartina cynosuroides* has been shown to be seasonal in the 10–30 cm zone but not at other depths, potentially indicating that little growth occurs at those depths (Schubauer and Hopkinson 1984). The depth affected by salt marsh plant roots varies depending on species and environmental conditions. In general, live roots of *Spartina alterniflora* have been shown to be prevalent in the top 15 cm of the sediment, with some roots and rhizomes extending to a depth of 25 cm in mineral marsh sediments, while in highly organic marsh sediments, significant biomass of roots and rhizomes are prevalent throughout the top 25 cm (Blum and Davey 2013). Researchers have measured live roots penetrating sediment up to 50 cm of depth, although the dry weight of material at this depth is a fraction of the biomass found at the 0–10 or 10–20 cm depths (Blum and Davey 2013). Therefore, it is likely that sediments below 25 cm are minimally impacted by salt marsh plant influence.

Keith-Roach et al. (2002) used PLFA analysis to examine seasonal changes in sediment community structure in a UK estuary at 32–35 cm depth. It had previously been shown that the 30–35 cm depth displayed seasonal cycling of Fe and Mn. The

analyses by Keith-Roach et al. (2002) demonstrated that the community was dominated by organisms with PLFA signatures associated with aerobic bacteria, consistent with Eh measurements indicating the sediments were mildly oxic all year at this site. Anaerobic signatures were shown to rise in abundance later in the year, along with a rise in dissolved Mn that has been observed previously at this depth (Keith-Roach et al. 2000), indicating a rise in activity of manganese-reducing organisms. No seasonal shifts in Fe were observed at this site (Keith-Roach et al. 2000). Koretsky and colleagues (2005) detected culturable aerobic bacteria down to 50 cm depth in a US marsh, as well as culturable MnRB and FeRB. Sulfate reducers were examined using sulfate reduction rate measurements and targeted 16S hybridization probes and were detected down to 50 cm, with a larger proportion of *Desulfovibrio* and *Desulfobacter* in samples taken during the summer (Koretsky et al. 2005). The community structure below 25 cm was found to be significantly different compared to other sampled depths, with decreased bacterial abundances of all taxa relative to shallower sediments (Koretsky et al. 2005).

8.4 Responses of Microbial Communities to Eutrophication

Anthropogenic eutrophication of coastal and marine environments can occur as a result of atmospheric deposition, agricultural runoff, and effluent from sewage treatment (Moseman-Valtierra et al. 2010; Vivanco et al. 2015). In particular, urbanization and agricultural development have raised nutrient levels in waters that move from terrestrial into coastal environments (Cloern 2001; Valiela and Bowen 2002). It has been estimated that nutrient levels entering coastal systems have increased tenfold since the industrial revolution and are predicted to continue to increase in the near future (Galloway et al. 2008; Howarth 2008). Eutrophication currently affects more than half of the estuaries in the United States (Howarth et al. 2000), and excessive concentrations of inorganic nitrogen and phosphorous in particular can produce harmful effects on ecosystems. Environmental effects of eutrophication on the physical structure of salt marshes include reduction in soil shear vane strength (Swarzenski et al. 2008; Turner 2011), changes in marsh plant biomass allocation (Xia and Wan 2008), shifts in dominant plant species (Valiela et al. 1985), increases in macroalgae that can smother grasses (Newton and Thornber 2013), and either increases (Rogers et al. 1998) or decreases (Langley and Megonigal 2010) in marsh elevation. Several studies (e.g., Turner et al. 2009; Morris and Bradley 1999) have shown that increasing nutrient concentrations affect the chemical composition of salt marsh sediment, including a loss of sediment carbon storage. This has raised concerns that eutrophication of salt marshes may contribute to rising levels of greenhouse gasses in the atmosphere, leading to a renewed interest in the effects of high concentrations of inorganic nutrients on marsh soil chemistry, primary productivity, and the microbial processes that cycle nutrients in the sediment.

8.4.1 *Effects of Eutrophication on Salt Marsh Ecosystem Services*

Salt marshes have long been recognized as pollution buffers, with the capacity to absorb land-introduced nutrients in runoff water via microbially mediated denitrification and plant biomass production (Valiela and Cole 2002). Indeed, salt marshes and other terrestrial wetlands appear to have an unparalleled capacity to trap and transform nutrients. Pioneering studies on experimentally fertilized plots in the Great Sippewissett Marsh in Massachusetts indicated that 60–94% of the added nitrogen was retained (Valiela et al. 1973), and even after 30 years of treatment, very little of this annually added nitrogen in these long-term fertilization plots was exported in tidal waters, indicating continued high retention of the inorganic nutrient (Brin et al. 2010). Marsh elevation and drainage appear to affect nutrient retention, since low-marsh areas with waterlogged sediments have been shown to be less efficient at capturing nutrients from terrestrial runoff than high-marsh areas, particularly during storm events (Oczkowski et al. 2015). Some of the added nitrogen becomes buried in the sediment (White and Howes 1994; Kinney and Valiela 2013), and some are exported via the tidal cycle, mainly in the form of NH_4^+ (Brin et al. 2010), while a majority is converted to atmospheric N_2 through the direct activities of denitrifying and the indirect activities of nitrifying bacteria through coupled nitrification-denitrification (Howes and Teal 1994; Hamersley and Howes 2005). Phosphate additions were also examined, and the fertilized Sippewissett plots retained 91–94% of the added phosphorous (Valiela et al. 1973). Although P tends to cause eutrophication issues in freshwater lakes and K can also contribute to eutrophication, studies performed on coastal systems indicate that P and K fertilization appear to have little effect and in general these systems are more likely to be negatively affected by excess N (Howarth et al. 2000). For this reason, the primary focus of this section will be on the effects of nitrogen-mediated eutrophication.

There is a growing body of evidence that indicates salt marshes can exhibit nonlinear responses to nutrient inputs. Plant biomass, which increases linearly with low nitrogen addition, often saturates at high nitrogen levels (Darby and Turner 2008), and levels of nitrogen incorporated into *Spartina* leaves and shoots reach a maximal threshold. It has been shown that plant competition shifts during N fertilization, with competition occurring mainly belowground at ambient nutrient concentrations but shifting to aboveground competition at high nutrient concentrations (Emery et al. 2001). Sediment organic N accumulation appears to increase exponentially in response to increasing fertilization (Vivanco et al. 2015), as does export of NO_3^- (Brin et al. 2010). However, most microbial processes appear to respond linearly to increasing levels of nitrogen. For example, tidal export of nitrogen in the form of NH_4^+ from ammonification shows a linear response with increasing N (Brin et al. 2010). This pattern appears to be reversible, with nitrogen-trapping activities restored once fertilization ceases (Brin et al. 2010). Vivanco et al. (2015) applied nitrogen gradients to three marshes on the West Coast of the United States and found that N mineralization decreased linearly and methane efflux

increased linearly. Similar effects of increased N on methane efflux have also been shown by other studies (Zhang et al. 2010; Irvine et al. 2012). It should be noted that some microbial processes demonstrate neutral responses to N addition; for example, nitrification (Vivanco et al. 2015) and methylotrophy (Irvine et al. 2012) were not significantly affected by increasing N concentrations.

Some microbial responses to increasing nitrogen appear to be site-specific. For example, in the West Coast study mentioned above, microbial respiration showed a neutral response to increasing nitrogen additions over a 14-month period (Vivanco et al. 2015); however, in other studies performed in the southeastern (Morris and Bradley 1999) or northeastern (Deegan et al. 2012) coastal United States, long-term exposure to elevated nitrogen was shown to increase bacterial respiration. In addition, a comparison of sediment inorganic nitrogen accumulation in three different marshes on the West Coast of the United States demonstrated different responses in sediment inorganic nitrogen accumulation with increasing nitrogen addition (Vivanco et al. 2015). It has been proposed that site-specific factors, such as the degree of carbon limitation of the sediments, directly impact microbial respiration and soil chemistry under conditions of eutrophication.

The microbial processes that have received the most attention in eutrophied salt marsh sediments are those involved in nitrogen cycling. In many cases, increasing nitrogen stimulates microbial sediment processes resulting in increased N_2O emission and nitrification with increasing nitrogen inputs while at the same time decreasing nitrogen fixation and saturating denitrification processes.

8.4.1.1 Effects of Fertilization on Nitrogen Fixation

Rates of nitrogen fixation have long been known to be affected by nitrogen loading (van Raalte et al. 1974), and nitrogenase enzyme activity in salt marsh sediments as measured by acetylene reduction assays has been shown to be inhibited by the presence of either ammonia or nitrate or both (Dicker and Smith 1980; Yoch and Whiting 1986). A more recent study performed in a salt marsh of the Tijuana Estuary showed that nitrogen fixation rates decreased significantly with additions of ammonium nitrate (Moseman-Valtierra et al. 2010). In this study, inhibition of sulfate-reducing bacteria by sodium molybdate indicated that SRB performed 70% of the nitrogen-fixing activities in the absence of eutrophication. Increased delivery of nitrogen to sediments decreases the need for the energetically expensive process of bacterial nitrogen fixation; however, not all studies demonstrate a decrease in nitrogen fixation following fertilization. In a short-term experiment following addition of ammonium nitrate, nitrogen fixation in a South Carolina marsh was found to increase at 2 weeks following fertilization; however at 8 weeks, nitrogen fixation rates were similar to controls (Lovell et al. 2001). The authors proposed that the diazotroph assemblage composition remained stable during increased nitrogen during the study period, an observation that is supported by molecular evidence from other salt marshes (Piceno and Lovell 2000; Bowen et al. 2012).

8.4.1.2 Effects of Fertilization on Nitrate Reduction and Ammonia Oxidation

In natural salt marsh systems, denitrification activity in salt marsh sediments increases as nitrogen levels increase, showing a sharp increase in late summer due to reduced competition with plants for NH_4^+ (Hamersley and Howes 2005). The ammonia fuels nitrification in oxic sediments, with the resulting nitrate diffusing into anoxic sediments to provide a substrate for denitrification (Reddy et al. 1989). Under experimental conditions with added nutrients, several studies have shown that rates of denitrification increase (Koop-Jakobsen and Giblin 2010; Deegan et al. 2012), and during the highest periods of activity, denitrification rates can be an order of magnitude greater in fertilized vs. unfertilized plots (Lindau and DeLaune 1991; Hamersley and Howes 2005) as well as in fertilized vs. unfertilized tidal creeks (Vieillard and Fulweiler 2012). Examination of stable isotope fractionation indicates that 47–80% of the added nitrogen is denitrified (Kinney and Valiela 2013). However, with excessive eutrophication, the linear relationship that has been observed between fertilizer concentration and denitrification approaches an asymptote, and as N continues to increase, denitrification actually declines (Valiela 1995). It is important to note that denitrification rates can be highly variable and may be site-specific (Wigand et al. 2004; Caffrey et al. 2007) and dependent on dominant vegetation patterns, salinity, and sampling time (Bañeras et al. 2012). Although denitrification can be very active in surface sediments, addition of nitrogen does not appear to affect denitrification rates in deeper sediments (Koop-Jakobsen and Giblin 2010).

The processes of denitrification and anammox result in the removal of nitrogen in the form of atmospheric N_2. Dissimilatory nitrate reduction to ammonia, however, results in the retention of nitrogen as ammonia, increasing the potential for excess nitrogen export from marsh environments (Koop-Jakobsen and Giblin 2010). It is important to note that DNRA rates are variable and can account for 0–60% of the nitrate reduced in salt marsh sediments (Ma and Aelion 2005; Tobias et al. 2001). A study comparing denitrification and DNRA in salt marsh surface sediments found that DNRA rates were consistent with denitrification rates in fertilized as well as unfertilized plots (Koop-Jakobsen and Giblin 2010). High sulfide concentrations inhibit both nitrification and denitrification (An and Gardiner 2002) and stimulate the growth of sulfide-oxidizing organisms that have been shown to be significant participants in DNRA in marine sediments (Burgin and Hamilton 2007). Examination of the effects of added nitrate on anammox indicates that this process shows little response to nitrogen addition (Peng et al. 2013).

8.4.1.3 Effects of Fertilization on Sediment Carbon Cycling

Many studies have detected increased microbial respiration in fertilized sediments compared to unfertilized controls (Morris and Bradley 1999; Wigand et al. 2009; Deegan et al. 2012). This pattern appears to hold true for marshes that are nitrogen

limited. However, Vivanco et al. (2015) showed that respiration was unaffected and sediment total C values did not change as a result of nitrogen addition in the West Coast US marshes, where sediments are generally carbon limiting rather than nitrogen limiting. Increased respiration is sometimes accompanied by decreased sediment carbon storage (Morris and Bradley 1999; Turner et al. 2009) as a result of eutrophication, and other times without carbon loss (Anisfeld and Hill 2012). In one study, carbon loss was observed from low-marsh *S. alterniflora*-vegetated sediments, but not from high-marsh *S. patens*-vegetated sediments (Wigand et al. 2009). It had been suggested that in situations with no visible carbon loss, other factors such as increased belowground plant productivity compensate for the increased CO_2 efflux resulting from increased root respiration (Anisfeld and Hill 2012) and potentially increased microbial respiration (see Deegan et al. 2012) in fertilized marsh plots.

Carbon can also be lost from salt marsh sediments through methanogenesis. Although methane fluxes are minor in marshes where sulfate reduction dominates, it has been suggested that addition of nitrogen may indirectly stimulate carbon-limited methanogens through stimulating plant production of labile carbon compounds in plant root exudates (Oremland and Polcin 1982). Vivanco et al. (2015) and Irvine et al. (2012) both showed that methane flux from salt marsh sediments increased linearly with nitrogen addition. The latter study also examined methylotrophy, which was unchanged with nitrogen addition (Irvine et al. 2012).

8.4.2 *Effects of Eutrophication on Microbial Community Structure*

Many controlled fertilization studies have shown that microbial communities associated with the rhizosphere of *Spartina* spp. have surprising resilience when faced with eutrophication. Bowen et al. (2009) examined DGGE patterns in a multi-year experiment of fertilized (N and P) vs. unfertilized *S. patens* and tall- and short-form *S. alterniflora* plots and found that nitrogen did not affect community structure. Of the five marsh environments sampled within four different marshes in this study, only the unvegetated marsh creek bank sediments colonized by filamentous algae showed a significant response to fertilization. The researchers proposed that shifts in carbon supply mediated by the algae resulted in increased diversity in the associated bacterial community (Bowen et al. 2009). Similarly, PLFA data from mesocosm experiments indicated that N addition did not affect community structure in *S. alterniflora*-vegetated sediments (Ravit et al. 2007). However, mesocosms vegetated with *P. australis* demonstrated significant shifts in microbial community structure as determined via PLFA, demonstrating increased diversity compared with unfertilized *P. australis* mesocosms (Ravit et al. 2007). Several short-term (Levine et al. 1998; Boyer and Zedler 1999; Bertness et al. 2002) and long-term (Kiehl et al. 1997; Emery et al. 2001; Fox et al. 2012) studies have shown shifts in the structure of salt marsh plant communities as a result of fertilization; therefore, postfertilization microbial community structure results

should be viewed with caution. Increased nitrogen may not show a direct effect on microbial community structure; however, indirect effects associated with shifts in dominant plant vegetation caused by fertilization may have lasting effects on the sediment microbial communities.

8.4.2.1 Effects of Fertilization on Diazotroph Community Structure

Specific groups of functional taxa may be more affected by increased fertilization than the microbial community as a whole. One group that has been investigated is the sediment nitrogen-fixing organisms. Nitrogenase consists of two multisubunit metalloproteins encoded by the *nif* genes, *nifHDK*, and these have been utilized as molecular targets to examine abundance and diversity of nitrogen-fixing organisms in the environment (Zehr and McReynolds 1989; Zehr et al. 2003). In an 8-week short-term fertilization study (Piceno and Lovell 2000), there was no detectable loss of *nifH* gene sequences from the *S. alterniflora* rhizosphere detected by DGGE analysis, although one sequence band decreased in intensity. In comparison with samples from a nearby long-term (>10 years) fertilization plot, only one DGGE sequence band was absent in fertilized plots compared to controls, though several bands from the fertilized plot samples were reduced in intensity (Piceno and Lovell 2000). Likewise, no change in diazotroph community composition was observed in TRFLP analysis during short-term (17 days) fertilization of *S. foliosa* sediments (Moseman-Valtierra et al. 2010), although in surface sediments diazotroph richness and evenness increased even as nitrogen fixation activity declined. This indicates that surface sediment communities may be more vulnerable to the effects of increasing nitrogen concentrations than rhizosphere organisms.

8.4.2.2 Effects of Fertilization on Denitrifier and Nitrifier Community Structure

Denitrification occurs due to the activities of phylogenetically diverse microbes. At the current time, the functional genes most often used to explore denitrification in soils are *nirK* which is a copper-containing nitrate reductase and the cytochrome cd1-type nitrate reductase *nirS* (Bañeras et al. 2012), both of which encode proteins that can independently catalyze the first step in the denitrification pathway. One study utilized both 16S pyrosequencing and a functional gene microarray targeting *nirS* to examine nutrient effects with both techniques showing no adverse effects of nutrient concentration on community composition (Bowen et al. 2011). In contrast, a subsequent study involving pyrosequencing of the *nirS* gene did detect differences in denitrifying communities in fertilized plots compared to controls (Bowen et al. 2013). Although bacterial abundance showed no changes in fertilized vs. unfertilized plots, the study by Bowen et al. (2013) identified differing patterns of denitrifying specialists vs. generalists, indicating that fertilization increases niche space for specialists, allowing increased genetic capacity for denitrification. The contrasting findings of these two studies were attributed to the

resolution of the techniques applied, with the earlier microarray study having lower resolution due to the reliance on database sequences for the development of the functional gene probes (Bowen et al. 2013). Kearns et al. (2015) analyzed *norB* (encoding a subunit of the nitric oxide reductase) and *nosZ* (encoding nitrous oxide reductase) sequences as indicators of nitrous oxide flux from denitrifying bacteria at the same marsh plots studied by Bowen et al. (2011, 2013). The number of unique *nosZ* sequences was found to increase with increasing fertilization though the overall abundance decreased, while the number of unique *norB* sequences remained relatively constant and the abundance of the gene as determined by qPCR was not affected (Kearns et al. 2015).

Organisms carrying out dissimilatory reduction of nitrate to ammonia (DNRA) belong to diverse taxonomic groups. At the current time, DNRA has been shown to occur due to the activities of a periplasmic nitrate reductase complex encoded by *napAB*, together with a nitrite reductase, either NrfA or Otr (Giblin et al. 2013). In several studies, PCR primers have been applied to detect *nrfA* (Mohan et al. 2004; Welsh et al. 2014) and have previously been used to examine DNRA communities in estuaries. In one such study involving benthic estuary sediments, real-time qPCR demonstrated a decline in *nrfA* transcripts together with *narG* (encoding a subunit of the cell membrane-localized nitrate reductase) and *nirS* across three sites, correlated with decreasing concentrations of nitrate (Dong et al. 2009). These molecular data matched nitrate reductase activity measurements recorded at the same three sites (Dong et al. 2009). These types of molecular tools may provide a valuable resource to examine anthropogenic effects on salt marsh sediment DNRA community structure in the future.

8.5 Responses of Salt Marsh Organisms to Heavy Metals

Salt marshes are widely accepted to be a sink for heavy metals. Although some metals such as Al, Ni, Fe, Mn, and Ag can originate from geochemical weathering of minerals, others such as Cu, Zn, Pb, Cd, Cr, and Hg are often from anthropogenic sources such as agricultural and road runoff, sewage effluent, atmospheric deposition, mining, manufacturing processes, and dredge spoils. The fate of heavy metals entering salt marsh environments depends on numerous factors, including the metal type, the sediment redox potential (especially as influenced by the activity of plant species and burrowing fauna), and the physical and geochemical composition of the sediment (reviewed in Williams et al. 1994). Sandy sediments with low organic matter retain metals poorly, while fine-grained sediments high in organic matter with clay components capable of carrying out ion exchange retain significant quantities of metals (Williams et al. 1994). The chemical environment determines the chemical speciation of heavy metals. In anaerobic environments where SRB generate high sulfide concentrations, metals often exist as precipitated metal sulfides. Metals can also complex to sediment organic material or can be bound to the mineral matrices (also known as the residual fraction; Tessier et al. 1979). In aerobic layers, metals

can be released from sulfides (Delaune and Smith 1985) as well as from organic material (Khalid et al. 1978), and the metals then either become bioavailable in the sediment pore water or adsorbed to Fe and Mn oxides and hydroxides.

Levels of metal contamination vary greatly between salt marshes (Otero and Macías 2002; Reboreda and Caçador 2008), and can also vary within a single marsh area (Almeida et al. 2004; Suntornvongsagul et al. 2007). One reason for intra-marsh variation is that metal concentrations can depend on the distance of the sampling site to tidal creeks, with sediments closer to tidal creeks having higher metal concentrations due to deposition of particulate bound metals brought in by the tides (Williams et al. 1994). In addition, gradients in metal concentration correlated to sampling depth are often observed, attributed to differing historical inputs of metals (Caçador et al. 1996; Cleary et al. 2012; Quillet et al. 2012; Chai et al. 2014).

Several studies have shown that metals are enriched in the rhizosphere of a variety of salt marsh plants as compared to nearby unvegetated sediments (Caçador et al. 1996; Doyle and Otte 1997; Almeida et al. 2004; Canário et al. 2007; Chai et al. 2014). Metal concentrations appear to be closely tied to seasonal shifts. During the spring and summer when plant growth is highest, the rhizosphere can become highly oxidized mobilizing metals and causing concentrations in sediment pore water to increase (Otero and Macías 2002). The activity of macrofauna can also play a role, and notably Hines et al. (1984) measured the highest level of interstitial water metal concentrations in the spring during periods of intense bioturbation. At this seasonal time, levels of metals in plant biomass also generally increase (Caçador et al. 2000; Weis et al. 2002), although metal uptake varies depending on the species of halophyte examined (Best et al. 2008; Caçador et al. 2009; Reboreda and Caçador 2007; Marques et al. 2011). During late autumn and winter as plant growth ceases, the sediments become more reduced and sulfide rich, leading to precipitation of metals (Caçador et al. 2000). On a daily time scale, photosynthetic plant activity promotes oxic sediment conditions during daylight hours, with more reducing conditions at night. The daily tidal cycle can also play a role, altering pore water salinity and potentially remobilizing metals such as Cd, Hg, and Zn that have strong affinities for chloride (Williams et al. 1994).

Although exposure to metals increases metal concentration in salt marsh plant tissues, few negative effects are generally observed in individual halophytes (Valiela 2015). However, in some cases there have been documented shifts in the distribution of dominant marsh plant species following metal contamination (Válega et al. 2008). A long-term study involving applications of metal-enriched sewage to salt marsh plots indicates that the metals appeared to have little effect on the growth rate and resulting biomass of halophytes, despite the accumulation of metals in halophyte tissues and their associated invertebrates (Giblin et al. 1980). Studies have documented the formation of iron plaques on halophyte roots which can be significantly enriched in Cd, Zn, Cu, and Pb compared to the surrounding sediment (Sundby et al. 1998). In some instances, these concretions appear to have a protective effect for the plant (Otte et al. 1989; Batty et al. 2000). In addition, several studies have provided evidence that many species of salt marsh plant preferentially accumulate metals in their root systems, protecting aboveground tissues (Weis and

Weis 2004; Reboreda and Caçador 2007; Canário et al. 2007; Chai et al. 2014). This in turn can affect cycling of metals in the sediments, as senescence of root and rhizome tissues can release metals back into the environment.

Rhizosphere bacteria associated with salt marsh halophytes have been investigated for their role in affecting the mobility of metals through the production of chelating agents, sulfides, and changing the chemical environment by shifting the pH or redox potential (Anjum et al. 2014). These rhizosphere bacteria may also assist phytoremediation of metals through the release of plant growth-promoting factors such as hydrolases and acid phosphatases. The following sections will describe what is currently known about the impact of heavy metals on sediment microbial communities and their ecosystem services.

8.5.1 Effects of Heavy Metals on Ecosystem Services

Salt marsh ecosystem services that involve symbiotic interactions between sediment microbes and salt marsh halophytes have been described in the above sections of this chapter. This section will take a closer look at services involving heavy metals in particular. These fall into two general categories: (1) services that assist with phytoremediation and phytostabilization of metals and (2) biogeochemical cycling services.

Plant growth-promoting services that assist with phytoremediation or phytostabilization in heavy metal-contaminated environments have been documented in several studies. The effect of arbuscular mycorrhizal fungi (AMF) in high-marsh environments has been investigated, and in some cases AMF colonization has been shown to be inversely correlated to metals such as Pb and Zn, indicating that the presence of AMF provides a protective function for the plant (though AMF colonization was negatively affected by the presence of metals; Carrasco et al. 2006). However, in other studies there has been no significant correlation between metals and the presence of AMF (Suntornvongsagul et al. 2007). Mesa et al. (2015) examined endophytic bacteria from *Spartina maritima* and found that the bacteria increased plant growth and decreased plant uptake of metals. Several of the bacterial strains cultivated from the contaminated environment in this study were found to be resistant to As, Cu, and Zn and displayed plant growth-promoting phenotypes including nitrogen fixation and phosphate solubilization. In another study, 22 different strains of bacteria isolated from contaminated *Spartina densiflora*-vegetated sediment were examined. Of these strains, most were highly resistant to Cu, and some were resistant to several metals such as Zn, Pb, As, and Cd. It was noted that 70% of the strains demonstrated one or more plant growth-promoting phenotypes and that a consortia of three of these strains inoculated onto *S. densiflora* seeds exhibited a significant antifungal protective effect on seed germination (Andrades-Moreno et al. 2014).

Regarding microbes that play a role in biogeochemical cycling, Capone et al. (1983) examined the effects of metals on microbial salt marsh processes including

methanogenesis, sulfate reduction, CO_2 production, and biomass in *S. alterniflora* sediments. The metals which they tested included chlorides of mercury, monomethyl mercury, lead, nickel, cadmium, iron, and copper; zinc sulfate; sulfides of lead and mercury; sodium molybdate and arsenite; and potassium mono- and dichromate. Many microbial processes were inhibited initially, followed by recovery and stimulation, likely due to decreased bioavailability following either precipitation or complexation of metals added during the experiment. Those authors found that methanogenesis was inhibited by methylmercury and $NaAsO_2$, while sulfate reduction was inhibited by all metals except Fe and Ni. In addition, decreases in microbial biomass were observed with the addition of Fe, Cd, Cu, Zn, and Cr (Capone et al. 1983). A more recent study by Quillet et al. (2012) examined the impact of metals on SRB activities. The researchers found molecular signatures of SRB down to a depth of 3.5 m, but *Desulfovibrio* appeared to be most active in the top 50 cm which contained the highest metal levels, suggesting that this group is more active than other SRB groups in metal-contaminated environments (Quillet et al. 2012).

Salt marsh sediment microbes produce a variety of extracellular enzymes that are involved in biogeochemical cycling. Phenol oxidase, beta-glucosidase, protease, and chitobiase are enzymes that are involved in the breakdown of complex carbon-containing compounds, while sulfatase and phosphatase are extracellular enzymes involved in the cycling of sulfur and phosphorous, respectively. Hydrolases are especially of interest, as they reduce the molecular weight of organic compounds affecting both the solubility of those compounds and potentially also the mobility of any attached metals (Reboreda and Caçador 2008). For example, Duarte and colleagues (2008a, 2009) found that the organic-bound fraction of metals decreased from summer to winter, correlated with increased activities of peroxidase, β-*N*-acetylglucosaminidase, and protease. The labile exchangeable fraction of metals decreased from spring to autumn, correlated to sulfatase activity. This sulfatase enzyme activity releases sulfide that likely contributes to metal precipitation (Duarte et al. 2009). Other studies have shown that metals can have an inhibitory effect on extracellular enzymatic activities such as dehydrogenase (an indicator of bacterial respiration) and some hydrolases such as phosphatase and β-glucosidase. Activity levels of these enzymes have been shown to be either lower or completely inhibited in anthropogenically impacted sediments as compared to unimpacted sediments (Ravit et al. 2003; Carrasco et al. 2006).

8.5.2 *Effects of Heavy Metals on Microbial Community Structure*

Pure-culture studies using complex media indicate that the bacterial strains that have colonized metal-contaminated rhizosphere samples are metal resistant. Andrades-Moreno et al. (2014) obtained multi-metal-resistant strains of *Pseudomonas*, *Bacillus*, and *Acinetobacter* from the *Spartina densiflora* rhizosphere. Cell counts of DAPI-

stained cells from two Portuguese estuarine systems differentially impacted by metals showed that microbial abundances were negatively impacted at higher metal concentrations (Machado et al. 2012).

Molecular techniques have also been implemented to examine microbial community structure in metal-contaminated environments. A DGGE analysis of vegetated and unvegetated sediments from two marshes with differing levels of metal contamination indicated that the presence of metals exerts a greater effect on microbial community structure than does either the presence or absence of plants (Andrades-Moreno et al. 2014). Another DGGE comparison of two highly contaminated marshes vs. one marsh with low contamination indicated that a significant amount of the variability in the community structure in the highly contaminated marshes was associated with metals (Pb/Fe in one and Ni/Fe, Cr/Fe, and Zn/Fe in the other), while in the third marsh, the variability in community structure was associated primarily with organic matter content (Machado et al. 2012). In their study, Machado et al. (2012) found that while some OTUs were present across all samples, other OTUs were site-specific indicating that the metals present in the samples may have effected a selection process for metal-resistant microbial communities. Cleary et al. (2012) found an association between the mercury content of sediments and bacterial community composition as determined by DGGE and PCA, although most of the variation was attributed to dominant plant species and sampling depth. Another study involving automated RNA intergenic spacer analysis (ARISA) conducted on mesocosms with added Cu indicated that Cu caused a shift in microbial community structure (Mucha et al. 2011).

A PLFA comparison between an anthropogenically impacted vs. an unimpacted site revealed lower diversity in the impacted site, though the actual metal concentrations of the samples were not measured in this study (Ravit et al. 2003). Cao et al. (2006) examined the effects of 20 different bioavailable metals using TRFLP and PLFA analysis of samples from two different marshes. After controlling for marsh spatial effects, several metals were found to be significantly associated with changes in community structure, as determined by partial canonical component analysis (pCCA), indicating that the metals likely acted as stressors on the microbial community. Of all the metals examined, Ni and Zn appeared to be the most consistent in their effect on TRFLP and PLFA profiles. In a follow-up study examining denitrifying bacteria specifically, metals appeared to influence both 16S and *nirS* TRFLP patterns, and some metals appeared to negatively correlate with denitrifying enzyme activity, indicating that the denitrifying community was impacted (Cao et al. 2008). In studies involving comparisons between sites, investigators have warned that a variety of other factors including differences in hydrogeology, geology, and organic matter content of sediments may also influence microbial community structure; therefore, caution is advised in interpreting data relating to the effects of metals on microbial community structure.

8.6 Conclusion

Salt marshes are beneficial and dynamic ecosystems, containing chemical and physical gradients that give rise to some of the most complex sediment microbial communities known. Current evidence indicates that the losses in salt marsh land area that have been documented over the last century appear to be due to human development as well as shifts in macroecology, particularly the ecology of herbivorous, burrowing macrofauna. However, the impact of anthropogenic compounds that are deposited into salt marsh sediments should not be overlooked. Studies have shown that excess nitrogen compounds introduced into salt marshes cause shifts in sediment nitrogen cycling and can negatively impact specific functional groups such as diazotrophic bacteria. Studies of metal-contaminated salt marshes indicate that, although the levels of organic matter and redox conditions in the sediments may decrease the bioavailability of metals due to the complexation of metals with sulfides and organics, metals can still often be detected in the interstitial waters, and increased levels of heavy metals negatively impact biogeochemical nutrient cycling and correlate with changes in microbial community structure. Since microbes play critical roles in the ecosystem services provided by salt marshes, an understanding of the factors that support or inhibit sediment microbial communities is essential for developing strategies to maintain the health of these wetland environments that act as sinks for terrestrial and aquatic pollutants.

Compliance with Ethical Standards

Conflict of Interest Jonna M. Coombs declares that she has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Chapter 9
Dirt and Disease: The Ecology of Soil Fungi and Plant Fungi That Are Infectious for Vertebrates

Christon J. Hurst

Abstract This chapter summarizes information regarding the fungi that naturally are found either in soil or associated with plants and also produce infectious disease in vertebrates. The main section contains what I consider to be the "Active" list, in which I present information for the 244 fungal genus names and their constituent species that presently either are known to or suspected of infecting vertebrates, mentioning the natural ecology of those genera along with the nature of the infections that they produce in vertebrates, and giving examples for each of their vertebrate host ranges. I also provide a separate listing of the 53 fungal genus names which no longer represent species that are infectious for vertebrates due to fungal taxonomic reassignments. The fact that fungal toxicoses can either mimic or obscure infectiousness is mentioned along with some appropriately representative fungal genera. I also separately list the fungal genera that have been indicated to infect vertebrates but for which I could not find corroborating evidence. Additionally, there is mention that soil and plant fungi which are infectious for invertebrates often have an indirectly deleteriously impact by reducing the food supply that is available for vertebrates.

9.1 Introduction

Fungi have an important environmental role as described well in Dighton and White (2017), but the fungi also are somewhat like a double edged sword that affects other life forms with both positive and negative results. Fungi in the soil decompose the complex into the simple as they saprotrophically recycle organic materials, including in the end ourselves. Fungi play a complex and ecologically invaluable beneficial role in plant ecology (Mohammadi et al. 2011) with an example being their potential role in resistance to other microbes as described by Barda et al. (2015), who discovered that a fungal species induces resistance to a bacterial species that affects

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tomato plants (see the entry for *Moesziomyces* in Sect. 9.4). Hamayun and his coresearchers (2017) discovered that the gibberellins produced by an endophytic fungus can rescue the growth of salt affected soybean (see the entry for *Porostereum* in Sect. 9.4). Providing yet another example, fungi have been found by Mestre et al. (2017) to affect the plant root/shoot ratio (see the entry for *Tausonia* in Sect. 9.4).

Soil and plant fungi interestingly have been suggested as a resource material for creating synthetic hydrophobic materials (Haneef et al. 2017) and happily neither of the plant fungi suggested for that purpose, *Ganoderma lucidum* which is utilized in traditional medicine and the oyster mushroom *Pleurotus ostreatus* which I can attest is quite edible, have been reported as infectious for vertebrates. Many of the soil fungi and plant fungi are eaten as food by invertebrates and vertebrates. And yet, some of those same fungi can cause lethal infections in animal species who consume the fungi either by intention as a food source (Salit et al. 2010) or by accident, as fungal contaminants of food (Benedict et al. 2016).

The degradative metabolic capabilities of soil fungi and plant fungi often are used intentionally, including the engineered application of saccharification processes for biodecomposition of plant materials (Brethauer et al. 2017, see the entry for Irpex in Sect. 9.4). You will find additional mentions of those purposes among the fungal genus and species entries in Sect. 9.4 of this chapter. It is unfortunate that infectious disease then results when the ecologically important growth and degradative recycling activity of fungi seems detrimentally to act too soon, and that is the subject of this chapter.

The organization of this chapter is as follows. Section 9.1 is, of course, my very brief general introduction to the subject. Section 9.2 of this chapter provides examples of fungal genera whose non-infectious pathogenicity mimics infectivity. Section 9.3 gives some examples of fungi whose infectiousness for invertebrate animals deleteriously impacts food resources that are consumed by vertebrates. Section 9.4 presents what I consider to be the "Active" list, those genera and species of soil fungi and plant fungi that infect vertebrates. My listing in Sect. 9.5 of the fungal genus and species names that historically have been significant from the perspective of infectious disease in vertebrates, but which no longer are on the "Active" fungal pathogen list due to taxonomic reassignments, should be helpful for all of us who study the role of ecology from the perspective of infectious disease. I additionally have included as Sect. 9.6 a mention of the fungal genera which others have listed as causing infectious disease in vertebrates, but for which I could not locate corroborating infectious disease information. A name to watch for in the future is *Chlamydosauromyces punctatus*, which is a dermatophyte of reptiles and might prove to be pathogenic.

9.2 Examples of Fungal Genera Whose Non-infectious Pathogenicity Mimics Infectivity

9.2.1 Examples of Systemic Toxicoses That Can Either Mimick or Obscure Fungal Infectivity for Vertebrates

There are a great many fungal genera which produce compounds that are toxic for vertebrates and some of those toxicities can either mimic or obscure fungal infectivity for vertebrates, as recently reviewed by Enyiukwu et al. (2018). I have provided a few examples of fungal genera which poduce such toxic effects.

9.2.1.1 Ergot

Ergot produces a toxicosis that causes vasoconstriction resulting in gangrene.

Claviceps **[known toxic affect upon: birds and mammals]**
Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Clavicipitaceae; Genus: *Claviceps*. The species *Claviceps purpurea* is a plant pathogen that seems not to cause infection in vertebrates. *Claviceps purpurea* produces ergot, which naturally causes a toxicosis termed ergotism in animals that ingest the fruiting structures of *Claviceps purpurea* while consuming either contaminated grasses and grains (Family Poaceae) or food products made from contaminated grain. Humans also ingest derivatives of the toxin, the alkaloid ergotamine, for its hallucinogenic properties. The vasoconstriction caused by ergot can produce a gangrene typically affecting more poorly vascularized parts of the body, such as hands and fingers, feet and toes. The symptoms of ergotism include vomiting, diarrhea, hallucinations, desquamation, edema, loss of peripheral sensation and weak peripheral pulse, which ultimately can result in loss of the affected tissues and death of the affected animal. Ergot alkaloids cause lesions of the liver in birds, and in mammals cause vasoconstriction. Ergot has been used medicinally by humans for the purpose of vasoconstriction, although with potentially dangerous outcomes.

9.2.1.2 Trichothecenes and Atranones

The trichothecenes cause symptoms that include hepatitis, pulmonary congestion, and sudden death, while atranones can cause pulmonary inflamation.

Paramyrothecium does not seem to be infectious for vertebrates but it produces the trichothecene roridin with known toxic affect upon mammals.
Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Stachybotryaceae; Genus: *Paramyrothecium*. *Paramyrothecium roridum* previously was named *Myrothecium roridum*, it is an endophytic pathogen that causes crown, leaf and stem rot on a variety of plants including melons as well as oher fruit and

vegetable crops, is particularly noted to affect bedding plants grown in greenhouses and indoor plants including perennials, it causes leaf spot on both *Coffea arabica* as well as *Coffea canephora*, and has been found as a contaminant of commercial plant culture medium. *Paramyrothecium roridum* produces trichothecene mycotoxins including roridin, a toxin that notedly is created when either this fungal species or *Albifimbria verrucaria* (previously named *Myrothecium verrucaria*) grow on rye-grass (presumably *Lolium*) and white clover *Trifolium repens* in pasture, and also when these fungal species grow on stored feeds. Roridin causes sudden death in sheep and cattle, an outcome that is associated with necropsy lesions of abomasitis, hepatitis, pulmonary congestion and edema. The toxicity associated with this fungal species may complicate the understanding of its possible infectiousness for vertebrates.

Stachybotrys is infectious but that is overshadowed by its toxicity, it produces the trichothecenes roridin and satratoxin, plus atranones, with known toxic affects upon birds and mammals.
Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Stachybotryaceae; Genus: *Stachybotrys*. The *Stachybotrys* are found in soil and cellulose-containing materials such as rotting grain, leaf debris, paper and wood products. *Stachybotrys* grows during the times when those substrate materials are soaking wet. *Stachybotrys chartarum* is the most notable species in this fungal genus. *Stachybotrys chartarum* can grow in the lungs as a infection of human, although the infectivity caused by *Stachybotrys chartarum* is overshadowed by its associated toxicosis. Growth of *Stachybotrys chartarum* requires a high moisture content and one of its more common habitats is cellulose-rich water damaged construction materials, with its original isolation having been from wallpaper collected at a home in Prague. *Stachybotrys chartarum* often is not harmful until it dries and the spores have gone airborne. Toxic effects associated with *Stachybotrys chartarum* result from the atranone and trichothecene compounds which it produces. The ensuing toxicoses include those from ingestion, inhalation, and dermal contact, which may be associated with moldy feed and moldy grain. In humans, horses and other animals the disease symptoms associated with *Stachybotrys chartarum* include irritation of the mouth, throat, and nose; shock; dermal necrosis; a decrease in leukocytes; internal hemorrhage; and nervous disorders including tremors. The effect of *Stachybotrys chartarum* can be fatal, including in large mammals such as horses. In cattle, *Stachybotrys chartarum* produces bovine hyperkeratosis and necrotic dermatitis, and also has been indicated to have association with mastitis and diarrhea which might be either infectious or allergic in nature. In chickens, *Stachybotrys chartarum* can cause a moldy feed toxicosis termed “poultry hemorrhagic syndrome”. The fungal genus *Stachybotrys* is mentioned in Sect. 9.4 of this chapter because of that infectivity. I suggest Andersen et al. (2003) for an example analysis and listing of *Stachybotrys* toxins.

9.2.2 Allergic Hypersensitivity

Allergic hypersensitivity can include hypersensitivity pneumonitis which may either mimic or obscure infectivity. This disease results from inhaled organic dusts causing inflammation of the alveoli. In humans, this disease often is associated with either occupation or hobbies and can be fatal. I have provided here some example fungal genera which cause allergic hypersensitivity.

Cryptostroma is not known to be infectious for vertebrates, but does cause allergic hypersensitivity that affects mammals.
Phylum: Ascomycota; Class: Sordariomycetes; Order: Xylariales; Family: (not assigned); Genus: *Cryptostroma*. The species *Cryptostroma corticale* is a plant pathogens that causes sooty bark disease and death of maples (Genus *Acer*) including *Acer pseudoplatanus.* The inhaled fungal spores cause in human a hypersensitivity alveolitis termed "Maple-bark disease", which is a potentially debilitating pneumonitis. There is uncertainty as to whether *Cryptostroma corticale* also is infectious.

Lycoperdon causes allergic hypersensitivity that affects mammals.
Phylum: Basidiomycota; Class: Agaricomycetes; Order: Agaricales; Family: Lycoperdaceae; Genus: *Lycoperdon*. The *Lycoperdon* are saprobes. *Lycoperdon pyriforme* is known as the stump puffball and it grows mainly on stumps and roots of dead trees, mulch, and buried banches. *Lycoperdon pyriforme* often appears to be growing on soil when decaying wood is beneath the soil surface. This fungal species is edible at an early stage of its growth. *Lycoperdon pyriforme* affects humans and dogs that have inhaled the fungal spores when playing near to puffballs. The disease which this causes is classified as a hypersensitivity pneumonitis rather that an infection and it can be fatal. The disease caused by *Lycoperdon pyriforme* does not seem to be infectious.

Serpula are infectious but that is overshadowed by their toxicity, with known affects upon mammals.
Phylum: Basidiomycota; Class: Agaricomycetes; Order: Boletales; Family: Serpulaceae; Genus: *Serpula*. *Serpula lacrymans* causes brown rot and dry rot damage to timber. *Serpula lacrymans* naturally and typically is found in spruce and other conifers. Harvesting of those infected trees for constructing buildings transports the fungus across borders and brings the fungus indoors. *Serpula lacrymans* affects humans by causing hypersensitivity pneumonitis (which is not infectious), and infections which result in pulmonary fibrosis, sinusitis, bronchitis, and pneumonia.

Note: The name *Serpula* also refers to a genus of calcareous tube worms of the Class Polychaete. The fungal genus *Serpula* is mentioned in Sect. 9.4 of this chapter because of that infectivity.

9.3 Examples of Fungi Whose Infectiousness for Invertebrate Animals Has Indirect Ecological Impact Upon Vertebrates

Fungi that infect invertebrates do have a strong indirect affect upon vertebrates by impacting vertebrate food supplies. I provide here some examples of soil and plant fungi that cause disease in decapod crustaceans, because that impact is obvious to all who might be reading this chapter. However, the impact of fungi upon other arthropods and non-arthropod food sources also critically affects the vertebrates who dietarily depend upon those invertebrates.

Acremonium species NJM 0672 is an animal pathogen that affects both the mantis shrimp *Oratosquilla oratoria* which are wild caught, and kuruma prawns *Penaeus japonicus* which are raised commercially, but thus far this fungal species seems not to affect either fish or other vertebrates.

Exophiala cancerae affects the *Scylla* mangrove crabs, and this fungal species causes lethargic crab disease in the swamp ghost crab *Ucides cordatus* which also is primarily found in mangrove (Family Rhizophoraceae) forests. These two crab groups serve as important food sources for some human populations.

Fonsecaea brasiliensis causes disseminated infection in human and also causes lethargic crab disease in the swamp ghost crab *Ucides cordatus* which is primarily found in mangrove forests. *Ucides cordatus* serves as an important food source for some human populations.

Fusarium oxysporum causes gill infections in kuruma prawn *Penaeus japonicus*. *Fusarium solani* additionally infects kuruma prawn *Penaeus japonicus* by producing hyphae and related tissue destruction in the gills, maxillipeds, pereiopods, thoracic body wall, thoracic central nerve and occasionally in the ventral thoracic artery. *Fusarium verticillioides* additionally affects crustaceans, as evidenced by this fungal species having caused gill lesions in kuruma prawn *Penaeus japonicus*.

I have not otherwise addressed in this chapter the subject of fungi that infect invertebrates except for a few bits of information that are included among the descriptions contained in Sect. 9.4, perhaps most notably represented by the entomopathogenic fungal species *Beauveria bassiana* and the entomopathogenic fungal genus *Cordyceps*.

9.4 The "Active" List: Genus and Species Names of Soil Fungi and Plant Fungi That Infect Vertebrates

When examining these entries, you will find instances when I indicate that a fungal genus 'possibly infects' some vertebrate group. Indication of possible infectivity usually signifies my having found reports of the fungal genus demonstrating infectivity in a particular group of vertebrates, but I feel that I must offer uncertainty regarding current relevance of that discovery. In most such cases, my indicating

possible infectivity results from the people who taxonomically identified the fungal isolates having determined only the fungal genus without specifying the fungal species. Incomplete taxonomic identification represents a problem when a fungal genus has lost some of its member species due to taxonomic reassignment, opening a possibility that the fungal species which caused a particular reported infection has been moved to a different fungal genus. There also are times when molecular identification techniques are used, but those identifications could be incorrect due to errors in the public databases. Stavrou et al. (2018) have reported on that type of error, and you will notice it mentioned in the entry for *Naumovozyma*. To gain understanding on how fungi are identified, I would suggest the book by Liu (2011) and I hope a new edition of that will be published.

Additionally, there are times when a fungus is found in a body location that suggests infection but uncertainty must exist about whether the ocurrence may have been commensal without pathogenicity versus a subclinical infection. Examples of that uncertainty are the discovery of fungi in the bile of seemingly healthy anuran amphibians. Volume 3 in this book series addressed the topic of commensal and symbiont microorganisms that opportunistically demonstrate pathogenicity (Hurst 2016).

9.4.1 Acaulium [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Microascales; Family: Microascaceae; Genus: *Acaulium*. Members of the genus *Acaulium* are found in caves and at least one species belonging to this genus, *Acaulium caviariforme*, which has been found on the cadavers of mammals in caves and mines, is suspected of being an obligate troglobite. The fungal genus *Acaulium* also causes disease in mammals. *Acaulium acremonium*, which previously was named *Scopulariopsis acremonium*, is saprotrophic and presumably found in soil, and it causes in human *Homo sapiens* invasive sinusitis and chronic pericardial infection that can include fungal presence in the pleural fluid.

Note: *Acaulium nigrum*, which also has been named *Penicillium nigrum*, now is *Microascus niger*.

9.4.2 Achaetomium [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: Chaetomiaceae; Genus: *Achaetomium*. Member species of the genus *Achaetomium* are rhizosphere fungi and some also cause disease in mammals. The two species *Achaetomium globosum* and *Achaetomium strumarium* often are mentioned as causing invasive infections of human, but specific disease details were not found.

9.4.3 Acremonium [Infects: Amphibians, Birds, Mammals, and Reptiles]

The species with this fungal genus name are plant pathogens and seem saprophytic, being isolated from rotting and dead plant material, and they also are isolated from soil. The *Acremonium* used to be part of the genus *Cephalosporium* and the genus name *Cephalosporium* still exists. The species of *Acremonium* currently are grouped into four genera, all belonging to the phylum Ascomycota. The genus *Acremonium* has been asssociated with a combined polymicrobial keratitis in human also caused by *Stemphylium* and alpha-*Streptococcus*, but those microbes were not identified at the level of species. *Acremonium*, similarly not identified at the species level, have been cultured from injured and repressed tissue regeneration sites in giant aquatic Ozark Hellbender salamander *Cryptobranchus alleganiensis bishopi*. The *Acremonium* associated with disease of vertebrates seeming are often not identified at the fungal species level. Only one of these four genera seems to contain species that specifically have been identified as pathogenic for vertebrates. *Acremonium* additionally produce fodder-associated toxicosis in mammals and poultry but this chapter focuses on infectious disease. I have numbered these four genera by the order that they are listed in the National Center for Biotechnology Information database.

Genus 1 [not shown to infect vertebrates]
Phylum: Ascomycota; Class: (not assigned); Order: (not assigned); Family: (not assigned); Genus: *Acremonium*. This genus contains *Acremonium species NJM 0672* which is an animal pathogen that affects both the mantis shrimp *Oratosquilla oratoria* which are wild caught, and Kuruma Prawns *Penaeus japonicus* which are raised commercially, but thus far this fungal species seems not to affect either fish or other vertebrates.

Genus 2 [infects: birds, mammals, and reptiles]
Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: (not assigned); Genus: *Acremonium*. The species *Acremonium egyptiacum*, *Acremonium exuviarum*, and *Acremonium sclerotigenum* belong to this same genus. The members of this genus can produce in human hyalohyphomycoses including skin infections, subcutaneous infections, mycetomas including eumycotic mycetoma, infections of the nails (onychomycosis), corneal ulcers, endophthalmitis, chronic sinus infections, arthritis, osteomyelitis, peritonitis, endocarditis, pneumonia, and cerebritis including meningitis. *Acremonium egyptiacum* and *Acremonium sclerotigenum* have been found as a combined infection in human complicating the course of aplastic anaemia. This fungal genus additionally produces infections in other mammals. *Acremonium sclerotigenum* has been isolated from an African ostrich egg *Struthio camelus* in association with termination of the embryogenesis. In a particular note, *Acremonium exuviarum*, which produces the toxic peptaibol acrebol, is a pathogenic dermatophyte in reptiles. Acrebol is a powerful inhibitor of the mitochondrial complex III, inhibiting the respiratory chain and causing ATP depletion.

Genus 3 [not shown to infect vertebrates]
Phylum: Ascomycota; Class: Sordariomycetes; Order: Glomerellales; Family: Plectosphaerellaceae; Genus: *Acremonium*.

Genus 4 [not shown to infect vertebrates]
Phylum: Ascomycota; Class: Leotiomycetes; Order: (not assigned); Family: (not assigned); Genus: *Acremonium*.

Notes: *Acremonium alabamense* now is named *Thielavia terrestris*; *Acremonium falciforme* which causes mycetomas in humans now is named *Fusarium falciforme*; *Acremonium kiliense* which produces mycetomas in humans now is *Sarocladium kiliense*; *Acremonium recifei* which produces mycetomas in humans now is *Xenoacremonium recifei*; and *Acremonium strictum* now is *Sarocladium strictum*.

9.4.4 Acrophialophora [Infects: Mammals]

Phylum: Ascomycota; Class: (not assigned); Order: (not assigned); Family: (not assigned); Genus: *Acrophialophora*. *Acrophialophora fusispora* is found in soil, air and on various plants, it affects human by causing keratitis (corneal infection), cerebral infections including brain abcess, plus pulmonary colonization and infection.

9.4.5 Albifimbria [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Stachybotryaceae; Genus: *Albifimbria*. *Albifimbria verrucaria* previously was named *Myrothecium verrucaria*. It causes leaf spot and pod blight, and can be found growing on rye-grass and white clover plants either in pasture or as stored feeds. *Albifimbria verrucaria* also is associated with sudden death in sheep *Ovis aries* and cattle *Bos taurus*, and has been associated with necropsy lesions related to abomasitis, hepatitis, pulmonary congestion and edema. In human, this fungal species causes allergic reactions and therefore may similarly affect other mammals. Interestingly, bilirubin oxidase from *Albifimbria verrucaria* (look for that information using this fungal species previous name *Myrothecium verrucaria*) catalyzes the oxidation of bilirubin to a colorless product. Perfusion of human blood containing bilirubin through filters packed with immobilized *Albifimbria verrucaria* bilirubin oxidase resulted in degradation of 90% of the bilirubin per pass, although toxicity and allergenicity associated with *Albifimbria verrucaria* may reason caution if this approach is used as an experimental treatment for the disease hyperbilirubinemia. Toxins produced by this fungal species include the trichothecene roridin.

9.4.6 *Allomyces* [Infects: Fish]

Phylum: Blastocladiomycota; Class: Blastocladiomycetes; Order: Blastocladiales; Family: Blastocladiaceae; Genus: *Allomyces*. The *Allomyces* are a genus of soil microbes. They mostly are isolated from soils in tropical countries, and commonly also are found in ponds, rice fields, and slow-moving rivers. *Allomyces anomalus* has been isolated from infections of the Gibelion catla *Catla catla*, clown knifefish *Chitala chitala*, snakehead murrel *Channa striata*, Jayanti rohu *Labeo rohita*, and great snakehead *Channa marulius*.

9.4.7 *Alternaria* [Infects: Birds, Fish, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Pleosporaceae; Genus: *Alternaria*. Members of the genus *Alternaria* are found in most outdoor environments. The *Alternaria* are common saprobes that thrive in damp soil consuming wood, decaying plants, and other dead organic debris including food stuffs. Some *Alternaria* are known plant pathogens and often found on weakened plants, with infected plants sometimes demonstrating a dark olive green to brown coloration and velvety texture. *Alternaria alternata*, in particular, is a noted cause of seed mold in carrot *Daucus carota*, and has been isolated from surface sterilized leaf segments of ethnopharmaceutically important medicinal herbs collected from Bhadra River Project Area in the Malnad region of Southern India suggesting a possible endophytic existance for the fungus. The *Alternaria* also have been found in outdoor air and they are disseminated as a dry spore through the action of wind. *Alternaria* has caused epidermal cysts in the comb of chicken *Gallus gallus* although the causative fungus for those cysts was not identified to the species level. The genus *Alternaria* has been noted as causing mycosis in the gilthead seabream *Sparus aurata* but that fungal isolate was not identified to species specificity. *Alternaria alternata* causes skin erosions and ulcerations in sharp tooth catfish *Clarias gariepinus*. *Alternaria tenuis*, which now is named *Alternaria alternata*, additionally has been identified as having caused infections of Gibelion catla *Catla catla*, clown knifefish *Chitala chitala*, snakehead murrel *Channa striata*, Jayanti rohu *Labeo rohita*, and great snakehead *Channa marulius*. *Alternaria alternata* causes shell necrosis in turtles. In terms of pathogenicity for mammals, *Alternaria alternata* causes keratitis and corneal ulcers in at least human, domestic cat *Felis catus* and horse *Equus caballus*. *Alternaria* more typically have been found as causative agents of subcutaneous lesions termed hyphomycosis both acting alone and as a mixed infection with the species *Phaeosclera dematioides* (see the listing for *Phaeosclera*). *Alternaria* also have been known to cause nail infections. Members of the genus *Alternaria* will attack the upper respiratory tract causing sinusitis and nasal lesions that can include erosion of the nasal septum. Most species of

Alternaria do not grow at 37 °C which fortunately imposes some limits on their internal pathogenicity for humans. Additional *Alternaria* species known to affect humans and other mammals are: *Alternaria atra* which previously was named *Ulocladium atrum* (affects human), *Alternaria botrytis* which previously was named *Ulocladium botrytis* (infects human), *Alternaria caespitosa* which previously was named *Botryomyces caespitosus* (infects human), *Alternaria chartarum* which previously was named *Ulocladium chartarum* (infects human), *Alternaria chlamydospora* (infects human), *Alternaria dianthicola* (infects human), *Alternaria infectoria* (infects dog and human), *Alternaria longipes* (infects human), and *Alternaria tenuissima* (infects human). *Alternaria* also produce trichothecene mycotoxins and are noted for causing toxicosis in poultry. As an allergen, the *Alternaria* in general have been known to cause both Type I and Type III symptoms as well as asthma in mammals.

9.4.8 Amesia [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: Chaetomiaceae; Genus: *Amesia*. *Amesia atrobrunnea*, which previously was named *Chaetomium atrobrunneum*, is an environmental microorganism for which the reservoirs are believed to include soil. The skin infections that this fungal species causes in humans are thought to result from direct contact with environmental reservoirs and accordingly farmers or children may have greater susceptibility.

Note: *Amesia* also is the name for a genus of moth in the Family Zygaenidae.

9.4.9 Anthopsis [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Chaetothyriales; Family: Cyphellophoraceae; Genus: *Anthopsis*. The species *Anthopsis deltoidea* has been isolated from horticultural soil in Italy and affects human by causing bursitis.

9.4.10 Aphanoascus [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Onygenaceae; Genus: *Aphanoascus*. The members of this genus typically are found in soil and feces. The species *Aphanoascus fulvescens* generally is considered to be a nonpathogenic commensal of the skin, but it has been determined to cause dermatophytosis which is a fungal infection of the skin and it thusly affects cat and horse.

Note: *Aphanoascus terreus*, which presently is named *Keratinophyton terreum*, is a dermatophyte found on the combs of chicken *Gallus gallus* but this fungal species is not know to be pathogenic for the birds on which it is found.

9.4.11 Apiotrichum [Infects: Mammals]

Phylum: Basidiomycota; Class: Tremellomycetes; Order: Trichosporonales; Family: Trichosporonaceae; Genus: *Apiotrichum*. The members of this genus are associated with soil. *Apiotrichum montevideense*, which previously was named *Trichosporon montevideense*, causes onychomycosis in human, Japanese macaque *Macaca fuscata*, and dog *Canis lupus familiaris*.

9.4.12 Apophysomyces [Infects: Mammals]

Phylum: Mucoromycota; Class: Mucoromycetes; Order: Mucorales; Family: Mucoraceae; Genus: *Apophysomyces*. The members of this genus typically are found in soil and decaying vegetation. The species *Apophysomyces elegans* causes ulcerative dermatitis (necrotizing fasciitis), which can become a systemic infection and possibly is associated with abortion in cattle and human.

9.4.13 Arachnomyces [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Arachnomycetaceae (The Arachnomyces typically are found in soil and rotting wood.); Genus: *Arachnomyces*. *Arachnomyces nodosetosus* which previously was named *Onychocola canadensis*, and *Arachnomyces kanei* which previously was named *Onychocola kanei*, both cause nail and skin infections in human.

9.4.14 Arnium [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: Lasiosphaeriaceae; Genus: *Arnium*. *Arnium leporinum*, which causes endocarditis in human, typically is found in dung which brings some uncertainty as to whether or not it truly is a soil microbe.

9.4.15 *Arthrinium* [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Xylariales; Family: Apiosporaceae; Genus: *Arthrinium*. *Arthrinium phaeospermum* causes cutaneous infections of humans. It is considered to be a cosmopolitan fungus which commonly is isolated from decomposing plant material and soil. This fungus is characterized as having a white coloration with the texture of wooly tufts or long soft hairs. It develops brown to black spore clusters and is disseminated as a dry spore by wind.

9.4.16 *Arthroderma* [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Arthrodermataceae; Genus: *Arthroderma*. Members of the genus *Arthroderma* are found in soil and on skin, and typically they affect the skin by causing a condition known as dermatophytosis. The species *[Arthroderma] racemosum*, previously named *Microsporum racemosum*, causes nail infections in human. *Arthroderma redellii*, which previously was named *Trichophyton redellii*, causes skin infections that resemble white-nose syndrome in hibernating bats and has been noted in the mouse-eared bat *Myotis velifer* and little brown bat *Myotis lucifugus*. *Arthroderma uncinatum* is a soil microbe that causes cutaneus infections in human.

Notes: *Arthroderma benhamiae* now has been *renamed Trichophyton benhamiae*; *Arthroderma gypseum*, previously also named *Achorion gypseum*, *Closterosporia gypsea*, *Gymnoascus gypseus*, *and Microsporum gypseum*, now is *Nannizzia gypsea*; *Arthroderma obtusum* has been renamed *Nannizzia nana*; *Arthroderma otae* has been renamed *Microsporum canis*; *Arthroderma simii* has been renamed *Trichophyton simii*; and *Arthroderma vanbreuseghemii* has been renamed *Trichophyton mentagrophytes*.

9.4.17 *Arthrographis* [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: (not assigned); Family: Eremomycetaceae; Genus: *Arthrographis*. *Arthrographis kalrae*, previously named *Oidiodendron kalrae*, occurs in soils and affects human by causing corneal infections, nail infections termed onychomycosis, soft tissue infections, sinusitis, ophthalmitis, knee joint infections following penetrating injury, fungemia, and pulmonary infections. *Arthrographis kalrae* also affects mice as an experimental infection.

Note: *Arthrographis cuboidea*, previously also named *Geotrichum microsporum* and *Oospora cuboidea*, currently is *Scytalidium cuboideum*.

9.4.18 Ascotricha [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Xylariales; Family: Xylariaceae; Genus: *Ascotricha*. *Ascotricha chartarum* is an environmental fungus that has been isolated from paper, linoleum, plaster, cardboard, cork, skin, soil, plant material and wood. *Ascotricha chartarum* affects humans by causing sinus infections that can include softening of the surrounding bone.

9.4.19 Aspergillus [Infects: Birds, Fish, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Eurotiales; Family: Aspergillaceae; Genus: *Aspergillus*. Members of the *Aspergillus* genus typically are found living saprophytically in mesophilic environments including decaying vegetation such as leaves, and in soil. *Aspergillus* also are found on stored food and feed products in tropical and subtropical regions. Some *Aspergillus* species are parasitic on insects, plants, and animals including human. Many members of the genus *Aspergillus* also produce neurotoxins which can complicate the concept of infections caused by these fungi. The production of trichothecene mycotoxins, which may be associated with the disease *Aspergillus* causes in humans and other animals, notably is dependent on both the fungal species as well as the fungal strain, and also can depend upon on the food source used by the fungus. Some *Aspergillus* toxins are carcinogenic in animal species including carcinogenicity for humans. *Aspergillus* have been reported very typically to cause opportunistic infections of the ears and eyes. Members of the genus *Aspergillus* cause subcutaneous mycotic nodules, produce both mastitis and foot infection, and can cause abscesses. This fungal genus additionally causes enteritis, disease in the both the upper as well as lower respiratory tracts including pneumonia, ocular infection, cerebral infection, myelitis (inflammation of the spinal cord), can be found in the bone marrow, causes urinary tract infections, and notably causes infection of the gutteral pouch in equids (horses, Family Equidae). *Aspergillus* also can produce systemic infections. There have been indications of *Aspergillus* causing disease in kangaroo *Macropus* but in those cases the fungi were not identified to the level of species. The effects of *Aspergillus* upon the unborn offspring of placental mammals result both from placentitis as well as directly infecting the fetus, causing abortion during the second and third trimesters of pregnancy as noticed particularly in bovines (members of the family Bovidae, subfamily Bovinae). *Aspergillus flavus* and *Aspergillus nidulans* are by themselves capable of causing abortion in cattle, and these same two *Aspergillus* species variously also have been found to coexist as part of mixed fungal infections with *Lichtheimia corymbifera*, *Rhizomucor pusillus*, and *Rhizopus oryzae* that caused abortion in cattle (see the listings for *Lichtheimia*, *Rhizomucor*, and *Rhizopus*. *Aspergillus amstelodami* has been reported to cause disease in cattle, and also

lung infection in a species of giant tortoise *Testudo elephantopis* but that tortoise species name no longer is recognized. *Aspergillus candidus* affects humans. *Aspergillus caninus*, which previously was named *Phialosimplex caninus*, causes fungal myelitis in dog. *Aspergillus chlamydosporus*, which previously was named *Phialosimplex chlamydosporus*, also affects dog. *Aspergillus clavatus* and *Aspergillus conjunctus* affect human. *Aspergillus deflectus* causes disease in both human and dog. *Aspergillus felis* causes disease in cat. *Aspergillus fischeri*, which previously was named *Neosartorya fischeri*, affects human notably causing bronchopulmonary mycosis. The species *Aspergillus flavus*, which affects both cattle and dog, produces mycetomas in humans. *Aspergillus flavus* also causes mycosis (fungal infection) in numerous groups of fish including tilapia *Oreochromis* and gilthead seabream *Sparus aurata*, and notably causes both skin erosions as well as ulcerations in sharp tooth catfish *Clarias gariepinus*. *Aspergillus flavipes* causes disease in dog. The species *Aspergillus fumigatus* is a thermophilic fungus found in mushroom compost (typically contains wheat straw, dried blood, horse manure and ground chalk that have been composted together). As a vertebrate pathogen, *Aspergillus fumigatus* causes epidermal cysts in chicken *Gallus gallus*, and mycosis in many fish species including gilthead seabream *Sparus aurata*, Gibelion catla *Catla catla*, clown knifefish *Chitala chitala*, snakehead murrel *Channa striata*, Jayanti rohu *Labeo rohita*, and the great snakehead *Channa marulius*. *Aspergillus fumigatus* also affects numerous mammalian species including human, domestic guinea pig, American bison *Bison bison*, sheep, goat *Capra hircus*, cattle, dog, cat, camel *Camelus*, capybara *Hydrochoerus hydrochaeris*, rabbit *Oryctolagus cuniculus*, red deer (sometimes called an elk) *Cervus elaphus*, and horse. *Aspergillus glaucus*, which previously was named *Eurotium herbariorum*, causes eye infections in human. *Aspergillus hiratsukae*, previously named *Neosartorya hiratsukae*, also affects humans. *Aspergillus japonicus* causes mycosis in tilapia *Oreochromis*. *Aspergillus nidulans* previously was named *Emericella nidulans*, and causes fungal infections in a number of fish species including Gibelion catla *Catla catla*, clown knifefish *Chitala chitala*, snakehead murrel *Channa striata*, Jayanti rohu *Labeo rohita*, and great snakehead *Channa marulius*. *Aspergillus nidulans* infects cats and cattle, in human it apparently causes both urinary tract infections and mycetomas, and also this fungal species produces the known carcinogenic toxin sterigmatocystin. *Aspergillus niger* has been isolated from surface sterilized leaf segments of ethnopharmaceutically important medicinal herbs collected from the Bhadra River Project Area in the Malnad region of Southern India, suggesting that the ecology of this fungal species includes an endophytic presence. As a pathogen of fish, *Aspergillus niger* causes skin erosions and ulcerations in sharp tooth catfish *Clarias gariepinus*, mycosis in gilthead seabream *Sparus aurata*, produces both internal and external infections in common carp *Cyprinus carpio*, and additionally causes fungal infections in Gibelion catla *Catla catla*, clown knifefish *Chitala chitala*, snakehead murrel *Channa striata*, Jayanti rohu *Labeo rohita*, and great snakehead *Channa marulius*. *Aspergillus niger* infects numerous mammalian species including alpaca *Vicugna pacos*, cat, cattle, cervids (Family Cervidae), dog, sheep, goat, and human. *Aspergillus ochraceus* and *Aspergillus panamensis* affect

human. *Aspergillus parasiticus* notably and very seriously affects both sugar cane *Saccharum* and corn *Zea mays subspecies mays*, and it causes mycosis in gilthead seabream *Sparus aurata*. *Aspergillus puniceus* affects humans. *Aspergillus rugulosus*, which previously was named *Emericella rugulosa*, causes abortion in cattle and also affects human. *Aspergillus sclerotialis*, which previously was named *Phialosimplex sclerotialis* and also has been named *Sagenomella sclerotialis*, affects dog. *Aspergillus tamarii* affects humans. *Aspergillus terreus* causes mycosis in both tilapia *Oreochromis* and in gilthead seabream *Sparus aurata*, plus it also infects human, dog, and cattle. *Aspergillus quadrilineatus*, which previously was named *Emericella quadrilineata*, causes sinusitis and skin infections in human. *Aspergillus spinulosporus* previously was named *Emericella echinulata*, and it causes disseminated infections in human. *Aspergillus thermomutatus*, which previously was named *Neosartorya pseudofischeri*, is noted for causing peritonitis and invasive gastrointestinal tract infection in human. *Aspergillus udagawae* previously was named *Neosartorya udagawae*, it causes chronic invasive infections of humans including those of the cornea and also produces chronic granulomatous disease. *Aspergillus versicolor* affects dog and human, and in particular *Aspergillus versicolor* causes disseminated aspergillosis.

9.4.20 Aureobasidium [Infects: Amphibians and Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Dothideales; Family: Saccotheciaceae; Genus: *Aureobasidium*. The species *Aureobasidium pullulans* typically is found in soil, fresh water, marine estuary sediments, air, limestone, and as mildew both on wood as well as finishes. *Aureobasidium pullulans* also is found on the aerial portion of plants including fruit, and has been isolated from surface sterilized leaf segments of ethnopharmaceutically important medicinal herbs collected from Bhadra River Project Area in the Malnad region of Southern India, which suggests that this fungal species may have an endophytic presence. On wood, this fungal species produces a cream to pink (when young) or dark brown (when old) coloration and a yeast-like texture. The fungal species *Aureobasidium pullulans* causes disseminated subclinical internal infections of anurans (the frogs and toads, Order Anura). *Aureobasidium pullulans* causes subcutaneous infections in cat that include nodule formation. As a infectious agent of humans, *Aureobasidium pullulans* has been linked to skin lesions and subcutaneous infections including keratosis, as well as causing fungemia, meningitis, peritonitis, and spleen abscess. In addition to producing infections, although *Aureobasidium pullulans* is not known to be a toxigenic agent it does cause hypersensitivity pneumonitis associated with usage of humidifiers and air conditioners. *Aureobasidium melanogenum* and *Aureobasidium proteae* also affect human.

9.4.21 *Auxarthron* [Infects: Birds and Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Onygenaceae; Genus: *Auxarthron*. The *Auxarthron* are soil microbes. *Auxarthron kuehnii*, which previously was named *Amauroascus kuehnii*, is a dermatophyte of birds although the possibility of disease being associated with that presence seems uncertain. Deer horn keratin is a substrate for *Auxarthron kuehnii*. *Auxarthron ostraviense* and *Auxarthron umbrinum* cause nail infections in humans.

9.4.22 *Barnettozyma* [Infects: Mammals]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales Family: Phaffomycetaceae; Genus: *Barnettozyma*. Members of the genus *Barnettozyma* are isolated from soil and tree fluxes in adddition to being found in water and animal dung. *[Candida] norvegica*, which has belonged to the genus *Candida* and previously was named *Paratorulopsis norvegica*, is considered to be an opportunistic pathogen of mammals. *[Candida] norvegica* is found in human sputum, is found in cattle milk, and causes mastitis in cattle.

9.4.23 *Basidiobolus* [Infects: Amphibians, Mammals, and Reptiles]

Phylum: Zoopagomycota; Class: Basidiobolomycetes; Order: Basidiobolales; Family: Basidiobolaceae; Genus: *Basidiobolus*. The *Basidiobolus* are found in decaying plant detritus including decaying leaf litter. The species *Basidiobolus ranarum* typically causes in human subcutaneous infections including skin ulcers and mycotic nodules, and also can cause endophthalmitis as well as systemic infections. *Basidiobolus ranarum* similarly affects other species of mammal including horse and dog. *Basidiobolus ranarum* has caused mycotic dermatitis in Wyoming toad *Anaxyrus baxteri*, and jaw tumor in Aldabra giant tortoise *Aldabrachelys gigantea*. *Basidiobolus* infections in human additionally can cause lung abcess, lesions on the liver, and intestinal infections that include ulcers in the cecum. *Basidiobolus ranarum* has been found in the intestinal contents of amphibians, fish, and reptiles, but without indication of associated pathogenicity. *Basidiobolus haptosporus* produces subcutaneous infections and also paranasal sinus infections in human. *Basidiobolus magnus* additionally affects human.

9.4.24 *Batrachochytrium* [Infects: Amphibians and Fish]

Phylum: Chytridiomycota; Class: Chytridiomycetes; Order: Rhizophydiales; Family: (not assigned); Genus: *Batrachochytrium*. Some chytrids are soil microbes so this possibly also is a soil microbial genus, although its natural environmental presence has yet to be determined. The *Batrachochytrium* can infect and proliferate on zebrafish *Danio rerio* tissue with the direct symptoms of infection including fin erosion, cell apoptosis and muscle degeneration. *Batrachochytrium dendrobatidis* causes a disease in amphibians that is called Amphibian chytridiomycosis and characterized by parakeratotic hyperkeratosis, which is a marked thickening of the stratum corneum, and hyperplasia of the epidermal layer, which is an abnormal increase in the number of cells of the epidermis. It is thought that these symptoms in amphibians possibly may impair cutaneous respiration and osmoregulation, and fungal toxicosis might be a compounding factor in death of the amphibian host. *Batrachochytrium* may be a commensal in reptiles. *Batrachochytrium* does infect the gastroentestinal tract of crayfish of the genus *Procambarus* and the species *Orconectes virilis*, in which the fungal zoosporangia grow colonially just below the gastrointestinal epithelial surface. These crayfish often are syntrophic with *Batrachochytrium* infected amphibians.

9.4.25 *Beauveria* [Infects: Fish, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Cordycipitaceae; Genus: *Beauveria*. The *Beauveria* are found in plant debris and soil, with some *Beauveria* species being known parasites of insects and for that reason they have been incorprated into pesticides. *Beauveria bassiana* is an entomopathogenic fungus that also infects several different groups of vertebrates. *Beauveria bassiana* infects embryos of the fish species inland silverside *Menidia beryllina* causing embryo rupture and death. Damage to the embryos presumably followed adherence of spores to the chorion, followed by spore germination and penetration of the germ tube into the embryos. *Beauveria bassiana* causes pulmonary lesions in American alligator *Alligator mississippiensis* and also causes pulmonary disease in giant galapagos tortoise *Chelonoidis niger galapagoensis*. In human, *Beauveria bassiana* causes disseminated infection.

9.4.26 *Bipolaris* [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Pleosporaceae; Genus: *Bipolaris*. The *Bipolaris* are a widespread fungal genus. They typically are plant pathogens found in subtropical and tropical regions and

frequently are associated with grasses, plant material, decaying food, and soil. *Bipolaris* also can cause cutaneous and subcutaneous infections of mammals. In human, members of the fungal genus *Bipolaris* cause subcutaneous infection, sinusitis, keratitis, ABPM (allergic bronchopulmonary mycosis due to coinfection with *Aspergillus*), pneumonia and disseminated infections. The following *Bipolaris* species cause disease in human: *Bipolaris bicolor*, *Bipolaris chloridis*, *Bipolaris cookei*, *Bipolaris drechsleri* (also affects cat), *Bipolaris eleusines*, *Bipolaris heveae*, *Bipolaris iridis*, *Bipolaris leersiae*, *Bipolaris oryzae* (previously was named *Cochliobolus miyabeanus*), *Bipolaris panici-miliacei*, *Bipolaris sacchari*, *Bipolaris setariae*, *Bipolaris sorghicola*, *Bipolaris sorokiniana*, *Bipolaris stenospila*, *Bipolaris tetramera*, *Bipolaris urochloae*, *Bipolaris victoriae*, *Bipolaris yamadae*, *Bipolaris zeae*, and *Bipolaris zeicola*.

Notes: *Bipolaris australiensis* now is *Curvularia australiensis*, and *Bipolaris hawaiiensis* now is *Curvularia hawaiiensis*.

9.4.27 Bjerkandera [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Polyporales; Family: Phanerochaetaceae; Genus: *Bjerkandera*. *Bjerkandera adusta* is an annual polypore fungus, widely distributed throughout the world, which is saprobic on the dead wood of deciduous trees and most commonly it appears on dead wood. *Bjerkandera adusta* also is found in Asian dust (Asian sand dust). *Bjerkandera adusta* additionally is a plant pathogen that causes white rot in live trees, and it is considered to be a "first fungus" that causes infection through bark damage. *Bjerkandera adusta* is one of the most important etiological fungi associated with chronic cough in human.

9.4.28 Blastobotrys [Infects: Mammals; Also Possibly Infects: Reptiles]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Trichomonascaceae; Genus: *Blastobotrys*. The *Blastobotrys* are found in soil. Both *Blastobotrys proliferans* and *Blastobotrys serpentis* cause invasive mycosis in human. There has been suggestion that *Blastobotrys* potentially also infects reptiles.

9.4.29 Blastomyces [Infects: Fish and Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Ajellomycetaceae; Genus: *Blastomyces*. The *Blastomyces* typically are found in

soil, decaying leaves, and wet decaying wood. Their spores can be inhaled and resultingly cause lung infections. The species *Blastomyces dermatitidis* causes pustular dermatitis, cellulitis, mastitis, endophthalmitis, lymphadenitis, and pneumonia, as well as systemic infections affecting the other internal organs of at least cat, dog, horse, and human. *Blastomyces dermatitidis* also has caused internally disseminated infection of a bottlenose dolphin, *Tursiops truncatus*, with that infection then having been transmitted to its attending veterinarian. *Blastomyces dermatitidis* also has been found associated with lesions in market fish. *Blastomyces parvus*, which previously has been named both *Emmonsia parva* and *Haplosporangium parvum*, causes pneumonic disease that affects humans and other mammals, perhaps most typically affecting wild rodents.

9.4.30 Boeremia [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Didymellaceae; Genus: *Boeremia*. *Boeremia exigua*, which previously was named *Phoma exigua*, is a plant pathogen that causes wet weather leaf blight and fruit spot, it also affects seedlings, plus it causes post-harvest rot of tubers and fruit. *Boeremia exigua* has caused fungal lung mass in a human.

9.4.31 Botryotrichum [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: Chaetomiaceae; Genus: *Botryotrichum*. The members of this genus grow on cellulose buried in soil. *Botryotrichum murorum*, which previously was *Chaetomium murorum*, causes phaeohyphomycosis in human.

9.4.32 Botrytis [Infects: Mammals]

Phylum: Ascomycota; Class: Leotiomycetes; Order: Helotiales; Family: Sclerotiniaceae; Genus: *Botrytis*. Most members of the genus *Botrytis* are important as plant pathogens and they are found virtually everywhere that plants are grown. *Botrytis* typically attack either weak plants or dying flowers, and in nature *Botrytis* help the recycling process of plant materials. As an example, *Botrytis cinerea* causes gray mold disease on various plant parts including the fruits of grape and strawberry *Fragaria x ananassa*. *Botrytis cinerea* may cause in human "winegrower's lung", a rare form of hypersensitivity pneumonitis. *Botrytis* also has caused necrotizing pulmonary granulomas in an otherwise apparently healthy human, but the fungal identification was specified only at the genus level.

9.4.33 *Byssochlamys* [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Eurotiales; Family: Thermoascaceae; Genus: *Byssochlamys*. *Byssochlamys* are part of the fungal community inhabiting large woody roots of healthy conifers. The members of this fungal genus typically are associated with food spoilage. The species *Byssochlamys spectabilis* more specifically is a thermophilic fungus found in soil, plants, and mushroom compost. *Byssochlamys* species can cause spoilage in canned fruit as a result of their heat-resistant ascospores. The species *Byssochlamys spectabilis* infects the nails, causes cutaneous infections, wound infections, mastitis, sinusitis, endophthalmitis, otitis media, osteomyelitis, and pyelonephritis in horse, dog, and goat. Additional information regarding the species *Byssochlamys spectabilis* can be found by searching for literature under its previous name, *Paecilomyces variotii*.

9.4.34 *Calyptrozyma* [Infects: Mammals]

Phylum: Ascomycota; Class: (not assigned); Order: (not assigned); Family: (not assigned); Genus: *Calyptrozyma*. The fungal species *Calyptrozyma arxii* is found in soil and associated with plant roots of the common reed *Phragmites australis*. *Calyptrozyma arxii* also has been isolated from the human lower oesophagus.

9.4.35 *Candida* [Infects: Amphibians, Birds, Fish, Mammals, and Reptiles]

Most species of *Candida* live on plants and rotting vegetation, typically degrading xylose, cellobiose, starch, and the aliphatic hydrocarbon components of plant cuticles. Some *Candida* are pathogens of plants. Some *Candida* species attack the insects that feed on plants. There are *Candida* which are either commensals or symbionts of additional invertebrate as well as vertebrate animal hosts. *Candida* infect amphibians, causing disseminated subclinical internal infections of anurans (Order Anura). Members of the *Candida* cause numerous diseases in mammals and that lengthy list includes arthritis, ascending urinary tract infections, dermatitis, endophthalmitis, enteritis, gastritis, mastitis, meningitis, necrotizing placentitis resulting in abortion, necrotizing stomatitis (necrotic periodontal disease), septicemia (fungemia is termed candidemia when caused by this genus, meaning *Candida* in the blood) and vaginitis. *Candida* species also can attack the oropharynx, bones, and lungs of mammals. There are *Candida* which are either commensals or symbionts of human. *Candida* have been isolated from pulmonary lesions of lizards (Superorder Lepidosauria) and chelonians (Order Testudines) but without identification at the fungal species level. The genus *Candida* has been divided, with some species remaining in the Family

Debaryomycetaceae and other species assigned to a group designated as being an unassigned genus of *Saccharomycetales* (*Saccharomycetales incertae sedis*). I have numbered these two genera by the order that they are listed in the National Center for Biotechnology Information database.

Genus 1 [infects: fish and mammals]
Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: (not assigned); Genus: *Candida*. The genus *Torulopsis* has been transferred to this genus of *Candida*. Under the previous taxonomic name *Torulopsis*, this *Candida* genus was identified as causing mycosis in the gilthead seabream *Sparus aurata* although that fungal identification was not species specific. The species *Candida africana*, which belongs to this genus, causes female genital infections in human.

Genus 2: [infects: amphibians, birds, fish, mammals, and reptiles]
Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Debaryomycetaceae; Genus: *Candida*. *Candida albicans* has caused oral infection in a pet parrot concurrent with oral infection in the birds human owner. *Candida albicans* also causes alimentary tract infections in birds, being known to affect at least chicken, turkey *Meleagris gallopavo*, and Common quail *Coturnix coturnix*. *Candida albicans* can colonize and invade zebrafish *Danio rerio* at multiple anatomical sites and kill the fish in a dose-dependent manner. *Candida albicans* is broadly infective of mammals including alpaca *Vicugna pacos*, cat, dog, pig *Sus scrofa*, horse, rabbit *Oryctolagus cuniculus*, kangaroo *Macropus*, sheep, goat, domestic guinea pig, and water buffalo *Bubalus bubalis*, plus *Candida albicans* causes mastitis in bovines and is found in mastitic bovine milk. *Candida albicans* very typically causes an infection of the throat that variously is called oropharyngeal candidiasis or thrush. *Candida albicans* also is one of the major causes of diaper dermatitis, often termed "diaper rash" and localized *Candida albicans* in the kidney can presented as a mass mimicking renal cell carcinoma. In reptiles, *Candida albicans* causes esophageal thrush, swelling of oral mucosa with pustules, miliary lesions of the liver, pneumonia, and enteritis. *Candida parapsilosis* is found naturally in rubber tree *Hevea brasiliensis* latex. *Candida parapsilosis* infects amphibians causing disseminated subclinical internal infections of anurans. *Candida parapsilosis* causes mastitis in cattle and is present in mastitic cattle milk, it also affects human causing keratitis including instances following corneal graft. *Candida tropicalis* causes disseminated subclinical internal infections of anuran amphibians; it infects kangaroo; causes mastitis in bovines and is found in mastitic cattle milk; also affects human including producing fungemia; and it has been loosely linked to rhinitis, tachypnea, and steatorrhea in reptiles. *Candida viswanathii* causes mastitis in cattle and is present in mastitic cattle milk.

Notes: *[Candida] auris* has been transferred to the genus *Clavispora*. *Candida catenulata* has been renamed *Diutina catenulata*. *Candida famata* has been renamed *Debaromyces hansenii*. *[Candida] glabrata*, previously named *Torulopsis glabrata*, now belongs to genus *Nakaseomyces*. *Candida guilliermondii* has been renamed *Meyerozyma guilliermondii*. *Candida humicola* which previously also was named

Asterotremella humicola and *Cryptococcus humicola*, now is *Vanrija humicola*. *Candida kefyr* and *Candida pseudotropicalis* have been combined into a single genus renamed *Kluyveromyces marxianus*. *Candida kruisii* has been renamed as *Teunomyces kruisii*. *[Candida norvegica]* has been moved to *Barnettozyma*. *Candida krusei* has been renamed *Pichia kudriavzevii*. *Candida rugosa* has been renamed *Diutina rugosa*. *Candida mesorugosa* has been renamed *Diutina mesorugosa*. *[Candida] pseudoaaseri* has been moved to the genus *Yamadazyma*. *Candida pseudorugosa* has been renamed *Diutina pseudorugosa*. *Candida vini*, previously also known as *Debaryomyces fluxorum*, *Debaryomyces fluxuum* and *Pichia fluxuum*, presently is named *Kregervanrija fluxuum*. *[Candida] zeylanoides* has been moved to *Kurtzmaniella*. *Torulopsis haemulonii* was renamed *Candida haemulonii* and then divided into *[Candida] haemulonis* and *[Candida] duobushaemulonis* with both of those species now belonging to the genus *Clavispora*.

9.4.36 Catenulostroma [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Capnodiales; Family: Teratosphaeriaceae; Genus: *Catenulostroma*. The genus *Catenulostroma* contains plant pathogens. *Catenulostroma castellanii*, which used to be named *Cladosporium castellanii*, affects human by causing the superficial dermatomycosis tinea nigra.

9.4.37 Cephalosporium [Infects: Reptiles]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: (not assigned); Genus: *Cephalosporium*. The *Cephalosporium* are soil fungi that also infect plants. *Cephalosporium* have been reported to cause pulmonary and disseminated infections in reptiles, however the fungal species was not determined and there is suggestion that the causative microbe may have been an *Acremonium*. This fungal genus also produces trichothecene mycotoxins, causing toxicosis particularly noted in poultry.

9.4.38 Cercospora [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Capnodiales; Family: Mycosphaerellaceae; Genus: *Cercospora*. Members of the genus *Cercospora* are widespread plant pathogens. They cause leaf spot on numerous plant species and are noted for causing purple, brown or black spots with decayed centers on field vegetables. *Cercospora apii* causes verrucous mycosis in human.

9.4.39 *Chaetomium* [Infects: Fish, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: Chaetomiaceae; Genus: *Chaetomium*. The *Chaetomium* often are found in soil enriched with either manure or cellulosic substrates including plant compost and straw. *Chaetomium* also grow on seeds, dung, and commonly are found growing in damp buildings, usually on the water damaged paper of sheetrock, as well as other paper and wood surfaces. *Chaetomium* have been isolated from surface sterilized leaf segments of ethnopharmaceutically important medicinal herbs collected from the Bhadra River Project Area in the Malnad region of Southern India, suggesting that this fungal genus may have an endophytic presence. *Chaetomium globosum* causes fungal infections in many species of fish, among them being the Gibelion catla *Catla catla*, clown knifefish *Chitala chitala*, snakehead murrel *Channa striata*, Jayanti rohu *Labeo rohita*, and great snakehead *Channa marulius*. In mammals, *Chaetomium globosum* causes cutaneous and subcutaneous skin infections and nail infections (onychomycosis) which can be invasive in dog, plus nail infections and also pneumonia in human. *Chaetomium globosum* causes scaling dermatitis in reptiles including red-eared slider turtle *Trachemys scripta elegans*, Mexican milksnake *Lampropeltis annulata*, and the Madagascar or Malagasy leaf-nosed snake *Langaha madagascariensis*. *Chaetomium perlucidum* produces lung and cerebral infections in human. *Chaetomium strumarium* also causes cerebral mycosis in human.

Notes: *Chaetomium* also are noted for causing toxicosis in poultry. *Chaetomium brasiliense* now is named *Ovatospora brasiliensis*.

9.4.40 *Chrysosporium* [Infects: Birds, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: (not assigned); Genus: *Chrysosporium*. The *Chrysosporium* are saprobic and typically found in soil and plant material where they hydrolyse cellulose. Members of the genus *Chrysosporium* often are keratinolytic which contributes to their pathogenicity in vertebrates. In mammals, the *Chrysosporium* typically cause dermatitis and foot rot. *Chrysosporium articulatum* infects human. *Chrysosporium keratinophilum* infects dog. *Chrysosporium longisporum* causes skin infections on reptiles. *Chrysosporium parvum* infects cat, cattle and horse. *Chrysosporium tropicum* infects dog and poultry. *Chrysosporium zonatum* infects human, notably causing pulmonary infection.

Notes: *Chrysosporium guarroi*, which infects reptiles, now is named *Nannizziopsis guarroi*. *Chrysosporium ophiodiicola*, which also infects reptiles, has been renamed *Ophidiomyces ophiodiicola*. *Chrysosporium parvum var. crescens*, previously also named *Ajellomyces crescens*, now is *Emmonsia crescens*. *Chrysosporium pannorum*, previously also named *Geomyces pannorum* and *Sporotrichum pannorum*, now is *Pseudogymnoascus pannorum*.

9.4.41 Cladophialophora [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Chaetothyriales; Family: Herpotrichiellaceae; Genus: *Cladophialophora*. The *Cladophialophora* typically are found in soil and decomposing plant materials including plant litter, trees and timber. Its member species can be found as plant pathogens, producing an olive to brown coloration on leaf surfaces of old and decaying plants. Some *Cladophialophora* species are more prevalent in subtropical and tropical forests. They are disseminated as a dry spore by the wind. Members of the genus *Cladophialophora* typically cause in mammals a chronic subcutaneous dermatitis that can disseminate to internal organs including the production of meningitis and brain abscesses. *Cladophialophora arxii* produces disseminated disease in human. *Cladophialophora bantiana* previously was named *Cladosporium trichoides* and there are suggestions that this species previously also was named *Xylohypha bantiana*. *Cladophialophora bantiana* is an opportunistic dermatophyte which can produce infections of the skin and disseminate to produce mycetomas of internal organs including cerebral abscesses, plus invade the adrenal glands, kidney, liver, and spleen. It has been known to affect alpaca, cat, dog and human. This fungal species additionally has been found contributing as a secondary infection to the bacterium *Ehrlichia canis* in dog. *Cladophialophora boppii*, which formerly was named *Taeniolella boppi*, infects human. *Cladophialophora carrionii* infects cattle, horse and human. *Cladophialophora devriesii* produces disseminated disease in human. *Cladophialophora emmonsii* infects cat and human. *Cladophialophora modesta*, *Cladophialophora mycetomatis*, *Cladophialophora samoensis*, and *Cladophialophora saturnica* infect human.

9.4.42 Cladorrhinum [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: Lasiosphaeriaceae; Genus: *Cladorrhinum*. The *Cladorrhinum* are endophytes. *Cladorrhinum bulbillosum* causes keratomycosis in human and horse.

9.4.43 Cladosporium [Infects: Amphibians, Fish, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Capnodiales; Family: Cladosporiaceae; Genus: *Cladosporium*. The *Cladosporium* typically are found on living, decaying and dead plant material, as well as on other decaying organic material, paint and textiles. Some members of this genus parasitize other fungi. *Cladosporium* can also grow inside damp buildings and are often found on shower

and window surfaces, usually feeding on soap and soil films, and I can attest they are persistent at that! *Cladosporium* is considered to be the most common mold in the outside air. In vertebrates, members of the genus *Cladosporium* cause ulcerative dermatitis, respiratory infections, and meningitis. The genus *Cladosporium* has been identified as causing mycosis in the gilthead seabream *Sparus aurata* but that fungal identification was not species specific. *Cladosporium* also has been identified as causing chromomycosis in frogs although that fungal identification was not species specific. The species *Cladosporium cladosporioides* has been isolated from surface sterilized leaf segments of ethnopharmaceutically important medicinal herbs collected from the Bhadra River Project Area in the Malnad region of Southern India, suggesting a possible endophytic existance for this fungal species. *Cladosporium cladosporioides* is known to opportunistically cause disease, typically dermatitis, of mammals including dog, sheep, cat, and giant panda *Ailuropoda melanoleuca*. At least in New Zealand, *Cladosporium cladosporioides* causes the skin disease 'black spot' of *Gekko*. *Cladosporium herbarum* causes skin erosions and ulcerations in sharp tooth catfish *Clarias gariepinus* and also infects human.

Cladosporium oxysporum infects human. *Cladosporium sphaerospermum* cause skin erosions and ulcerations in sharp tooth catfish *Clarias gariepinus* and infects human.

Notes: *Cladosporium elatum* now is *Ochrocladosporium elatum*. *Cladosporium werneckii* now is *Hortaea werneckii*.

9.4.44 Clavispora [Infects: Mammals]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Metschnikowiaceae; Genus: *Clavispora*. The members of this genus include plant pathogens. *Clavispora lusitaniae* typically is associated with cactus and necrotic plant tissues. This fungal species also has been isolated from materials of plant origin including cornmeal, citrus peel, fruit, and fruit juices. *Clavispora lusitaniae* additionally is found in bird manure which may represent a natural reservoir for this fungal species, and *Clavispora lusitaniae* is an opportunistic pathogen of invertebrate animals. In mammals, the species *Clavispora lusitaniae* causes invasive infections including fungemia and meningitis of human. *Clavispora lusitaniae* has been isolated from milk of cows with mastitis, suggesting that *Clavispora lusitaniae* may have caused the mastitis. Interestingly, this fungal species has been found as a natural caecal inhabitant of pig, where its potential role as an opportunistic pathogen remains to be explored. Additional information can by found by researching under *Candida lusitaniae*, which was the previous name of *Clavispora lusitaniae*. *[Candida] auris* causes nosocomial ear infections in human and those infections can be invasive. *Torulopsis haemulonii* was renamed *Candida haemulonii* and then divided into *[Candida] duobushaemulonis* and *[Candida] haemulonis*, which can cause deep cutaneous infections of human.

9.4.45 Coccidioides [Infects: Birds, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: (not assigned); Genus: *Coccidioides*. The members of this genus are soil microbes that also have evolved by interacting with their animal hosts. Members of the genus *Coccidioides* can cause ocular infections, lymphadenopathy which can disseminate, and pneumonia. Intestinal infection by *Coccidioides* has been reported in coypu *Myocastor coypus* but information regarding species level identification of the fungus could not be found. *Coccidioides immitis* infects a wide range of mammals, including cat, cattle, dog, horse, camel, llama *Lama glama*, rodents (Order Rodentia), black rhinoceros *Diceros bicornis*, and human. *Coccidioides immitis* also has caused respiratory infection in Sonoran gopher snake *Pituophis catenifer affinis*, and in chicken. *Coccidioides posadasii* similarly affects a wide range of mammals including dog, cat, cattle, sheep, horse, human, and llama, and it also has been reported in red coachwhip snake *Masticophis flagellum piceus*.

9.4.46 Cochliobolus [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Pleosporaceae; Genus: *Cochliobolus*. *Cochliobolus pallescens*, which previously was named *Curvularia pallescens*, causes leaf spot of eggplant, and perhaps most notably causes leaf spot of sugarcane *Saccharum* in addition to affecting other plants growing as weeds in sugarcane fields. *Cochliobolus pallescens* causes invasive and necrotic skin infections in human.

Notes: *Cochliobolus lunatus* now is named *Curvularia lunata*, *Cochliobolus miyabeanus* now is named *Bipolaris oryzae*, and *Cochliobolus spicifer* which produces mycetomas in humans now is *Curvularia spicifera*.

9.4.47 Cokeromyces [Infects: Mammals]

Phylum: Mucoromycota; Class: Mucoromycetes; Order: Mucorales; Family: Mucoraceae; Genus: *Cokeromyces*. The species *Cokeromyces recurvatus* is found in decaying organic material, typically vegetation and feces. *Cokeromyces recurvatus* also is found normally in the gastrointestinal tract and cervix of many mammalian species including cat, dog, and human. It causes pneumonia including lung nodules, enteritis including intestinal perforation, urogenital infections including chronic hemorrhagic cystitis, and can invade the pleural as well as peritoneal fluids in association with peritonitis.

9.4.48 Colletotrichum [Infects: Mammals and Reptiles]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Glomerellales; Family: Glomerellaceae; Genus: *Colletotrichum*. The members of this genus typically have a symbiotic endophytic existance and some are phytopathogens, including the production of anthracnose in cactus (Family Cactaceae). However, the *Colletotrichum* also can cause infections of many animals. *Colletotrichum* infections of mammals typically result in subcutaneous lesions including those involving the foot, and they also can cause eye infections. An instance of subcutaneous *Colletotrichum* infection which also affected the lung and kidney has been reported in cat but the fungal species was not determined. Among the *Colletotrichum* species known to infect humans are *Colletotrichum acutatum*, *Colletotrichum coccodes*, *Colletotrichum crassipes*, *Colletotrichum dematium*, *Colletotrichum gloeosporioides* which in addition notably also causes Umbel blight in carrot *Daucus carota*, and *Colletotrichum graminicola*. *Colletotrichum acutatum* also has been found to cause disseminated infection in an Atlantic ridley sea turtle *Lepidochelys kempii*. *Colletotrichum fioriniae* is an entomopathogen.

9.4.49 Conidiobolus [Infects: Mammals]

Phylum: Zoopagomycota; Class: Entomophthoromycetes; Order: Entomophthorales; Family: Ancylistaceae; Genus: *Conidiobolus*. The *Conidiobolus* typically can be found as saprotrophs in leaf litter. As a pathogen of vertebrates, *Conidiobolus* characteristically cause subcutaneous infections and they also infect the nasal mucosa. However, they additionally can produce pharyngeal masses, systemic infections and gastrointestinal ulcerations. Those species belonging to this microbial genus which have been indicated as causing disease in mammals are: *Conidiobolus coronatus* which infects dog, horse, llama and causes disseminated infection in human; *Conidiobolus incongruus* which infects sheep, red deer, and human; *Conidiobolus lamprauges* which infects horse and causes disseminated infection in human, and *Conidiobolus thromboides* which also infects human. *Conidiobolus thromboides* additionally is an entomopathogen used as a biological agent to control aphids.

9.4.50 Coniochaeta [Infects: Fish and Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Coniochaetales; Family: Coniochaetaceae; Genus: *Coniochaeta*. The *Coniochaeta* are found in water and soil. *Coniochaeta* typically also inhabit woody plants by living independently on the outside of lichens, growing as saprotrophs on leaves, and existing as root

endophytes. The *Coniochaeta* additionally are associated with tree wounds, and can penetrate to feed on the host plant resulting in their association with necrotic lesions, plus the *Coniochaeta* are found living on dead wood. Members of the genus *Coniochaeta* cause subcutaneous infections in mammals that include nodule formation, have been found in the bone marrow as an association with osteomyelitis, and can cause abortion. *Coniochaeta cateniformis*, previously named *Lecythophora cateniformis*, affects mammals including dog and cat. *Coniochaeta hoffmannii*, previously named *Lecythophora hoffmannii*, affects dog, cattle and human. *Coniochaeta mutabilis*, previously named *Lecythophora mutabilis*, affects zebrafish *Danio rerio* by occluding the oral cavity and gills leading to starvation and asphyxiation. *Coniochaeta mutabilis* also infects human. *Coniochaeta polymorpha* has been found in endotracheal aspirates of human.

9.4.51 Coprinopsis [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Agaricales; Family: Psathyrellaceae; Genus: *Coprinopsis*. *Coprinopsis cinerea* previously was named *Coprinus cinereus*, it is an edible mushroom which grows in soil and is associated with wood debris, including growth around tree stumps and on buried wood. This fungal species also affects humans by causing invasive wound infections including skin and underlying soft tissue, as well as producing disseminated infections that can involve the small intestine, brain and heart. Nearly all patients develop fatal multiorgan failure.

9.4.52 Cordyceps [Infects: Reptiles]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Cordycipitaceae; Genus: *Cordyceps*. The *Cordyceps* typically are endoparasitoids, parasitic mainly on insects and other arthropods. Some *Cordyceps* are parasitic on other fungi, and they can be found in soil with their fruiting bodies often sprouting from buried insects. *Cordyceps fumosorosea* previously was named *Paecilomyces fumosoroseus*, and the presumed cause of pulmonary disease in an Aldabra tortoise *Aldabrachelys gigantea*.

9.4.53 Corynespora [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Corynesporascaceae; Genus: *Corynespora*. *Corynespora cassiicola* mostly is known as a endophytic and necrotrophic plant pathogen which causes disease

affecting a wide variety of plants, perhaps most notably the cultivated rubber tree *Hevea brasiliensis*. The plant disease called 'corynespora leaf fall' is indeed one of the most economically significant fungal infections of rubber trees. *Corynespora cassiicola* infects humans, causing indurated plaques, mycetomas including nodules, plus erosions and necrotic ulcers on the legs.

9.4.54 Cryptococcus [Infects: Birds, Fish, Mammals, and Reptiles]

Phylum: Basidiomycota; Class: Tremellomycetes; Order: Tremellales; Family: Cryptococcaceae; Genus: *Cryptococcus*. The *Cryptococcus* typically are found in soil and rotting wood, as well as being found on the leaves, flowers, and woody trunks of *Eucalyptus* trees. *Cryptococcus* also are found in bird guano. *Cryptococcus* species have been found to cause localized invasive infection of the upper respiratory tract in parrots (Family Psittacidae), and invasive subcutaneous infection in pigeons (Family Columbidae) but those fungal identifications were not species specific. The genus *Cryptococcus* has been identified as causing mycosis in the gilthead seabream *Sparus aurata* but that fungal identification also was not species specific. In mammals, members of the genus *Cryptococcus* generally cause mastitis and subcutaneous infections which include nodule formation, both upper and lower respiratory infections, and they can become internally disseminated resulting in such complications as encephalitis and meningitis. A *Cryptococcus* species also has been found to cause disease in an Eastern water skink *Eulamprus quoyii*. *Cryptococcus depauperatus* affects humans. *Cryptococcus gattii* has caused pneumonia and disseminated in dease in kiwi *Apteryx australis mantelli*. *Cryptococcus gattii* also infects numerous mammalian species including cat, dog, goat, llama, alpaca, donkey (ass) *Equus asinus*, and human. *Cryptococcus neoformans* previously has been named *Filobasidiella neoformans* and *Torula histolytica*, it produces fungemia in zebrafish *Danio rerio*. *Cryptococcus neoformans* also infects a wide range of mammalian species including pig, goat, sheep, dog, cat, horse, domestic guinea pig, cattle, and water buffalo. In human, *Cryptococcus neoformans* causes granulomas of the lung, fungemia, central nervous system infections including encephalitis and meningitis, and infects burn wounds. *Cryptococcus neoformans* has caused pneumonia and meningoencephalitis in green anaconda *Eunectes murinus*. *Cryptococcus neoformans var. grubii* has produced disseminated infection including the lung and cerebrum of a western gorilla *Gorilla gorilla*. The *Cryptococcus* also infect a broad range of invertebrate animal hosts.

Notes: *Cryptococcus albidus* has been renamed *Naganishia albida*. *Cryptococcus chernovii* has been renamed *Filobasidium chernovii*. *Cryptococcus flavescens* has been renamed *Papiliotrema flavescens*. *Cryptococcus heveanensis* now is *Kwoniella heveanensis*. *Cryptococcus humicola*, which previously also was named *Asterotremella humicola* and *Candida humicola*, now is *Vanrija humicola*.

Cryptococcus laurentii has been renamed *Papiliotrema laurentii*. *Cryptococcus liquefaciens* now is *Naganishia liquefaciens*. *Cryptococcus magnus* has been renamed *Filobasidium magnum*. *Cryptococcus* is the name of two genera, one of those is this listed genus of Basidiomycota, the other is a genus of Arthropoda.

9.4.55 Cunninghamella [Infects: Mammals]

Phylum: Mucoromycota; Class: Mucoromycetes; Order: Mucorales; Family: Cunninghamellaceae; Genus: *Cunninghamella*. The *Cunninghamella* typically are saprotrophic and found in a wide variety of ecological niches including soil, they also can cause root rot. *Cunninghamella bertholletiae* is collagenolytic and it often causes infections of human following trauma injuries associated with thorns and splinters. The consequences of *Cunninghamella bertholletiae* infection in human can include nail infections and pneumonia, plus this fungal species also has been isolated from human lung, heart and tibia. *Cunninghamella bertholletiae* also affects other mammals, with an example being sinus infection and lung nodules in bottlenose dolphin *Tursiops truncatus*. *Cunninghamella blakesleeana* produces sepsis with multiorgan failure in human. *Cunninghamella echinulata* has caused fatal rhinocerebral infection in human. *Cunninghamella elegans* causes pulmonary infections in human.

9.4.56 Curvularia [Infects: Birds, Mammals, and Reptiles; Also Possibly Infects: Fish]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Pleosporaceae; Genus: *Curvularia*. The *Curvularia* are endophytic fungi and opportunistic pathogens of plants typically infecting grasses, and they can enter plants through the cut tips of grass blades. *Curvularia* also can grow as saprobes in soil and on plant debris. *Curvularia* are fairly common molds in the outside air and can grow inside of damp buildings where they often are found on wood products. Many species of this mold genus are in addition known to be allergenic. *Curvularia* has produced necrotizing conjunctivitis and intralesional fungal hyphae as a mixed flora infection with *Aspergillus* in gopher tortoise *Gopherus polyphemus*, although the causative fungi were not identified at the species level. In human and other mammals the *Curvularia* often cause subcutaneous infections, but *Curvularia* also can cause in mammals abortion, ABPM (allergic bronchopulmonary mycosis due to coinfection with *Aspergillus*), mycetoma incuding eumycetoma, granulomatous encephalitis, keratitis (corneal infection), onychomycosis, peritonitis, sinusitis, and disseminated infections. Various species that now belong to the genus *Curvularia* have had previous membership in the genera *Bipolaris* and *Drechslera*, such that appropriate information on these fungi can be found by researching under those other genus

names. *Curvularia aeria* infects human. *Curvularia australiensis*, which was *Bipolaris australiensis*, has produced in human a chronic allergic fungal sinusitis complicated by invasion into the frontal lobe producing a fungal brain mass. *Curvularia australis*, *Curvularia brachyspora*, *Curvularia clavata*, *Curvularia coicis*, and *Curvularia crustacea* infect human. *Curvularia geniculata* infects cattle, causes mycetoma in human, and has caused mycetoma in an Eclectus parrot *Eclectus roratus roratus*. *Curvularia hawaiiensis*, which previously was named *Bipolaris hawaiiensis*, is a pathogen of Bermuda grass *Cynodon dactylon*. *Curvularia hawaiiensis* causes cutaneous phaeohyphomycosis in the Carribean manatee *Trichechus manatus manatus*. In human, *Curvularia hawaiiensis* has caused subungual hyperkeratosis and intracranial mass. *Curvularia inaequalis* causes fungal peritonitis in humans undergoing continuous ambulatory peritoneal dialysis. *Curvularia kusanoi* also infects human. *Curvularia lunata*, previously named *Cochliobolus lunatus*, has been isolated from surface sterilized leaf segments of ethnopharmaceutically important medicinal herbs collected from the Bhadra River Project Area in the Malnad region of Southern India, suggesting an endophytic existance for this fungal species. *Curvularia lunata* has been found in tissues of the spotted snakehead *Channa punctata* but the information found did not mention specific details of an associated disease. *Curvularia lunata* infects mammals including cat. In human, *Curvularia lunata* causes mycetomas, has produced fungal peritonitis in patients undergoing continuous ambulatory peritoneal dialysis, and caused endophthalmitis following a penetrating inury. *Curvularia neoindica*, *Curvularia nodulosa*, *Curvularia ovariicola*, *Curvularia papendorfii* which previously was named *Bipolaris papendorfii*, *Curvularia portulacae*, and *Curvularia senegalensis* all infect human. *Curvularia spicifera*, which previously also has been named *Bipolaris spicifera*, *Cochliobolus spicifer*, and *Drechslera spicifera*, produces mycetomas in human and additionally infects a wide range of other mammals among which are cat, dog, horse, and cattle. *Curvularia verruculosa* also infects human.

Note: *Curvularia pallescens* now is *Cochliobolus pallescens*.

9.4.57 Cutaneotrichosporon [Infects: Amphibians, Mammals, and Reptiles]

Phylum: Basidiomycota; Class: Tremellomycetes; Order: Trichosporonales; Family: Trichosporonaceae; Genus: *Cutaneotrichosporon*. The *Cutaneotrichosporon* are soil microbes. *Cutaneotrichosporon cutaneum*, which previously was named *Trichosporon cutaneum*, affects numerous mammalian species including dog, cattle and human. In human, *Cutaneotrichosporon cutaneum* causes dermatitis, throat infection, endocarditis subsequent to abuse of intravenous drugs, and is found in urine. *Cutaneotrichosporon cutaneum* causes disseminated subclinical internal infections of anurans (Order Anura). *Cutaneotrichosporon cutaneum* causes dermatitis in reptiles and has been isolated from conspecific bite-induced subcutaneous hematomas in

Carolina anole *Anolis carolinensis*. *Cutaneotrichosporon jirovecii*, which was named *Trichosporon jirovecii*, causes bronchotracheitis in dog. *Cutaneotrichosporon mucoides*, previously named *Trichosporon mucoides*, causes nail infections in human.

9.4.58 Cyphellophora [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Chaetothyriales; Family: Cyphellophoraceae; Genus: *Cyphellophora*. The *Cyphellophora* have been isolated from plant roots, and they can grow as epiphytic colonies on many types of plants including bamboo (Family Poaceae, Tribe Bambuseae). Members of the genus *Cyphellophora* also can affect plants by colonizing and blemishing the epicuticular wax layer of fruit causing the disease called sooty blotch and flyspeck, which is economically damaging. In human, the *Cyphellophora* typically are associated with superficial lesions. *Cyphellophora europaea*, previously named *Phialophora europaea*, potentially affects human by colonizing and infecting skin and nails. *Cyphellophora fusarioides*, in addition to colonizing and infecting human skin and nails, has been isolated from bronchoalveolar lavage fluid of a human following heart bypass surgery. *Cyphellophora guyanensis*, *Cyphellophora laciniata*, *Cyphellophora oxyspora* which previously was named *Phialophora oxyspora*, and *Cyphellophora pluriseptata* also potentially affect human by colonizing and infecting skin and nails. *Cyphellophora reptans*, which previously was named *Phialophora reptans*, is one of many keratinophilic fungi isolatable from public parks soil and potentially it also affects humans by colonizing and infecting skin and nails. The species *Cyphellophora suttonii* lives as a commensal on the skin and nails of human, has been isolated from ulcerating skin lesions of human, and in dog this fungal species causes subcutaneous lesions including those of the ear.

9.4.59 Cystobasidium [Infects: Mammals; Also Possibly Infects: Amphibians]

Phylum: Basidiomycota; Class: Cystobasidiomycetes; Order: Cystobasidiales; Family: Cystobasidiaceae; Genus: *Cystobasidium*. The *Cystobasidium* grow on other fungi, lichens, and wood. *Cystobasidium minutum*, previously named *Rhodotorula minuta*, does in particular live on and cause the decay of wood, as well as living in dead wood, leaves, sticks, and other organic debris. *Cystobasidium minutum* also attacks human and sheep causing nail infection, and in human this fungal species produces systemic infections including those which affect the kidney. *Cystobasidium minutum* is found in bile of the anuran amphibian *Kaloula pulchra* but a disease association for that finding remains uncertain.

9.4.60 Debaryomyces [Infects: Mammals]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Debaryomycetaceae; Genus: *Debaryomyces*. As a genus, the *Debaryomyces* are salt tolerant and typically marine, but they also can be found in cheeses and sausages, and are associated with invertebrate animal hosts. *Debaryomyces* have been isolated from coal mine soil. *Debaromyces hansenii*, previously named *Candida famata*, is found naturally in latex of the rubber tree *Hevea brasiliensis*. *Debaromyces hansenii* is very salt tolerant, it multiplies more rapidly when the salt concentration reaches 1 M NaCl and grows in environments as high as 4 M NaCl. *Debaromyces hansenii* can grow epiphytically on desert plants. A psychrotolerant *Debaryomyces hanseni* strain from fermented leaves of a tea plant *Camellia sinensis* is used as a probiotic for cultured fish and crustaceans, and *Debaromyces hansenii* naturally is part of salmonid intestinal microbiota. *Debaromyces hansenii* infects human thereby causing acute zonal occult retinopathy, mediastinitis, invasive catheter-related infections including fungemia and peritonitis (in a peritoneal dialysis patient). *Debaromyces hansenii* also infects horse, causes systemic infections in rat, and causes mastitis in bovines including the fungus being found in mastitic bovine milk. It has been suggested that the fungal species *Debaryomyces nepalensis* can behave as an opportunistic pathogen in human, and curiously this species has been found in the oral cavity of an apparently healthy dog such that the fungus was presumed to have commensal status for the dog.

Note: *Debaryomyces fluxuum*, previously also known as *Candida vini*, *Debaryomyces fluxorum*, and *Pichia fluxuum*, now is named *Kregervanrija fluxuum*.

9.4.61 Diaporthe [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Diaporthales; Family: Diaporthaceae; Genus: *Diaporthe*. The *Diaporthe* are filamentous, endophytic plant pathogens. *Diaporthe longicolla*, which previously was named *Phomopsis longicolla*, causes cutaneous infections in human. *Diaporthe phaseolorum* causes cutaneous infections and also the chronic subcutaneous disease known as eumycetoma in human. *Diaporthe raonikayaporum* causes subcutaneous lesions including nodules in human. *Diaporthe sojae* causes cutaneous lesions in human.

9.4.62 Dichotomophthora [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Pleosporaceae; Genus: *Dichotomophthora*. *Dichotomophthora portulacae* spreads

through the stems and roots of plants causing black stem rot. As a pathogen of vertebrates, *Dichotomophthora portulacae* causes keratitis in human.

9.4.63 Dichotomopilus [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: Chaetomiaceae; Genus: *Dichotomopilus*. The *Dichotomopilus* are endophytic and saprobic fungi which can be found both on healthy living and decayed fallen branches. *Dichotomopilus funicola*, previously named *Chaetomium funicola*, is an endophyte found in the rhizosphere and it produces the apigenin flavone glucoside vitexin. As a pathogen of vertebrates, *Dichotomopilus* funicola causes superficial and deep cutaneous lesions in human.

9.4.64 Didymella [Infects: Mammals and Reptiles]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Didymellaceae; Genus: *Didymella*. The *Didymella* are plant pathogens. *Didymella gardeniae*, previously named *Peyronellaea gardeniae*, causes subcutaneous lesions with abcess in human. *Didymella glomerata*, which previously has been named both *Phoma glomerata* and also *Peyronellaea glomerata*, causes leaf blight and crown rot as a plant pathogen. *Didymella glomerata* infects the ear pinna of goat causing a pathological condition, it also causes cutaneous lesions in common chameleon *Chamaeleo chamaeleon*. *Didymella gardeniae*, previously named *Peyronellaea gardeniae*, causes subcutaneous lesions with abcess in human. *Didymella heteroderae*, previously named *Phoma heteroderae*, has been isolated from peripheral lung tissue of an asthmatic human.

9.4.65 Diutina [Infects: Birds and Mammals]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: (not assigned); Genus: *Diutina*. *Diutina* have been isolated from soil. *Diutina catenulata*, which previously was named *Candida catenulata*, has been isolated from soil and its discovery as a contaminant of dairy products suggests a possible association with mastitis, it also is known to cause fungemia in human. *Diutina mesorugosa* previously was named *Candida mesorugosa* and causes urinary tract infection in human. *Diutina rugosa*, previously named *Candida rugosa*, has been found in sea water and beach sand. As a pathogen of mammals, *Diutina rugosa* has caused opportunistic gastrointentinal infection in turkey, uremia and catheter-related fungemia in human, plus mastitis in dairy cattle and correspondingly is found as a

contaminant in cattle milk. *Diutina pseudorugosa* previously was named *Candida pseudorugosa*, in humans this fungal species is found in sputum and causes fungemia.

9.4.66 Emarellia [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Trematosphaeriaceae; Genus: *Emarellia*. Nothing seems known about the natural ecology of this fungal genus. The fact that this genus causes the fungal disease "black-grain mycetoma", also known as "eumycetoma", may suggest a natural ecology for the *Emarellia*. The other fungal genera which cause this same disease syndrome (*Falciformispora*, *Madurella*, *Medicopsis*, *Nigrograna*, *Pseudochaetosphaeronema*, and *Trematosphaeria*) are tropical and subtropical soil inhabitants and plant pathogens. For that reason, I will include the genus *Emarellia* on my list of soil fungi and plant fungi, presuming that when the natural ecology of *Emarellia* eventually is understood it will be found to include residence in soil and possibly plant pathogenicity. Both *Emarellia grisea* and *Emarellia paragrisea* cause black-grain mycetomas, also known as eumycetoma, in human. That is a debilitating, chronic, fungal infection endemic in India and Indonesia, parts of Africa, plus South America and Central America. These infections typically follow traumatic implantation of saprophytic fungi, and in the absence of appropriate treatment the infection frequently requires radical surgery including possible amputation.

9.4.67 Emergomyces [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Ajellomycetaceae; Genus: *Emergomyces*. The *Emergomyces* can be found in soil. *Emergomyces africanus*, which is found both in soil and in rooftop air sampling, has been identified as infecting small terrestrial mammals in Africa. Humans infected by *Emergomyces africanus* most commonly present with widespread skin lesions, pulmonary disease, and systemic mycosis. *Emergomyces canadensis* causes systemic infections in humans involving blood, skin, cervix, lung, and lymph node. *Emergomyces pasteurianus*, which previously was named *Emmonsia pasteuriana*, causes in human disseminated cutaneous infections, and those infections affect multiple cutaneous sites but thus far seem not to include internal dissemination.

9.4.68 *Emmia* [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Polyporales; Family: Irpicaceae; Genus: *Emmia*. *Emmia lacerata* previously was named *Ceriporia lacerata*, it causes white rot on wood and bronchopulmonary mycosis in human.

9.4.69 *Emmonsia* [Infects: Mammals; Also Possibly Infects: Amphibians]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Ajellomycetaceae; Genus: *Emmonsia*. The *Emmonsia* are soil fungi. *Emmonsia* has been identified as causing skeletal intramuscular (leg) infection of American bullfrog *Rana catesbeiana*, but without fungal identification at the species level which allows consideration that the infectious agent may have been *Emmonsia parva*, now named *Blastomyces parvus* which is known to infect that frog species. The *Emmonsia* cause pulmonary disease as well as disseminated disease that can seem common in wild rodents, and it also infects both horse and rabbit. *Emmonsia crescens*, previously also named *Ajellomyces crescens* and *Chrysosporium parvum var. crescens*, is primarily a rodent pathogen which causes cutaneous mycoses and disseminated mycosis affecting shrews, moles, voles, and water voles *Arvicola amphibius*. In human, *Emmonsia crescens* perhaps most typically causes lung infections. We honor with pride the memory of medical mycologist Chester Wilson Emmons.

Notes: *Emmonsia parva*, which affects humans, other mammals and amphibians including American bullfrog *Rana catesbeiana*, previously also was named *Haplosporangium parvum* and now is named *Blastomyces parvus*. *Emmonsia pasteuriana*, which causes disseminated cutaneous infections in human, has been renamed *Emergomyces pasteurianus*.

9.4.70 *Epicoccum* [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Didymellaceae; Genus: *Epicoccum*. The *Epicoccum* typically disseminate as a dry spore through wind action and commonly are isolated from air, plant debris, soil and food. They also are endophytic and cause leaf spot. *Epicoccum nigrum* is an important endophytic fungus of sugarcane. *Epicoccum nigrum* is found on the skin and saliva of humans, and it causes both hypersensitivity pneumonitis as well as intramuscular abcess in human. The fluorescent stain epicocconone is extracted from *Epicoccum nigrum*. *Epicoccum sorghinum* previously was *Phoma sorghina*, and as a pathogen of human it causes skin lesions.

9.4.71 *Epidermophyton* [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Arthrodermataceae; Genus: *Epidermophyton*. The species *Epidermophyton floccosum* has been isolated from soil. *Epidermophyton floccosum* usually infects only the non-living cornified layers of epidermis and sometimes causes severe onychomycosis, although it also can cause keratitis. In human, *Epidermophyton floccosum* additionally is known for complicating interdigital infections, is one of the major causes of diaper dermatitis "diaper rash", and it can cause invasive infections. *Epidermophyton floccosum* has also been found to affect the Caucasian squirrel *Sciurus anomalus*, and that rodent species therefore might serve as a zoonotic reservoir for this fungal species.

9.4.72 *Exophiala* [Infects: Amphibians, Fish, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Chaetothyriales; Family: Herpotrichiellaceae; Genus: *Exophiala*. The *Exophiala* commonly are found as saprotrophs, often growing either on plants and decaying wood or in soil enriched with organic wastes. *Exophiala* can be associated with plant roots. They also are found growing in water and on wet surfaces. The *Exophiala* typically seem to cause infections that begin on the outer surfaces of animals, including skin and environmentally exposed surfaces such as gills, but *Exophiala* also can cause internal infections. *Exophiala* have been found to affect wild caught spotted whiting *Sillaginodes puntuta* and to cause ulcerative skin lesions in the Japanese flounder *Paralichthys olivaceus*, but without identification of those fungi at the species level. In mammals, members of the genus *Exophiala* often are found on the feet and nails, and notably produce abscesses. Subcutaneous infections produced by *Exophiala* include nodule formation and tumor-like cysts that are termed mycetomas, and the subcutaneous infections can become progressively granulomatous involving not only the skin and subcutaneous tissues but additionally becoming invasive of underlying muscle and bone. The members of this genus also infect the nasal cavity of mammals and can invade the lung, disseminating with systemic consequences that include causing abortion. Additionally, although more rarely, the *Exophiala* can attack the cornea. *Exophiala angulospora* affects the common seadragon also called weedy seadragon *Phyllopteryx taeniolatus*, and causes both skin and spleen infections of the lumpfish *Cyclopterus lumpus*. *Exophiala aquamarina* affects the leafy seadragon *Phycodurus eques* causing lesions in the skin, skull and other bone. *Exophiala aquamarina* also affects Winter flounder *Pseudopleuronectes americanus*, little tunny *Euthynnus alletteratus*, lumpfish *Cyclopterus lumpus*, and sand lances *Ammodytidae*. *Exophiala angulospora* and *Exophiala aquamarina* target the blood vessels of fish thus allowing systemic necrotizing lesions, and

those lesions have been observed mostly in the extradural sinus, gill, kidney, skeletal muscle, skin, spinal cord, and swim bladder, plus less often in the heart, liver, mesentery, muscle coats and serosa of the intestine, and spleen. *Exophiala asiatica* affects human. *Exophiala attenuata* affects cat and also human. *Exophiala bergeri* affects human. *Exophiala cancerae* causes skin and nail infections in human, infects the liver of the green toad *Anaxyrus debilis* and yes, as you may have guessed from suggestion by its species name, *Exophiala cancerae* does infect crab. *Exophiala cancerae* infects mangrove crabs *Scylla*, and *Exophiala cancerae* causes lethargic crab disease in the swamp ghost crab *Ucides cordatus* which also is primarily found in mangrove (Family Rhizophoraceae) forests and serves as an important food source for some human populations, thus ecologically having an indirect effect upon humans. *Exophiala castellanii* causes skin infections in human. *Exophiala dermatitidis* used to be named *Wangiella dermatitidis*, it affects cat, dog, cattle, human, and also causes disseminated subclinical internal infections of anurans. *Exophiala equina* affects horse. In human, *Exophiala equina* is found in sputum, causes keratitis, nail infections, and produces dialysis related infections. *Exophiala equina* also has caused infection of Galapagos tortoise *Chelonoidis niger galapagoensis* which had spread to the animals lungs and eyes. *Exophiala halophila* causes skin and nail infections in humans. *Exophiala hongkongensis* is a relatively recently discovered microbe which causes an infection of the toenails termed dermatophytic onychomycosis in human and may eventually be found to affect livestock mammals. *Exophiala hongkongensis* may be the same species as *Phialemoniopsis hongkongensis* although both of these species names currently are recognized (see the listing for genus *Phialemoniopsis*). *Exophiala jeanselmei*, which is known to infect cat, cattle, and the Eastern box turtle *Terrapene carolina carolina*, produces mycetomas in human and has as well caused double fungal infections of the human skin concurrent with *Phialemoniopsis endophytica*. *Exophiala lecanii-corni* infects human. *Exophiala mesophila* infects human causing nasal and sinus infections plus produces cysts and can infect hip joint. *Exophiala moniliae*, *Exophiala oligosperma*, and *Exophiala opportunistica* infect human, typically causing skin and nail infections. *Exophiala phaeomuriformis* used to be named *Sarcinomyces phaeomuriformis*, in human it causes cutaneous, subcutaneous, and deep tissue infections. *Exophiala pisciphila* infects frogs (order Anura). *Exophiala pisciphila* attacks cranial, cutaneous, muscle and visceral tissues in numerous salmonid species (Family Salmonidae), causes cutaneous ulcers in American plaice *Hippoglossoides platessoides*, infects the big-belly seahorse also called the potbelly seahorse *Hippocampus abdominalis*, and also infects smooth dogfish *Mustelus canis*. In channel catfish *Ictalurus punctatus*, *Exophiala pisciphila* causes lesions that are cutaneous and also visceral with a predilection for the kidney. *Exophiala pisciphila* additionally causes skin papules and nail infections in human. *Exophiala psychrophila* infects Atlantic salmon *Salmo salar*. *Exophiala salmonis* has caused ulcers in a captive European blind cave salamander *Proteus anguinus*. *Exophiala salmonis* infects numerous species of salmonids in hatcheries, including causing cerebral lesions in cutthroat trout *Oncorhynchus clarkii*. *Exophiala salmonis* also infects human. *Exophiala spinifera* infects cat and humans.

Exophiala xenobiotica infects the gill, heart and kidney of cultured hard-tail jack *Pseudocaranx dentex*. *Exophiala xenobiotica* also infects human.

9.4.73 Exserohilum [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Pleosporaceae; Genus: *Exserohilum*. The *Exserohilum* are soilborne and typically considered to be plant pathogens. In mammals, they cause mycetomas which can disseminate. *Exserohilum fusiforme*, *Exserohilum gedarefense*, *Exserohilum longirostratum*, and *Exserohilum mcginnisii* are known to affect human. *Exserohilum rostratum* is the best known mammaliam pathogen in this genus. *Exserohilum rostratum* previously was named *Drechslera rostrata* and also *Setosphaeria rostrata*. As a plant pathogen, *Exserohilum rostratum* causes leaf spots as well as crown rot and root rot in grasses (Family Poaceae). *Exserohilum rostratum* also can be found in soil including the mobile surface layer of saharan desert soil, and on textiles in subtropical and tropical regions. In humans and other mammals, *Exserohilum rostratum* causes subcutaneous infection and mycetomas, the infections include cutaneous granulomas as well as necrotic lesions of the skin which can very quickly become quite large, and corneal ulcers that can fulminantly disseminate resulting in meningitis. Additional complications of *Exserohilum rostratum* infection in human are brain abscess, chromoblastomycosis, eumycetoma, and pneumonia. *Exserohilum rostratum* produces necrotic lesions in the nasal cavities and lymph nodes of cattle. *Exserohilum turcicum* similarly affects human.

9.4.74 Falciformispora [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Trematosphaeriaceae; Genus: *Falciformispora*. *Falciformispora senegalensis* previously was named *Leptosphaeria senegalensis*. *Falciformispora senegalensis* has been isolated from soil and causes mycetomas in human, often those are infections that follow traumatic implantation of saprophytic fungi, and frequently these infections require either radical surgery or even amputation in the absence of appropriate antifungal treatment. *Falciformispora tompkinsii* previously was named *Leptosphaeria tompkinsii* and also produces mycetomas in human.

9.4.75 Filobasidium [Infects: Mammals]

Phylum: Basidiomycota; Class: Tremellomycetes; Order: Filobasidiales; Family: Filobasidiaceae; Genus: *Filobasidium*. The *Filobasidium* have been isolated from

soil. *Filobasidium chernovii*, previously named *Cryptococcus chernovii*, causes nasal infection in human. *Filobasidium magnum*, previously named *Cryptococcus magnus*, causes granulomatous dermatitis, panniculitis, myositis, and lymphadenitis in cat, and it produces nasal infection in human. *Filobasidium uniguttulatum* causes meningitis in human.

Note: *Filobasidiella neoformans*, which previously also has been named *Filobasidiella neoformans* and *Torula histolytica*, currently is named *Cryptococcus neoformans*.

9.4.76 Fonsecaea [Infects: Amphibians and Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Chaetothyriales; Family: Herpotrichiellaceae; Genus: *Fonsecaea*. The members of this genus are saprotrophs typically found in soil and on living plants including trees. They have been isolated from rotting wood including tree stumps, woodpiles and fence posts. Members of the genus *Fonsecaea* are known to produce ulcerations localized to the skin and subcutaneous tissues, but they also can cause cerebral abscess. *Fonsecaea brasiliensis* causes disseminated infection in human and also causes lethargic crab disease in the swamp ghost crab *Ucides cordatus* which is primarily found in mangrove forests and serves as an important food source for some human populations. Thusly, *Fonsecaea brasiliensis* infections in crustaceans have an ecologically indirect effect upon humans. *Fonsecaea compacta*, previously named *Rhinocladiella compacta*, causes chromoblastomycosis in human. *Fonsecaea monophora* causes chromoblastomycosis and additionally causes cerebral infection in human. *Fonsecaea multimorphosa* produces cerebral infection in cat. *Fonsecaea nubica* causes chromoblastomycosis in human. *Fonsecaea pedrosoi* is found in soil, it causes generalized granulomatous infections including granulomas in the internal organs of many toad species among which is the marine toad *Rhinella marina*, previously named *Bufo marinus*. *Fonsecaea pedrosoi* causes skin infections in cat and dog, plus it also affects human causing both keratitis and the chronic subcutaneous infection variously termed chromoblastomycosis, verrucous dermatitis, Fonseca's disease and Pedroso's disease.

9.4.77 Fusarium [Infects: Amphibians, Fish, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Nectriaceae; Genus: *Fusarium*. Members of the genus *Fusarium* include endophytic parasites that are plant pathogens, and saprophytic soil organisms, that variously are associated with root rot, stem rot, fruit rot, and vascular wilt. Some *Fusarium* species

are common on commodities such as rice, bean, and soybean, and are notable as producers of mycotoxins including trichothecenes. *Fusarium* can cause food-associated mycotoxicosis problems in vertebrates and that includes severe loses in poultry, but my focus for this chapter is infectious disease rather than toxicosis. Although I would imagine that *Fusarium* infections do exist in birds, I could not find reference to that. The genus *Fusarium* has been identified as causing mycosis (fungal infection) in the gilthead seabream *Sparus aurata*, Gibelion catla *Catla catla*, clown knifefish *Chitala chitala*, snakehead murrel *Channa striata*, Jayanti rohu *Labeo rohita*, and great snakehead *Channa marulius*, but those fungal identifications were not species specific. In mammals, members of the genus *Fusarium* cause dermatitis that can become granulomatous in nature and these infections may include skin ulcers as well as subcutaneous nodule formation. Fusarial infections may cause ulceration of the cornea. *Fusarium* infections also can produce invasive and disseminated disease with pulmonary involvement plus they can attack the brain resulting in meningoencephalitis. Hemodialysis graft infection in human has been attributed to *Fusarium incarnatum* - *Fusarium equiseti* species complex, which could have represented either *Fusarium incarnatum* or *Fusarium equiseti*. *Fusarium chlamydosporum* has caused catheter-associated fungemia in human. *Fusarium dimerum*, previously named *Microdochium dimerum*, has been found to cause eye infections, endocarditis, and disseminated infections in human. *Fusarium equiseti* may have been associated with hemodialysis graft infection in human (see above). *Fusarium falciforme*, which causes mycetomas in human and keratitis in horse, previously was named *Acremonium falciforme*. *Fusarium incarnatum*, previously named *Fusarium semitectum*, has caused the chronic shell infection termed necrotizing scute disease in both free-living and also captive Texas gopher tortoise *Gopherus berlandieri* resulting in extensive discoloration and blemishes of the carapace. *Fusarium lichenicola*, previosly named *Cylindrocarpon lichenicola*, causes cutaneous infections and mycetomas in human. *Fusarium neocosmosporiellum*, which was named *Neocosmospora vasinfecta*, causes systemic infection in human including ulcerous lesions of the foot that can disseminate to include lung infiltration. *Fusarium oxysporum* has been isolated from surface sterilized leaf segments of ethnopharmaceutically important medicinal herbs collected from the Bhadra River Project Area in the Malnad region of Southern India, suggesting an endophytic presence for this fungal species. *Fusarium oxysporum* has caused dermatitis in Wyoming toad *Anaxyrus baxteri*, previously named *Bufo baxteri*. *Fusarium oxysporum* affects fish and has caused kidney infection in red seabream *Pagrus major*. *Fusarium oxysporum* affects cat, including the production of granulomatous pododermatitis. In human, *Fusarium oxysporum* produces mycetomas, causes keratitis including outbreaks associated with the use of contact lenses, endophthalmitis, onychomycosis, cutaneous and subcutaneous infections, arthritis, sinusitis, and disseminated infection. There also has been a possible association of *Fusarium oxysporum* with neonatal anencephalia in human. Infections by *Fusarium oxysporum* can be complicated by the production of fungal toxins that cause infertility. *Fusarium oxysporum* causes gill infections in kuruma prawn *Penaeus japonicus*, which represents an indirect ecological effect of this fungus by impacting

a valuable food resource for vertebrates including the fish and humans which consume that prawn species. *Fusarium proliferatum* causes dermatitis in Wyoming toad *Anaxyrus baxteri*, previously named *Bufo baxteri*. *Fusarium proliferatum* also affects cat and human, including the production of soft tissue infections at the site of puncture wounds caused by plants. *Fusarium sacchari* is a pathogen of sugarcane and causes mycotic keratitis among sugarcane farmers. *Fusarium solani* has caused dermatitis in the Wyoming toad *Anaxyrus baxteri*, previously named *Bufo baxteri*. *Fusarium solani* causes skin erosions and ulcerations in sharp tooth catfish *Clarias gariepinus*, and *Fusarium solani* species complex has caused both dermatitis as well as systemic mycosis in lined seahorses *Hippocampus erectus*. *Fusarium solani* has caused meningoencephalitis in dog, keratitis in horse, and also affects numerous other mammalian species including goat and sheep. *Fusarium solani* is perhaps the *Fusarium* species most notable for causing infectious disease of humans including keratitis, onychomycosis and mycetomas. *Fusarium solani* also affects reptiles, including its having been responsible for mass mortalities in nests of Loggerhead turtle *Caretta caretta*. *Fusarium solani* additionally infects kuruma prawn *Penaeus japonicus* by producing hyphae and tissue destruction due to fungal infection in the gills, maxillipeds, pereiopods, thoracic body wall, thoracic central nerve and occasionally in the ventral thoracic artery. *Fusarium sporotrichioides* has caused chronic ulcerative dermatitis in dog, and diabetic foot wound in human. *Fusarium verticillioides*, which previously was named *Fusarium moniliforme*, is yet another *Fusarium* species that produces dermatitis in Wyoming toad *Anaxyrus baxteri*, previously named *Bufo baxteri*. *Fusarium verticillioides* produces mycetomas in humans. *Fusarium verticillioides* also has caused mycotic pneumonia in an American alligator *Alligator mississippiensis*. *Fusarium verticillioides* additionally infects crustaceans, as evidenced by this fungal species having caused gill lesions in kuruma prawn *Penaeus japonicus*. *[Nectria] haematococca*, which occurs as a saprophyte in diverse habitats as well as being a plant pathogen, now is part of the *Fusarium solani* species complex. *[Nectria] haematococca* has been reported to affect human and also to be a pathogen of other animals, but no specific details could be found regarding those affects.

9.4.78 Galactomyces [Infects: Mammals and Reptiles]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Dipodascaceae; Genus: *Galactomyces*. *Galactomyces candidus*, previously also named *Galactomyces geotrichum*, typically is found in plants, soil, water, sewage, cereals and dairy products, and it is noted as a cause of sour rot in carrot and other vegetables. In mammals, the species *Galactomyces candidus* causes mastitis, attacks the tonsils, and causes chronic infection of the lungs, mouth and intestine. These infections can become disseminated and pyogranulomatous resulting in pneumonia, hepatitis, and nephritis, which affects among others dog, sheep, goat, pig, cattle, and water buffalo. *Galactomyces candidus* also affects human by causing invasive

cutaneous infections, disseminated infections, and post operative fungal endopthalmitis. *Galactomyces candidus* additionally affects a wide range of reptile species by causing dermal mycosis including dermal pustules, perhaps most notably in snakes including the dusky pigmy rattlesnake *Sistrurus miliarius barbouri*.

9.4.79 Geotrichum [Infects: Amphibians, Birds, Fish, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Dipodascaceae; Genus: *Geotrichum*. The *Geotrichum* are soil microbes and plant pathogens, among them being *Geotrichum citri - aurantii* which causes a common post harvest fungus disease of *Citrus* known as sour rot. *Geotrichum* are notable for their use in the production of lipases and are used in lipolytic fermentations, including *Geotrichum candidum* which is common in soil and on plants, is used in cheese and yogurt production, and is used in fish fermentations. The lipolytic activity of *Geotrichum* species might be associated with their pathogenicity for vertebrates. *Geotrichum* has been identified as causing mycosis in the gilthead seabream *Sparus aurata* but that fungal identification was not species specific. *Geotrichum candidum* infects the lung tissues of frogs. *Geotrichum candidum* has been presumed to cause severe enteritis of guineafowl chicks (Family Numididae) and also infects the genus *Buteo* (hawks and buzzards). *Geotrichum candidum*, which can be found in human sputum and feces, causes disease in human by producing invasive cutaneous infections, post operative fungal endopthalmitis, and disseminated infections including abcesses that can involve heart, kidney, lung, liver, spleen, peripancreatic soft tissue, hilar and retroperitoneal lymph nodes, and the bone marrow. *Geotrichum candidum* causes tonsillitis in pigs, bovine mastitis, and has been noted as a possible cause of bovine abortion. *Geotrichum candidum* causes dermal mycosis including dermal pustules in lizards and in snakes, including affecting the dusky pigmy rattlesnake *Sistrurus miliarius barbouri*, and *Geotrichum candidum* is presumed to similarly be disease producing in all reptile species.

Notes: *Geotrichum capitatum*, which affects human and previous also has been named *Blastoschizomyces capitatus*, *Dipodascus capitatus*, *Saprochaete capitata*, and *Trichosporon capitatum*, has been renamed *Magnusiomyces capitatus*. *Geotrichum clavatum*, which affects human, has been renamed *Saprochaete clavata*. *Geotrichum microsporum*, previously also named *Arthrographis cuboidea* and *Oospora cuboidea*, now is *Scytalidium cuboideum*.

9.4.80 Gloniopsis [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Hysteriales; Family: Hysteriaceae; Genus: *Gloniopsis*. The natural ecology of *Gloniopsis*, including *Gloniopsis praelonga*, includes growth both on live and dead woody plants. The fungal genus *Gloniopsis* has produced subcutaneous lesions including abcess and aponeurotic cyst in human, but the causative fungus was not identified at the species level.

9.4.81 Graphium [Infects: Mammals]

The *Graphium* are filamentous fungi found in plant debris including woody substrates, manure, soil, and polluted water. They cause infections of mammals and also produce allergic reactions in the lungs of mammals. My focus in this chapter is infections, and so I will not further consider the allergenic nature of *Graphium*. *Graphium* species have been divided into three genera and I have numbered these three genera by the order that they are listed in the National Center for Biotechnology Information database.

Genus 1 [Infects: Mammals]
Phylum: Ascomycota; Class: Sordariomycetes; Order: Microascales; Family: (not assigned); Genus: *Graphium*. The species *Graphium eumorphum* causes keratitis in human. *Graphium fructicola* causes systemic mycosis in dog.

Genus 2 [Infects: Mammals]
Phylum: Ascomycota; Class: Sordariomycetes; Order: Microascales; Family: Graphiaceae; Genus: *Graphium*. The species *Graphium basitruncatum* affects human by causing infections that often occur after either severe local trauma or after aspiration of polluted water. *Graphium basitruncatum* infections in human include ophthalmic infections, subcutaneous infections, white grain mycetoma, invasion into the central nervous system, invasive sinusitis, pneumonia and fungemia.

Genus 3 [not shown to infect vertebrates]
Phylum: Ascomycota; Class: Leotiomycetes; Order: Helotiales; Family: (not assigned); Genus: *Graphium*. Of the three *Graphium* fungal genera, only this one seems not to contain species that are infectious for vertebrates.

Note: *Graphium* also is the genus name for a group of butterflies belonging to the Family Papilionidae.

9.4.82 Gymnascella [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Gymnoascaceae; Genus: *Gymnascella*. The *Gymnascella* live naturally in a variety of habitats including being found in soil. *Gymnascella hyalinospora* infects human, causing pulmonary infection and also having caused peritonitis in a patient receiving peritoneal dialysis.

9.4.83 Gymnoascus [Possibly Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Gymnoascaceae; Genus: *Gymnoascus*. The *Gymnoascus* are rhizosphere microbes. *Gymnoascus demonbreunii* has been isolated from dog, and *Gymnoascus reesii* has been isolated from human, but causal association of those fungal detections with infection in the mammalian species remains uncertain.

Note: *Gymnoascus gypseus*, previously also named *Achorion gypseum*, *Arthroderma gypseum*, *Closterosporia gypsea*, and *Microsporum gypseum*, now is *Nannizzia gypsea*.

9.4.84 Helminthosporium [Infects: Fish and Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Massarinaceae; Genus: *Helminthosporium*. The *Helminthosporium* are plant pathogens that cause leaf spot and affect tubers. *Helminthosporium* species also have been isolated from submerged wood in streams. The genus *Helminthosporium* has been found to cause mycosis in the gilthead seabream *Sparus aurata*, and sinus infections in human, but those causal fungi were not identified to the species level.

9.4.85 Histoplasma [Infects: Birds, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Ajellomycetaceae; Genus: *Histoplasma*. The species *Histoplasma capsulatum*, previously also named *Ajellomyces capsulatus*, is a common organism that both is found in and grows in soil, particularly soil that contains large amounts of either bird or bat droppings. It often is present in moist, shaded environments such as woods, caves, and cellars. *Histoplasma capsulatum* also is found in rotting excrement from bats and birds. Typically for immuncompetent hosts, the fungal species *Histoplasma capsulatum* which is very broadly infective of mammals often will produce a

transiently noticed although potentially never eliminated granulomatous pulmonary infection, and in that regard the pulmonary disease histoplasmosis which this fungus produces is similar to the illness tuberculosis which is caused by the bacterial species *Mycobacterium tuberculosis*. Histoplasmosis has other names including Darlings disease, named for Samuel Taylor Darling who first identified the illness. *Histoplasma capsulatum* also causes conjunctivitis and opthalmic infection, the latter of which can result in permanent blindness and can be an occupational disease for people who wash windows and window ledges. This fungus causes sinus infections, can invade the skin producing subcutaneous mycotic nodules, and is capable of causing disseminated infections that can be granulomtous and lethal including attacking the central nervous system and causing endocarditis. The fungal species *Histoplasma capsulatum* has been considerd the most common endemic fungal infection in North America and it is all too well know in geographical areas such as the Ohio River Valley in the United States of America, which is where I live. Here, it has a particularly noted association with the cloacal excrement of rock pigeon *Columba livia* because the fungus grows in that excrement. In particular, my home city of Cincinnati, Ohio, once was referred to as being the Histoplasmosis Capital of North America with more than 86% of its residents being serologically positive for the organism. Histoplasmosis likely could have caused the blindness of the "Bird Woman" character who sells crumbs to feed pigeons in the play and movie "Mary Poppins", as based upon the books of Pamela Lyndon Travers. *Histoplasma capsulatum* infects chickens experimentally, it has been identified as infecting many species of mammal including cat, dog, llama, horse, kangaroo, cattle, and sheep, and it is mentioned as rare in reptiles.

9.4.86 Hormographiella [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Agaricales; Family: Psathyrellaceae; Genus: *Hormographiella*. I will guess that *Hormographiella* can be found in soil because members of the order Agaricales are gilled mushrooms and typically do reside in soil. The species *Hormographiella aspergillata* infects human, causing pulmonary infections which may include pulmonary abcess, and those infections may be accompanied by cerebral as well as ocular involvement.

9.4.87 Hortaea [Infects: Amphibians, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Capnodiales; Family: Teratosphaeriaceae; Genus: *Hortaea*. The *Hortaea* are plant pathogens. *Hortaea werneckii*, which previously was named *Cladosporium werneckii*, is a saprophytic

fungus believed to occur in compost, humus, wood in humid tropical as well as subtropical regions, and in soil. *Hortaea werneckii* is notably halotolerant and survives on salted fish. *Hortaea werneckii* has been isolated from frog kidney. *Hortaea werneckii* affects humans and is one of the fungi that produce tinea nigra, which is a non-invasive skin infection evidenced as patchy dark brown to black discoloration on the soles of the feet and the palms of the hand. The infection caused by *Stenella araguata* produces a similar effect in human (see the listing for *Stenella*). *Hortaea werneckii* also infects reptiles, having caused the integumentary lesions known as lymphohistiocytic proliferative syndrome (PIX disease) in American alligator *Alligator mississippiensis*.

9.4.88 Humicola [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: Chaetomiaceae; Genus: *Humicola*. The *Humicola* are plant pathogens and soil fungi that also have been isolated from karst caves. *Humicola fuscoatra* is found on tomato plants, has been identified as a cause of peritonitis infection associated with peritoneal dialysis in humans, and has been found in diseased coral. *Humicola grisea* causes a wilt disease of plants and hypersensitivity pneumonitis in humans, but does not seem to have been found infectious for either humans or other vertebrates.

9.4.89 Hymenochaete [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Hymenochaetales; Family: Hymenochaetaceae; Genus: *Hymenochaete*. *Hymenochaete porioides*, previously named *Cyclomyces tabacinus*, grows as a leathery bracket on live and dead trees and it also grows on bamboo (Family Poaceae, Tribe Bambuseae). *Hymenochaete porioides* causes deep tissue infections in human.

9.4.90 Hyphopichia [Infects: Mammals and reptiles]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Debaryomycetaceae; Genus: *Hyphopichia*. The *Hyphopichia* are associated with rotting wood. *Hyphopichia burtonii*, which previously was named *Pichia burtonii*, interestingly is known for causing chalky mould spoilage of bakery products. *Hyphopichia burtonii* has caused cutaneous mycosis in a Barbastelle bat *Barbastella barbastellus*, enteritis in a central bearded dragon *Pogona vitticeps*, and this fungal species also has been isolated from the lungs of an unidentified lizard species.

9.4.91 Ilyonectria [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Nectriaceae; Genus: *Ilyonectria*. *Ilyonectria destructans* previously has been named *Cylindrocarpon destructans* and also *Neonectria radicicola*, it is a soil-borne organism that causes root rot of plants. *Ilyonectria destructans* produces mycetoma in human.

9.4.92 Irpex [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Polyporales; Family: Irpicaceae; Genus: *Irpex*. *Irpex lacteus* is a common bracket or crust fungus associated with the rotting of wood and it mainly inhabits angiosperm branches. Proteinases produced by *Irpex lacteus* have been used as milk clotting enzymes in cheesemaking, this fungus also has been used for biological treatments to enhance saccharification of beech wood (Brethauer et al. 2017), processing of corn plant residues (corn stover), and processing straw of bread wheat *Triticum aestivum*. *Irpex lacteus* causes pulmonary abcess in human.

9.4.93 Juxtiphoma [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Didymellaceae; Genus: *Juxtiphoma*. *Juxtiphoma eupyrena*, which previously was named *Phoma eupyrena*, is a plant pathogen known to infect several tree species, perhaps most notably Douglas-fir *Pseudotsuga menziesii* and golden rain tree *Koelreuteria paniculata*. *Juxtiphoma eupyrena* has been known to cause leaf spot on both *Aloe vera* and water lettuce *Pistia stratiotes*, plus it causes stalk rot of grains. As an infectious agent of vertebrates, *Juxtiphoma eupyrena* causes skin lesions in human.

9.4.94 Kluyveromyces [Infects: Fish and Mammals]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Saccharomycetaceae; Genus: *Kluyveromyces*. *Kluyveromyces marxianus* is found naturally in soil and colonizes plants including corn. *Kluyveromyces marxianus* also represents two previous species names, *Candida kefyr* and *Candida pseudotropicalis*. Under the name *Candida pseudotropicalis*, this species was found to be associated with fungal infections of market fish. *Kluyveromyces*

marxianus causes candidiasis including female genital infections, upper respiratory infections, and fungemia in human. *Kluyveromyces marxianus* also has been found in human as a part of biofilms on prosthetic heart valves, catheters, and indwelling devices including pacemakers. *Kluyveromyces marxianus* causes mastitis in cattle and is found in cattle milk.

9.4.95 Knufia [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Chaetothyriales; Family: Trichomeriaceae; Genus: *Knufia*. Members of the genus *Knufia* can be found in soil and on plants. *Knufia epidermidis*, which previously was named *Coniosporium epidermidis*, produces infections in human that present as black pigmented macular skin lesions.

9.4.96 Kodamaea [Infects: Mammals; Also Possibly Infects: Fish]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Metschnikowiaceae; Genus: *Kodamaea*. *Kodamaea ohmeri*, which previously was named *Pichia ohmeri*, is found naturally in latex of the rubber tree *Hevea brasiliensis*, and also has been isolated from fish inestines but without indication of associated pathogenicity for the fish. *Kodamaea ohmeri* infects human in various ways, including the production of oral infections, fungemia, fungemia-associated phlebitis, cellulitis, native valve endocarditis, funguria, peritonitis, and it has been isolated from central venous catheters. *[Candida] mesenterica*, which now belongs to the genus *Kodamaea*, is isolated from fungal basidiocarps, which are the sporocarps of basidiomycetes (Phylum Basidiomycota). *[Candida] mesenterica* also has been found in intestines of beetles that feed on fungal basidiocarp, and *[Candida] mesenterica* has been mentioned in association with human but without clear indication of associated infection.

9.4.97 Kregervanrija [Infects: Mammals]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Pichiaceae; Genus: *Kregervanrija*. Members of the *Kregervanrija* are found in soil and also have been found in natural fermentations of wine and cider. The species *Kregervanrija fluxuum*, in addition to being found in soil, is one of the organisms that cause spoilage of beer and wine, most typically those wines with low-alcohol

content. *Kregervanrija fluxuum* previously has been named *Candida vini*, *Debaryomyces fluxorum*, *Debaryomyces fluxuum*, and *Pichia fluxuum*, it affects human by causing the medical condition candidiasis which most typically is a fungal infection of moist areas such as mouth and vagina.

9.4.98 Kurtzmaniella [Infects: Mammals; Also Possibly Infects: Fish]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Debaryomycetaceae; Genus: *Kurtzmaniella*. The species *[Candida] zeylanoides*, which has been moved to *Kurtzmaniella*, is isolated from soil and it also is part of the normal female genital tract flora for the Arabian camel *Camelus dromedarius*. As an infectious agent, *[Candida] zeylanoides* causes mastitis in bovines and is found in mastitic bovine milk, it also causes fungemia and endocarditis in human, plus it has been found as a contributing microbe for a skin infection of a beached Southern right whale *Eubalaena australis*. *[Candida] zeylanoides* colonizes fish but apparently not with indication of disease.

9.4.99 Kwoniella [Possibly Infects: Amphibians]

Phylum: Basidiomycota; Class: Tremellomycetes; Order: Tremellales; Family: Cryptococcaceae; Genus: *Kwoniella*. The *Kwoniella* are isolated from soil and tree bark. *Cryptococcus heveanensis*, which now is *Kwoniella heveanensis*, has been found in bile of the anuran amphibian *Duttaphrynus melanostictus* but the possibility of that presence having a disease association remains unclear.

9.4.100 Lasiodiplodia [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Botryosphaeriales; Family: Botryosphaeriaceae; Genus: *Lasiodiplodia*. The *Lasiodiplodia* tend to be endophytic, phytopathogenic fungi that cause plant rot and dieback. *Lasiodiplodia theobromae* is a plant pathogen which causes stem canker, rotting and dieback in many plant species notably including the post harvest fungus disease of *Citrus* known as stem-end rot, and it also causes bot canker of grapevine *Vitis*. *Lasiodiplodia theobromae* infects human including causing necrosis in cutaneous burn lesions, keratitis, onychomycosis, and corneal ulcer.

9.4.101 Lichtheimia [Infects: Birds and Mammals]

Phylum: Mucoromycota; Class: Mucoromycetes; Order: Mucorales; Family: Lichtheimiaceae; Genus: *Lichtheimia*. The *Lichtheimia* are found in soil and also are associated with plants. The species *Lichtheimia corymbifera*, previously also named *Absidia corymbifera*, *Mucor corymbifer*, and *Mycocladus corymbifer*, is a thermophilic fungus that usually inhabits soil. *Lichtheimia corymbifera* is associated with decomposing plant material including leaves, mushroom compost, plant seeds including cacao *Theobroma cacao*, and possibly contributes to the decay of grass as well as hay in agricultural settings. *Lichtheimia corymbifera* produces lung infection in chicken. *Lichtheimia corymbifera* causes mastitis, lymphangitis, and also abortion in cattle. *Lichtheimia corymbifera* has been found in both aborted cattle fetus and also the placenta. In horse, *Lichtheimia corymbifera* causes cutaneous infection, abortion, and systemic infection. In human, mucormycosis caused by *Lichtheimia corymbifera* typically involves deep infections including those which are rhinocerebral, plus it infects the bronchorespiratory tract, and it can cause abortion. That outcome of abortion in human caused by *Lichtheimia corymbifera* also has been noted for instances of mixed fungal infections that included this species and members of the genus *Aspergillus* (see the listing for *Aspergillus*). *Lichtheimia corymbifera* has been administered as a probiotic to humans but with a result of leukemia plus infection of the appendix and liver. *Lichtheimia corymbifera* can cause in human necrotic ear infections and also can be found in the human central nervous system, lung, liver, kidney and spleen, and it can be found simultaneous to infection with *Aspergillus fumigatus*. *Lichtheimia corymbifera* additionally causes pneumonia in alpaca *Vicugna pacos* and affects llama. *Lichtheimia hongkongensis* causes rhinocerebral, gastrointestinal, and cutaneous inections in human. *Lichtheimia ornata* causes wound infections in human. *Lichtheimia ramosa* causes wound infections and also disseminated infections in human.

Note: The ability of *Lichtheimia hyalospora*, previously named *Absidia hyalospora*, to grow at 37 Celsius has resulted in consideration that *Lichtheimia hyalospora* eventually may be found pathogenic for human.

9.4.102 Lomentospora [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Microascales; Family: Microascaceae; Genus: *Lomentospora*. The species *Lomentospora prolificans*, previously named *Scedosporium inflatum* and *Scedosporium prolificans*, has been found in the soil of both house plants and greenhouse plants, including the planting soils of benjamin fig also called the Java fig *Ficus benjamina* and Schefflera plants *Schefflera actinophylla*, previously named *Brassaia actinophylla*, that were in hospitals. A general disease association exists between this fungus species and subcutaneous lesions caused by either splinters or plant thorns. *Lomentospora*

prolificans additionally causes arthritis and degenerative osteomyelitis in horse. In human, *Lomentospora prolificans* infection typically follows trauma and remains localized, characteristically may include bone and joint involvement, and a corneal infection has occurred following injury from a lawn trimmer. Disseminated *Lomentospora prolificans* infections in human can include endocarditis and fungemia. *Lomentospora prolificans* has been isolated from cat without apparent disease association.

9.4.103 Lophophyton [Infects: Birds and Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Arthrodermataceae; Genus: *Lophophyton*. The species *Lophophyton gallinae* previously has been named *Microsporum gallinae* and *Trichophyton gallinae*. Although this fungal species has not been specifically identified either in soil or as a plant pathogen, I have placed this genus and species among my list of included fungi because many members of the fungal Family Arthrodermataceae do seem naturally to reside in soil wherein they presumably are involved with keratin degradation. *Lophophyton gallinae* causes dermatophytosis, which is a skin infection. In birds, this is called fowl favus, which presents as "white comb" lesions affecting the comb and wattles of chickens and other fowl. *Lophophyton gallinae* skin infections of mammals often are called ring worm, typically localized, and most commonly observed either on the head as tinea capitis or on other areas of the body as tinea corporis. Such *Lophophyton gallinae* infections often affect human, mice *Mus*, squirrel (Family Sciuridae), cat, dog and monkey (Order Primates, Infraorder Simiiformes). Humans can acquire these infections as a result of handling infected animals and from shared clothing. In human, *Lophophyton gallinae* has been found to form severe dissemination on the skin instead of small localized lesions.

9.4.104 Macrophomina [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Botryosphaeriales; Family: Botryosphaeriaceae; Genus: *Macrophomina*. The *Macrophomina* are endophytic including being found in roots, they notably cause Ashy stem (charcoal rot) of both cotton *Gossypium hirsutum* and numerous other plants. *Macrophomina phaseolina*, for which infections of human cause keratitis and cutaneous lesions, causes disease symptoms on flowering dogwood *Cornus florida* and critically causes charcoal root rot in soybean *Glycine max*.

9.4.105 Madurella [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: (not assigned); Genus: *Madurella*. The *Madurella* are soil fungi. *Madurella fahalii* causes mycetoma in human. The species *Madurella mycetomatis* is a soil fungus that seems limited to tropical and subtropical regions. *Madurella mycetomatis* produces in human chronic cutaneous and subcutaneous granulomatous infections that can involve fascia. *Madurella mycetomatis* also has been identified as causing abdominal mycetoma in dog. *Madurella tropicana* also causes mycetoma in human.

Note: *Madurella grisea*, which produces mycetomas in human, has been renamed *Trematosphaeria grisea*.

9.4.106 Magnusiomyces [Infects: Mammals]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Dipodascaceae; Genus: *Magnusiomyces*. The *Magnusiomyces* typically are found in wood, but they also can be isolated from soil, beach sand, poultry feces, wood pulp, plants, water, and air. *Magnusiomyces capitatus* previously has been known as *Blastoschizomyces capitatus*, *Dipodascus capitatus*, *Geotrichum capitatum*, *Saprochaete capitata*, and *Trichosporon capitatum*. *Magnusiomyces capitatus* is a saprobe that can colonize human skin, mucosa of the oral cavity and esophagitis, attack the respiratory tract including production of pneumonia, cause gastrointestinal infections including pancolitis which is a form of ulcerative colitis, invade the pleural space, cause fungemia, and can be found in urine. Disseminated infections in human caused by *Magnusiomyces capitatus* can lead to death from multiple organ failure including fungal presence in the heart, brain, spleen, liver, lungs and kidneys. *Magnusiomyces capitatus* is known to cause both mastitis as well as abortion in cattle and horse.

9.4.107 Malassezia [Infects: Mammals; Also Possibly Infects: Birds]

Phylum: Basidiomycota; Class: Malasseziomycetes; Order: Malasseziales; Family: Malasseziaceae; Genus: *Malassezia*. *Malassezia* ribosomal DNA (rDNA) has been reported from soil and members of this genus also are found associated with marine invertebrates. *Malassezia brasiliensis* and *Malassezia psittaci* have been isolated from birds, but as yet there seems no indication that those fungal species cause disease in birds. The *Malassezia* very customarily live on the skin of mammals where they consume naturally excreted oils and fats. Members of this fungal species typically cause the benign dermal condition called dandruff which is an exfoliative

dermatosis, but they can infect the skin surrounding nails and claws causing the condition termed paronychia. The *Malassezia* also cause otitis externa, otitis media, and otitis interna. The mammalian host range for some *Malassezia* species is lengthy, and I often have included only a few examples to represent the host range. *Malassezia arunalokei* infects human. *Malassezia caprae* infects goat. *Malassezia cuniculi* infects rabbit. *Malassezia dermatis* infects dog and human. *Malassezia equina* infects horse and human. *Malassezia furfur* infects cat, cattle, dog, goat, pig, guinea pig, horse, and human. *Malassezia globosa* infects cat, cattle, goat, horse, sheep and human. *Malassezia japonica* infects human. *Malassezia nana* infects cat and cattle. *Malassezia obtusa* infects cat, goat, horse, and human. *Malassezia pachydermatis* infects dog, cat, cattle, goat, human, and has caused exfoliative dermatitis in the Indian rhinoceros *Rhinoceros unicornis* for which this fungal species was named. *Malassezia restricta* infects cat, goat, horse, sheep and human. *Malassezia slooffiae* infects cat, cattle, horse, and human. *Malassezia sympodialis* infects cat, cattle, goat, sheep, and human. *Malassezia vespertilionis* infects bats (Order Chiroptera). *Malassezia yamatoensis* infects human. Additional information can be found by researching members of this fungal genus under the previously assigned genus name *Pityrosporum*.

9.4.108 Marasmius [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Agaricales; Family: Marasmiaceae; Genus: *Marasmius*. The *Marasmius* often feed on soil organic matter, they sometimes produce fairy rings, and some of its member species form mushrooms that are edible by humans. *Marasmius palmivorus* previously was named *Marasmiellus palmivorus*, it attacks living crepe- myrtle trees *Lagerstroemia indica* and causes bronchopulmonary mycosis in human.

9.4.109 Mariannaea [Infects: Amphibians]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Nectriaceae; Genus: *Mariannaea*. Many of the *Mariannaea* are soil microbes, some are endophytic, and some have been isolated from freshwater. *Mariannaea elegans* attacks and kills the embryos in eggs of the terrestrial four-toed salamander *Hemidactylium scutatum*. *Mariannaea elegans* has been proposed as a biological control agent to prevent sapstain in wood and wood products. Interestingly, *Mariannaea camptospora*, which thus far does not seem to infect vertebrates, produces the prenylated phenylpropanoid antibacterial compounds Marianin A and Marianin B.

9.4.110 Medicopsis [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Neohendersoniaceae; Genus: *Medicopsis*. The species *Medicopsis romeroi*, previously named *Pyrenochaeta romeroi*, is a saprophyte mostly found either in soil or associated with plants. *Medicopsis romeroi* causes mycetomas, subcutaneous lesions including nodules, and abcesses in human.

9.4.111 Memnoniella [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Stachybotryaceae; Genus: *Memnoniella*. The *Memnoniella* commonly are found in soil, dead plant material in tropical countries, very wet wood, paper, and the paper side of wallboard, all of which are habitats well suited for the cellulolytic lifestyle of this fungal genus. *Memnoniella echinata* previously was named *Stachybotrys echinata*, and in human it causes both sinus infection and pneumonia, as well as idiopathic pulmonary hemosiderosis. It is worth noticing that *Memnoniella echinata* produces the orally administered antifungal mitosis-inhibitor griseofulvin, which is used when antifungal creams have not worked for treating some types of fungal infections of the nails and skin, plus griseofulvin additionally is used to treat dermatophytic fungal infections that cover large body surface areas. Griseofulvin, named for the species *Penicillium griseofulvum*, is ineffective topically.

9.4.112 Metarhizium [Infects: Fish, Mammals, and Repiles]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Clavicipitaceae; Genus: *Metarhizium*. The *Metarhizium* are characterized as naturally growing in soil and as being entomopathogenic. *Metarhizium anisopliae*, previously named *Entomophthora anisopliae*, naturally grows in soil and can endophytically attack plant roots. *Metarhizium anisopliae* attacks insects, typically beetle larvae (Order Coleoptera), and it causes keratitis as well as chronic sinusitis in human. *Metarhizium anisopliae* is used as a biological insecticidal treatment for birds to reduce levels of disease-vectoring insects. *Metarhizium granulomatis* causes dermatitis, glossitis and disseminated visceral infection in veiled chameleon *Chamaeleo calyptratus*. *Metarhizium marquandii*, which previously was named *Paecilomyces marquandii*, causes renal mycosis of Mozambique tilapia *Oreochromis mossambicus* and also causes cellulitis of human. *Metarhizium viride* has been associated with fatal systemic mycoses of lizards including veiled chamelion *Chamaeleo calyptratus*, panther chameleon *Furcifer pardalis*, and central bearded dragon *Pogona vitticep*.

9.4.113 *Metschnikowia* [Infects: Fish and Mammals]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Metschnikowiaceae; Genus: *Metschnikowia*. The *Metschnikowia* can be found in soil and on plants. Some *Metschnikowia* species are pathogens of aquatic invertebrates including crabs and prawns of the Order Decapoda, and common water fleas *Daphnia* of the Order Diplostraca. *Metschnikowia bicuspidata* causes disease of aquatic invertebrates and has been found to cause fatal systemic infections in which the fungus could be isolated from the kidneys of Chinook salmon *Oncorhynchus tshawytscha*, with the association that those fish had been feeding upon live adult brine shrimp *Artemia franciscana*. The species *Metschnikowia pulcherrima* occurs naturally on plant buds, floral parts and fruits. *Metschnikowia pulcherrima* has invertebrate animal hosts in addition to causing dermal infections of mammals (dog and human). This genus presumably was named for one of my heroes in biology, Ilya Ilyich Mechnikov, who in 1908 was awarded the Nobel Prize in Physiology or Medicine.

9.4.114 *Meyerozyma* [Infects: Amphibians, Fish, and Mammals]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Debaryomycetaceae; Genus: *Meyerozyma*. The species *Meyerozyma guilliermondii* is a soil microorganism which previously was named *Candida guilliermondii*. *Meyerozyma guilliermondii* causes subclinical internal infections of anurans (Order Anura). Under its previous name of *Candida guilliermondii*, this species has been found associated with lesions in market fish. *Meyerozyma guilliermondii* also is found in mastitic cattle milk, causes mastitis as well as abortion, and in these ways it affects cattle, horse, and human.

9.4.115 *Microascus* [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Microascales; Family: Microascaceae; Genus: *Microascus*. The *Microascus* are soil saprophytes and plant pathogens. Some species of *Microascus* have been isolated from human clinical sources such as cases of onychomycosis, cutaneous lesions, and mycetomas. *Microascus cinereus* causes suppurative cutaneous granuloma, lower respiratory tract infection, and also brain abcess in human. *Microascus cirrosus* causes disseminated fungal infection in human. *Microascus gracilis*, previously named *Scopulariopsis gracilis*, also causes disease in human, having been isolated from deep tissue fluids, as well as having been found in the upper and lower respiratory

tracts. *Microascus niger*, previously named both *Acaulium nigrum* and *Penicillium nigrum*, has been listed as a pathogen of human but with no associated specific details. *Microascus trigonosporus* causes pulmonary infection of human.

Notes: *Microascus brevicaulis* now is *Scopulariopsis brevicaulis*. *Microascus manginii* now is *Scopulariopsis candida*.

9.4.116 Microsphaeropsis [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: (not assigned); Genus: *Microsphaeropsis*. The *Microsphaeropsis* are plant pathogens. A suggested association was found between this fungal genus and disease in horse, although the possible clinical nature of that interaction was uncertain. The species *Microsphaeropsis arundinis* normally is an endophytic inhabitant of plants. *Microsphaeropsis arundinis* opportunistically causes dermal infections including ulcerations, most typically in diabetic mammals. *Microsphaeropsis arundinis* infects cat and dog, plus it notably causes skin and soft tissue infections in human. *Microsphaeropsis olivacea* is an endophytic fungus of the cyprus species *Pilgerodendron uviferum*, plus *Microsphaeropsis olivacea* causes skin infections in human and interestingly produces a cerebroside.

9.4.117 Microsporum [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Arthrodermataceae; Genus: *Microsporum*. The *Microsporum* naturally reside in soil wherein they presumably are involved with keratin degradation. Members of the genus *Microsporum* cause tinea capitis, tinea corporis, subcutaneous infections which can include nodule formation, and nail infections. The members of this genus have broad host ranges and I am lsting only a few examples of the mammalian species that they infect. *Microsporum audouinii* infects human. *Microsporum canis*, previously named *Arthroderma otae*, infects cat, dog, horse, domestic guinea pig *Cavia porcellus*, human, and presumably also infects kangaroo *Macropus*. *Microsporum distortum* infects dog and human. *Microsporum equinum* infects horse and human. *Microsporum ferrugineum* infects human. *Microsporum rivalieri* infects horse and human.

Notes: *[Microsporum] boullardii*, which infects human, now is assigned to the genus *Nannizzia*. *Microsporum cookei*, which is a dermatophyte of reptiles, now is named *Paraphyton cookei*. *Microsporum fulvum*, which infects human, now is named *Nannizzia fulva*. *Microsporum gallinae*, which infects birds and human, previously also was named *Trichophyton gallinae* and now has been renamed *Lophophyton gallinae*. *Microsporum gypseum*, previously also named *Achorion gypseum*, *Arthroderma gypseum*, *Closterosporia gypsea*, and *Gymnoascus gypseus*,

now is named *Nannizzia gypsea*, it is a saprophyte found in soil and as a dermatophyte infects kangaroo, dog, cat, cattle, horse, sheep, goat, guinea pig, water buffalo, and human. *Microsporum incurvatum*, which infects human, now is *Nannizzia incurvata*. *Microsporum persicolor*, which infects dog, kangaroo and human, has been renamed *Nannizzia persicolor*. *Microsporum praecox*, which infects horse and human, has been renamed *Nannizzia praecox*. *Microsporum racemosum*, which infects human and donkey, has been renamed *[Arthroderma] racemosum*.

9.4.118 Moesziomyces [Infects: Mammals]

Phylum: Basidiomycota; Class: Ustilaginomycetes; Order: Ustilaginales; Family: Ustilaginaceae; Genus: *Moesziomyces*. The *Moesziomyces* are epiphytes and cause smut. The natural ecology of *Moesziomyces aphidis*, previously named *Pseudozyma aphidis*, has an interesting aspect in that this fungal species induces salicylic-acid-independent resistance to *Clavibacter michiganensis* in tomato plants *Solanum lycopersicum* (Barda et al. 2015). *Moesziomyces aphidis* causes fungemia with invasive fungal pneumonia in human. *Moesziomyces bullatus* also causes fungemia in human.

9.4.119 Monascus [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Eurotiales; Family: Aspergillaceae; Genus: *Monascus*. *Monascus* is one of the fungal groups that produce nephrotoxins. The species *Monascus ruber* is found in soil and also associated with many plants. In human, *Monascus ruber* causes onychomycosis, and also has caused invasive gastric infection associated with consumption of dried and salted fish.

Note: *Monascus* also is the name for a genus of trematoda.

9.4.120 Moniliella [Infects: Mammals]

Phylum: Basidiomycota; Class: Moniliellomycetes; Order: Moniliellales; Family: Moniliellaceae; Genus: *Moniliella*. It could be surmised that the natural ecology of *Moniliella* might be related to plant seeds and plant oils, because the species *Moniliella suaveolens* normally is associated with oils and oil-based foods including press cake made from seeds. Interestingly, *Moniliella suaveolens* causes spoilage of margarine. *Moniliella suaveolens* is known to cause subcutaneous infections of cat and human.

9.4.121 Monocillium [Infects: Mammals]

Phylum: Ascomycota; Class: (not assigned); Order: (not assigned); Family: (not assigned); Genus: *Monocillium*. The species *Monocillium indicum* normally is found in soil. As an opportunistic pathogen of mammals, it causes respiratory infections, lymphadenopathy, and splenitis of dog.

9.4.122 Mortierella [Infects: Mammals and Reptiles]

Phylum: Mucoromycota; Class: Mortierellomycetes; Order: Mortierellales; Family: Mortierellaceae; Genus: *Mortierella*. Members of the genus *Mortierella* typically are isolated from soil where they live on decaying leaves and other organic material. *Mortierella* additionally live on fecal pellets and exoskeletons of arthropods. *Mortierella* also are colonizers on the surface of roots, plus they are found in sugarcane, damaged grasses, and vegetable seeds. *Mortierella* have been mentioned as causing phycomycosis in reptiles, which involves lesions most frequently observed in the skin, digestive, genital and respiratory tracts, plus invasion of blood vessels leading to necrosis of adjacent tissue. The mention of *Mortierella* infecting reptiles was not species specific with regard to either the fungus or the reptiles. In mammals, members of the genus *Mortierella* typically cause lung infections and abortion. *Mortierella polycephala* causes pulmonary mycosis in cattle. *Mortierella wolfii* causes meningoencephalitis in cattle, systemic infection following abortion in cattle, and invasive disease including growth in the liver of human.

9.4.123 Mucor [Infects: Amphibians, Birds, Fish, Mammals, and Reptiles]

Phylum: Mucoromycota; Class: Mucoromycetes; Order: Mucorales; Family: Mucoraceae; Genus: *Mucor*. Members of the genus *Mucor* are saprophytes typically isolated from plants and they decay organic matter in soil. As pathogens, the *Mucor* characteristically cause ulcerative dermatitis and nasal nodules, rhinocerebral mucormycosis, and systemic infections resulting in meningitis. *Mucor* species have been found to cause fungal infections in Gibelion catla *Catla catla*, clown knifefish *Chitala chitala*, snakehead murrel *Channa striata*, Jayanti rohu *Labeo rohita*, and great snakehead *Channa marulius*, although those identifications were not at the fungal species level. Frogs and toads infected by *Mucor amphibiorum* have fungi disseminated through their internal organs and skin. *Mucor amphibiorum* also causes ulcerative skin disease in platypus *Ornithorhynchus anatinus*. *Mucor circinelloides* infects numerous species including cattle, pig, platypus, reptiles, and it notably causes

skin as well as invasive maxillofacial disease of human. *Mucor hiemalis* causes skin erosions and ulcerations in sharp tooth catfish *Clarias gariepinus*. *Mucor hiemalis* also affects platypus and causes skin infections of human. *Mucor irregularis*, previously named *Rhizomucor variabilis*, is a pathogen affecting eggs of the four-toed salamander *Hemidactylium scutatum*. *Mucor ramosissimus* has been associated with dermatitis and feather loss in birds. *Mucor ramosissimus* also produces cutaneous lesions in human. In reptiles, *Mucor ramosissimus* causes both cutaneous lesions, necrosis of digits, and osteomyelitis which can be fatal.

Note: *Mucor corymbifer*, previously also named *Absidia corymbifera* and *Mycocladus corymbifer*, now is *Lichtheimia corymbifera*.

9.4.124 Mycocentrospora [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Capnodiales; Family: Mycosphaerellaceae; Genus: *Mycocentrospora*. The *Mycocentrospora* are plant pathogens. *Mycocentrospora acerina* infects many plants including the production of crown rot in carrot and celery *Apium graveolens*, and it causes "Ugly disease" in peonies *Paeonia*. *Mycocentrospora acerina* causes verrucous facial lesions in human.

9.4.125 Mycoleptodiscus [Infects: Mammals]

Phylum: Ascomycota; Class: (not assigned); Order: (not assigned); Family: (not assigned); Genus: *Mycoleptodiscus*. The *Mycoleptodiscus* are opportunistically endophytic plant pathogens that notably cause crown and root rot of alfalfa *Medicago sativa*. *Mycoleptodiscus indicus* causes subcutaneous infections in dog, and this fungal species also infects human by causing subcutaneous infections that include nodule formation which can be accompanied by invasion of both synovial fluid and bursa.

9.4.126 Myrmecridium [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Myrmecridiales; Family: Myrmecridiaceae; Genus: *Myrmecridium*. The *Myrmecridium* are soil saprophytes. *Myrmecridium schulzeri* seems to be the same species as *Ramichloridium schulzeri* (also see the listing for *Ramichloridium*), although currently both taxonomic names are recognized). *Myrmecridium schulzeri* is a soil saprophyte and it has been isolated from plant detritus. *Myrmecridium schulzeri* also has been found in bronchoscopy

fluid of human, presumably from bronchoalveolar lavage, and it causes erosive lesions of the tongue that in human are termed “Golden Tongue” syndrome.

9.4.127 Naganishia [Infects: Mammals; Also Possibly Infects: Amphibians]

Phylum: Basidiomycota; Class: Tremellomycetes; Order: Filobasidiales; Family: Filobasidiaceae; Genus: *Naganishia*. The *Naganishia* naturally reside in high altitude soils. *Naganishia albida* previously was named *Cryptococcus albidus*, it causes lung, cerebrospinal fluid, and blood infections in many mammalian species including horse, cat, dog and human. *Naganishia albida* additionally infects burn wounds in human. *Naganishia liquefaciens*, previously named *Cryptococcus liquefaciens*, has been found in bile of the anuran amphibians *Duttaphrynus melanostictus*, *Hoplobatrachus rugulosus*, *Kaloula pulchra*, and *Sylvirana faber*, although there was no indication of an associated disease in those amphibians.

9.4.128 Nakaseomyces [Infects: Amphibians, Birds and Mammals; Also Possibly Infects: Reptiles]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Saccharomycetaceae; Genus: *Nakaseomyces*. The *Nakaseomyces* are soil microbes. *[Candida] glabrata* previously was named *Torulopsis glabrata*, it is able to survive and even replicate inside macrophage, and it is one of the species which causes the infection syndrome known as candidiasis. *[Candida] glabrata* causes disseminated subclinical internal infections of Amazonian anurans. *[Candida] glabrata* causes the disease called Torulopsis in poultry, in which the liver gets enlarged and reveals yellowish-white with well defined nodules of variable size. *[Candida] glabrata* causes abortion and mastitis in bovines, has been found in mastitic milk, and it also affects dog. The range of disease syndromes which *[Candida] glabrata* causes in human include: cutaneous infection, oral infection, oesophagitis, pneumonia, urinary tract infection, fungemia, and vaginitis. *[Candida] glabrata* also has caused bronchopneumonia in a bottlenose dolphin *Tursiops truncatus*. *[Candida] glabrata* seems to be a cloacal commensal of birds and reptiles, but considering that it is infectious for birds, this fungal species presumably could also be infectious for reptiles. Brunke and coauthors (2014) interestingly noted that the pathogenicity associated with *[Candida] glabrata* can relate to a single point mutation.

9.4.129 Nannizzia [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Arthrodermataceae; Genus: *Nannizzia*. The *Nannizzia* are soil organisms that additionally are dermatophytes which produce skin infectious in a broad range of mammals. I am listing only some examples of the host range for the *Nannizzia* species. *Nannizzia nana* which previously was named *Arthroderma obtusum*, infects cattle. *Nannizzia fulva* previously was named *Microsporum fulvum*, it is found in soil and infects human. *Nannizzia gypsea*, which previously has been named *Achorion gypseum*, *Arthroderma gypseum*, *Closterosporia gypsea*, *Gymnoascus gypseus*, and *Microsporum gypseum*, is a saprophyte found in soil and also is a dermatophyte which infects kangaroo, dog, cat, cattle, horse, sheep, goat, guinea pig, water buffalo, chinchillas (Family Chinchillidae), and human. *Nannizzia incurvata* which previously was named *Microsporum incurvatum* causes the skin infection "cat favus" in cat. *Nannizzia persicolor*, previously named *Microsporum persicolor*, infects dog, kangaroo, and human. *Nannizzia praecox*, which previously was *Microsporum praecox*, infects horse and human. *[Microsporum] boullardii* now is assigned to the genus *Nannizzia*, and it infects human.

9.4.130 Nannizziopsis [Infects: Mammals and Reptiles]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Nannizziopsiaceae; Genus: *Nannizziopsis*. The *Nannizziopsis* potentially are soil microorganisms because they are members of the fungal Order Onygenales, and at least one member species, *Nannizziopsis mirabilis*, has been isolated from soil. All of these listed *Nannizziopsis* species are keratinophilic microfungi that primarily cause skin infections in reptiles of the Order Crocodylia and Order Squamata. It is important to consider that *Nannizziopsis* infections in humans may come from handling infected reptiles! The disease which *Nannizziopsis* cause in reptiles can be characterized as a fatal cutaneous mycosis, it often is called yellow fungus disease, and typically includes dermatitis as well as cellulitis. Initial hyphae proliferation occurrs in the outer epidermal stratum corneum, with subsequent invasion of the deeper epidermal strata and dermis. A spectrum of lesions has been observed ranging from liquefactive necrosis of the epidermis to granulomatous inflammation in the dermis. The *Nannizziopsis* have broad host ranges and they are: *Nannizziopsis arthrosporioides* whose hosts include water dragon *Physignathus*; *Nannizziopsis barbata* whose host range includes bearded dragon *Pogona barbata*; *Nannizziopsis chlamydospora* known to affect at least central bearded dragon *Pogona vitticeps*; *Nannizziopsis crocodili* whose hosts include Australian saltwater crocodile *Crocodylus porosus*; *Nannizziopsis dermatitidis* whose hosts include chameleon *Chamaeleo*; *Nannizziopsis draconii* whose hosts include central bearded dragon *Pogona vitticeps*; *Nannizziopsis guarroi* (previously was named *Chrysosporium*

guarroi) for which host species include bearded dragon *Pogona* and Common green iguana *Iguana iguana*; *Nannizziopsis hominis* (also infects human, involving lymph nodes, heart, lungs, spleen, and kidneys); *Nannizziopsis infrequens* (also causes lung infection of human); *Nannizziopsis obscura* (also causes disseminated infections in human and abcesses including those of the brain); *Nannizziopsis pluriseptata*; whose hosts include skinks *Oligosoma*; and *Nannizziopsis vriesii* (also causes brain abcess in human). *Nannizziopsis vriesii* produces in particular a dermatomycosis that appears to be contagious called "*Chrysosporium* anamorph of *Nannizziopsis vriesii*", abbreviated CANV, which readily infects a very wide range of reptiles including Australian saltwater crocodile *Crocodylus porosus*, Leopard gecko *Eublepharis macularius*, and bearded dragon *Pogona barbata*, presumably spreading either through contact with infected individuals or transfer of the fungus indirectly via fomites. The signs of CANV include dense tufts of sporulating hyphae on the skin surface. Histology of CANV typically shows multifocal coagulation necrosis of the epidermis including marked heterophilic infiltration but without involvement of the underlying dermis.

9.4.131 Naumovozyma [Possibly Infects: Mammals]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Saccharomycetaceae; Genus: *Naumovozyma*. The members of this genus are associated with rotting wood. *Naumovozyma dairenensis*, previously also named *Naumovia dairenensis* and *Saccharomyces dairensis*, is found in fermented milk and may contribute to the spoilage of mayonnaise based salads. *Naumovozyma dairenensis* has been reported to affect humans but no disease specifics were found, and that reporting may have been a genomic misidentification (Stavrou et al. 2018).

9.4.132 Nectria [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Nectriaceae; Genus: *Nectria*. The *Nectria* cause several common canker and dieback diseases, especially in hardwood trees, and can invade wood that has been damaged by other factors such as animals, insects including beech scale *Cryptococcus fagisuga*, freezing and hail. *Nectria inventa*, previously named *Verticillium lateritium*, causes wilt of fruit including possibly watermelon *Citrullus lanatus subspecies vulgaris*. *Nectria inventa* has been reported to cause keratomycosis in human.

Notes: *[Nectria] haematococca*, which occurs as a saprophyte in diverse habitats, as a plant pathogen and also as a pathogen of animals, now is part of the *Fusarium solani* species complex (Genus *Fusarium*). The name *Nectria* also belongs to a genus of seastars.

9.4.133 Neocucurbitaria [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Cucurbitariaceae; Genus: *Neocucurbitaria*. The Cucurbitariaceae are plant pathogens and either necrotrophic or saprobic on woody plants. In human, members of the fungal genus *Neocucurbitaria* typically cause keratitis, onychomycosis, subcutaneous infection, and eumycetoma. *Neocucurbitaria cava*, which previously was named both *Phoma cava* and *Pleurophoma cava*, produces subcutaneous infections in human. *Neocucurbitaria keratinophila* previously was named *Pyrenochaeta keratinophila*, and it notably produces keratitis in human. *Neocucurbitaria unguis-hominis*, previously named *Pyrenochaeta unguis-hominis*, is a dematiaceous fungus which produces skin and nail infections in human.

9.4.134 Neodeightonia [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Botryosphaeriales; Family: Botryosphaeriaceae; Genus: *Neodeightonia*. The species *Neodeightonia subglobosa* previously was named *Botryosphaeria subglobosa* and *Sphaeropsis subglobosa*. *Neodeightonia subglobosa* presumably is endophytic, because it has been isolated from surface sterilized leaf segments of ethnopharmaceutically important medicinal herbs collected from the Bhadra River Project Area in the Malnad region of Southern India. *Neodeightonia subglobosa* infects human by producing keratomycosis (fungal keratitis) associated with injury due to bamboo splinters.

9.4.135 Neopestalotiopsis [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Xylariales; Family: Sporocadaceae; Genus: *Neopestalotiopsis*. The *Neopestalotiopsis* are plant pathogens that have been associated with: trunk disease, leaf spotting and post-harvest fruit rot of grape *Vitis*; root, crown and fruit rot of strawberry *Fragaria*; and dieback in blueberry *Vaccinium darrowii*. *Neopestalotiopsis clavispora*, which infects human by producing fungal keratitis, previously was named *Pestalotiopsis clavispora*.

9.4.136 Neoscytalidium [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Botryosphaeriales; Family: Botryosphaeriaceae; Genus: *Neoscytalidium*. The *Neoscytalidium* typically are

latent pathogens involved with branch canker and dieback of woody plants, notably causing that disease in *Citrus* fruit trees. Members of the genus *Neoscytalidium* produce keratinase, with that enzymatic activity likely contributing to some of the infections which this genus characteristically causes in mammals. Those infections typically produce cutaneous and subcutaneous disease affecting the feet and nails, although *Neoscytalidium* also can be invasive to produce subcutaneous infections, disseminated infections as well as fungemia. The fungal species *Neoscytalidium dimidiatum*, previously named *Scytalidium dimidiatum*, is a plant pathogen that causes dieback of *Albizia lebbeck*. *Neoscytalidium dimidiatum* is known to infect humans by causing: onychomycosis in green tea *Camellia sinensis* leaf pluckers; subcutaneous mycetomas that may be recalcitrant; sinus infection; pulmonary infections; and fungemia. *Neoscytalidium dimidiatum* also infects dog, and causes pulmonary infections in non-livestock mammals including Risso's dolphin *Grampus griseus*. *Neoscytalidium hyalinum* infects dog, and also infects human including the production of pulmonary infection.

9.4.137 Neotestudina [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Testudinaceae; Genus: *Neotestudina*. The *Neotestudina* have been isolated from soil in tropical areas. The species *Neotestudina rosatii* infects human by producing mycetomas.

9.4.138 Neurospora [Infects: Fish and Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: Sordariaceae; Genus: *Neurospora*. The *Neurospora* are a genus of soil fungi which can grow on cellulose, they also are found in lumber yards, plywood factories, on steamed logs, on areas of burned grass including those beside railways and roads, and on the stubble of burned sugarcane fields. *Neurospora* species cause fungal infections in many fish, including Gibelion catla *Catla catla*, clown knifefish *Chitala chitala*, snakehead murrel *Channa striata*, Jayanti rohu *Labeo rohita*, and great snakehead *Channa marulius*. *Neurospora sitophila* has caused in human endophthalmitis following cataract extraction.

9.4.139 Nigrograna [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Nigrograna ceae; Genus: *Nigrograna*. The species *Nigrograna mackinnonii*

previously was named *Pyrenochaeta mackinnonii* and also has been named *Biatriospora mackinnonii*, it can be found in soil and is endophytic. *Nigrograna mackinnonii* infects human, typically by causing mycetoma, cutaneous phaeohyphomycosis and subcutaneous cysts. Such infections follow traumatic implantation of saprophytic fungi and frequently require either radical surgery or amputation in the absence of appropriate antimicrobial treatment.

9.4.140 Nigrospora [Infects: Fish and Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Trichosphaeriales; Family: Trichosphaeriaceae; Genus: *Nigrospora*. The *Nigrospora* are plant pathogens that can grow endophytically, and they commonly are isolated from leaves. The genus *Nigrospora* has been identified as causing mycosis in the gilthead seabream *Sparus aurata* but that fungal identification was not species specific. *Nigrospora oryzae* is a fungal endophyte in rice *Oryza sativa*, often is found in decaying plant material and soil, and it has been isolated from surface sterilized leaf segments of ethnopharmaceutically important medicinal herbs collected from the Bhadra River Project Area in the Malnad region of Southern India. *Nigrospora oryzae* additionally is a dematiaceous mold which affects human by producing skin infections. *Nigrospora sphaerica* affects human by causing superficial skin infection, onychomycosis (nail infection), and corneal ulcer.

9.4.141 Nodulisporium [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Xylariales; Family: Xylariaceae; Genus: *Nodulisporium*. The *Nodulisporium* are endophytes, they have been noted to produce sinus disease and brain infection (cerebral phaeohyphomycosis) in human but without identification of the causative fungal species. The species *Nodulisporium sylviforme*, which presumably is not infectious for vertebrates, notably produces the mitotic inhibitor taxol which has been used in anticancer chemotherapy.

9.4.142 Ochrocladosporium [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: (not assigned); Genus: *Ochrocladosporium*. The *Ochrocladosporium* are found in soil and associated with black mold on plants. *Ochrocladosporium elatum*, formerly named *Cladosporium elatum*, infects human sinuses and has been identified in a stain of human sinus tissue.

9.4.143 *Ochroconis* [Infects: Amphians, Fish, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Venturiales; Family: Sympoventuriaceae; Genus: *Ochroconis*. The *Ochroconis* are endophytes whose natural habitat additionally includes soil and decaying leaves. *Ochroconis cordanae* infects human, with the most common anatomical sites of isolation from human being the lower respiratory tract followed by superficial and deep tissues. The species *Ochroconis humicola*, previously named *Scolecobasidium humicola*, has been found growing endophytically on the roots of tomato *Solanum lycopersicum*, and it also has an environmental life on the skin of animals. *Ochroconis humicola* has caused granulomatous dermatitis in eastern spadefoot toad *Scaphiopus holbrookii*. *Ochroconis humicola* has proven infectious for numerous fish species among which are: several members of the Family Salmonidae, including *Ochroconis humicola* having been isolated from kidney of coho salmon *Oncorhynchus kisutch*; having been the cause of phaeohyphomycosis in rainbow trout *Oncorhynchus mykiss*, and being associated with muscular black spot disease of Atlantic salmon *Salmo salar*; mycoses of the false kelpfish *Sebastiscus marmoratus* and the Short-horn sculpin also called scorpion fish *Myoxocephalus scorpius*; having producing phaeohyphomycosis in walking catfish *Clarias batrachus*; causing erosive and ulcerative lesions at the base of the dorsal fin in red seabream *Pagrus major*; and creating open ulcers on the dorsal surface which included fungal growth in the underlying musculature of cultured devil stinger *Inimicus japonicus*. *Ochroconis humicola* is an oppportunistic pathogen of mammals, as demonstrated by having causing subcutaneous infections in cat and attacking the nasal tissue of human. *Ochroconis humicola* additionally infects reptiles, as evidenced by this fungal species having been isolated from granulomatous, superficially ulcerated, papular foot lesions in an eastern box turtle *Terrapene carolina carolina*. *Ochroconis mirabilis* causes subcutaneous infection in human. *Ochroconis musae* causes subcutaneous infection in human. *Ochroconis ramosa* also has been isolated from human clinical specimens but I could not find specific details on that discovery. *Ochroconis tshawytschae* causes kidney infections of chinook salmon *Oncorhynchus tshawytscha* and subcutaneous infection in human.

Note: *Ochroconis gallopava*, which previously also was named *Dactylaria gallopava*, now has the taxonomic name *Verruconis gallopava* and it is extremely deleterious in birds.

9.4.144 *Oidiodendron* [Infects: Mammals and Reptiles]

Phylum: Ascomycota; Class: Leotiomycetes; Order: (not assigned); Family: Myxotrichaceae; Genus: *Oidiodendron*. The *Oidiodendron* are endophytic and found in roots, additionally they are found in soil, peat, wood, and decomposing

materials. *Oidiodendron* has been reported as causing necrotizing encephalitis in Common green iguana *Iguana iguana*, but without identification of the fungal species except a statement that it was not *Oidiodendron cerealis*. *Oidiodendron cerealis* causes dermatitis (neurodermatitis) in human.

Note: *Oidiodendron kalrae* which infects mammals including human, but seemingly does not infect reptiles, now is named *Arthrographis kalrae*.

9.4.145 Ophidiomyces [Infects: Reptiles]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Onygenaceae; Genus: *Ophidiomyces*. The species *Ophidiomyces ophiodiicola*, previously named *Chrysosporium ophiodiicola*, is believed to persist saprophytically in soil aside from its keratinophilic association with living hosts. *Ophidiomyces ophiodiicola* infects reptiles, particularly snakes, and it causes Snake Fungal Disease (SFD) which results in severe morbidity and mortality. Snake Fungal Disease involves facial swelling and can progress from the nasal cavity internally via the eyes, where it causes eye infections, and via the throat to the lungs where it causes pneumonia. The fungus additionally spreads externally along the neck, body, and tail causing skin swelling, nodules and ulcerations.

9.4.146 Ophiostoma [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Ophiostomatales; Family: Ophiostomataceae; Genus: *Ophiostoma*. The *Ophiostoma* are plant pathogens. *Ophiostoma piceae* causes sapstain in timber, although in human it causes disseminated infections involving the lung, brain, kidney and bone marrow. This genus very notably includes the Dutch elm disease pathogens *Ophiostoma novo-ulmi* and *Ophiostoma* ulmi, Dutch elm disease is a lethal vascular wilt that affects American elm *Ulmus americana*.

9.4.147 Ovatospora [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: Chaetomiaceae; Genus: *Ovatospora*. *Ovatospora brasiliensis* previously was named *Chaetomium brasiliense*, it is a saprophyte associated with soil and presumably also with decaying plant materials. In humans, *Ovatospora brasiliensis* causes otitis externa.

9.4.148 Paecilomyces [Possibly Infects: Fish]

The *Paecilomyces* typically are soil microbes. I am not certain if *Paecilomyces* presently belongs on the "active" list, but because of that uncertainty I will include it here. The genus *Paecilomyces* has been identified as causing mycosis in the gilthead seabream *Sparus aurata* but identification of that fungus was not done to the species level, and quite possibly that may have been either *Paecilomyces lilacinus* or *Paecilomyces marquandii* which are fish pathogens that have been renamed and moved to oher genera (see below). All of the other members of this genus which are known to infect vertebrates also have been renamed and moved to other genera (likewise, see below). The genus *Paecilomyces* has been divided into three genera, of which only either one, or indeed none, still may contain a species that is pathogenic for vertebrates and that would be the unnamed fish pathogen. I have numbered these three genera by the order that they are listed in the National Center for Biotechnology Information database.

Genus 1: [not shown to infect vertebrates]
Phylum: Ascomycota; Class: Eurotiomycetes; Order: Eurotiales; Family: Thermoascaceae; Genus: *Paecilomyces*.

Genus 2: [not shown to infect vertebrates]
Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Clavicipitaceae; Genus: *Paecilomyces*.

Genus 3: [not shown to infect vertebrates]
Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: (not assigned); Genus: *Paecilomyces*.

Notes: *Paecilomyces crustaceus*, previously also named *Dactylomyces crustaceus*, has been renamed *Thermoascus crustaceus*. *Paecilomyces lilacinus*, which causes a wasting disease that affects both Mozambique tilapia *Oreochromis mossambicus* and Tilapia aurea *Melanochromis auratus*, infects human and may cause dermatomycosis as well as systemic mycosis in aquatic reptiles, it now is named *Purpureocillium lilacinum*. *Paecilomyces marquandii*, which causes renal mycosis of Mozambique tilapia *Oreochromis mossambicus*, and which also causes cellulitis of human, has been renamed *Metarhizium marquandii*. *Paecilomyces variotii*, which infects human and other vertebrates, now is *Byssochlamys spectabilis*.

9.4.149 Papiliotrema [Infects: Mammals]

Phylum: Basidiomycota; Class: Tremellomycetes; Order: Tremellales; Family: Rhynchogastremataceae; Genus: *Papiliotrema*. The *Papiliotrema* grow both endophytically and on the surface of plants, including leaves, they degrade a large

variety of plant structural compounds and produce a phytase. In mammals, the *Papiliotrema* tend to produce subcutaneous infections that include nodule formation. *Papiliotrema flavescens* previously was named *Torula flavescens* and *Cryptococcus flavescens*, it is present on grape berries, produces subcutaneous disease in dog, and is found in bird fecal samples which suggests that pet birds may be a carrier of the pathogen. *Papiliotrema laurentii* previously was named *Cryptococcus laurentii*, it is a soil organism that also is endophytic, and its association with apple *Malus domestica* results in this fungal species being found in apple cider. *Papiliotrema laurentii* has shown pathogenicity for mammals by causing bone lesions associated with subclinical visceral leishmaniasis in a dog, and in human this fungal species causes: cutaneous infection in addition to infecting burn wounds, fungemia, meningitis, lung abcess, and pneumonia which can include pleural effusion. *Papiliotrema laurentii* seems to be an oral commensal of the yellow-spotted Amazon River turtle *Podocnemis unifilis* with no indication of associated disease.

9.4.150 Paracoccidioides [Infects: Birds and Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: (not assigned); Genus: *Paracoccidioides*. The *Paracoccidioides* are soil microbes. The species *Paracoccidioides brasiliensis* commonly is associated with soils in which *Coffea* are cultivated and *Paracoccidioides brasiliensis* also can be grown in animal excrement. There is serological evidence that *Paracoccidioides brasiliensis* infection occurs naturally in free range chicken *Gallus gallus*. In mammals, *Paracoccidioides brasiliensis* causes a progressive mycosis of which the initial infection usually occurs either in the lungs or through traumatic implantation, after which the infection may spread to include skin, mucous membranes, gastrointestinal tract, lymph nodes, glands, and essentially all internal organs resulting in organomegaly. Among mammals, *Paracoccidioides brasiliensis* causes disease in: dog; human; rabbit; small wild rodents (Order Rodentia) of the Genera *Abrawayaomys*, *Akodon, Euryoryzomys*, *Thaptomys, Oligoryzomys,* and *Sooretamys*; produces thymic atrophy in house mouse *Mus musculus*; affects short-tailed opossums *Monodelphis*; and has caused cutaneous granulomas in bottlenose dolphins *Tursiops truncatus*. *Paracoccidioides lutzii* also infects human by causing fatal fungemia, systemic granulomatous infection with visceral involvement, and has been noted to have tegumentary involvement that diagnostically was confused with leprosy.

9.4.151 Paraconiothyrium [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Didymosphaeriaceae; Genus: *Paraconiothyrium*. The *Paraconiothyrium* typically

are inhabitants of wood and soil, endophytic pathogens, and hyperparasites on other fungi. *Paraconiothyrium cyclothyrioides* causes cutaneous and subcutaneous infections including nodules and abcesses in human, and also has caused both skin and soft tissue infection of human in association with *Phaeoacremonium parasiticum*. *Paraconiothyrium fuckelii*, which previously was named *Coniothyrium fuckelii*, has specifically been associated with stem cankers of apple trees and it causes liver infection of humans.

9.4.152 Paranannizziopsis [Infects: Reptiles]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Nannizziopsiaceae; Genus: *Paranannizziopsis*. The *Paranannizziopsis* presumably reside naturally as soil organisms, but as pathogens of reptiles the *Paranannizziopsis* cause dermatomycosis affecting lizards and snakes. *Paranannizziopsis australasiensis* has been noted in at least the bearded dragon *Pogona barbata*, tuatara *Sphenodon punctatus*, and tentacled snake *Erpeton tentaculatum*. *Paranannizziopsis californiensis*, and *Paranannizziopsis crustacea*, infect at least the tentacled snake *Erpeton tentaculatum*.

9.4.153 Paraphyton [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Arthrodermataceae; Genus: *Paraphyton*. The species *Paraphyton cookei*, previously named *Microsporum cookei* and *Nannizzia cajetani*, is a soil microbe and also a dermatophyte that infects humans, rodents, and dogs.

9.4.154 Parathyridaria [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Thyridariaceae; Genus: *Parathyridaria*. The *Parathyridaria* are plant pathogens. *Parathyridaria percutanea*, which previously was named *Roussoella percutanea*, causes subcutaneous cysts and bursitis in human.

9.4.155 Parengyodontium [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Cordycipitaceae; Genus: *Parengyodontium*. The *Parengyodontium* are found in

soil and on plant debris. *Parengyodontium album*, which previously was named *Engyodontium album*, affects at least human and cattle by producing cutaneous infections, brain abcess, keratitis, fungaemia, native valve endocarditis, and also bioprosthetic valve (bovine) endocarditis in human.

9.4.156 Penicillium [Infects: Birds, Fish, Mammals and Reptiles; Also Possibly Infects: Amphibians]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Eurotiales; Family: Aspergillaceae; Genus: *Penicillium*. The *Penicillium* typically live in the soil where they process dead and decaying organic matter. The trichothecene toxins produced by *Penicillium* can influence the infections which this genus causes in vertebrates. The genus *Penicillium* has been isolated from injured and repressed tissue regeneration sites in Ozark hellbender salamander *Cryptobranchus alleganiensis bishopi* but unfortunately the fungal identification was not species specific, and since some of the *Penicillium* have taxonomically been reasigned into other genera and I could find no additional mentions of *Penicillium* having caused infection of amphibians, I will consider this infection in Ozark hellbender as possibly having been caused by a species that has remained in the genus *Penicillium*. *Penicillium* species cause fungal infections in Gibelion catla *Catla catla*, clown knifefish *Chitala chitala*, snakehead murrel *Channa striata*, Jayanti rohu *Labeo rohita*, and great snakehead *Channa marulius*. Members of the genus *Penicillium* cause a canine pneumonia and also have been associated with bone lesions of a dog with osteomyelitis. *Penicillium* additionally have been recovered from lesions of the lungs, stomach, liver, and pancreas of the Indefatigable Island giant tortoise *Chelonoidis porteri*. *Penicillium aurantiogriseum* has been found to cause skin erosions and ulcerations in sharp tooth catfish *Clarias gariepinus*. *Penicillium brevicompactum* causes pneumonia in dog, and systemic disease in Aldabra giant tortoise *Aldabrachelys gigantea*. *Penicillium canis* causes osteomyelitis in dog. *Penicillium camemberti* and *Penicillium capsulatum* cause intestinal invasion and disseminated disease including cerebral infection in human. *Penicillium chrysogenum* is used as a starter culture in food preservation notably the fermented, cured sausage called salami, even though *Penicillium chrysogenum* can cause allergic reactions in people who come into contact with that meat product, and presumably that usage was the cause of mycosis that infected the lung, liver, and kidney of a grey parrot *Psittacus erithacus*. *Penicillium chrysogenum* has been found to cause skin erosions and ulcerations in sharp tooth catfish *Clarias gariepinus*. *Penicillium chrysogenum* causes intestinal invasion and disseminated disease including cerebral infection in human. *Penicillium chrysogenum* also has been noted to cause lethal systemic mycosis in green iguana *Iguana iguana*, digit necrosis in skink *Scincidae*; and shell exfoliation in red-eared slider turtle *Trachemys scripta elegans*. *Penicillium citrinum* has caused pneumonia with pericarditis in human. *Penicillium commune* has caused a combined pulmonary

and cerebral infection in human. *Penicillium decumbens* causes paravertebral infection in human. *Penicillium digitatum* causes opportunistic pneumonia in human. *Penicillium griseofulvum* has been found in the lungs, air sacs, liver and other tissues of toucanet *Aulacorhynchus*. *Penicillium oxalicum* has caused invasive mycosis affecting the spleen, liver and lung of human. *Penicillium roquefortii* causes fungus ball maxillary sinusitis disease in human.

Notes: *Penicillium marneffei* has been renamed *Talaromyces marneffei*.

Penicillium nigrum, which also has been named *Acaulium nigrum*, now is *Microascus niger*. *Penicillium piceum*, which causes pulmonary nodule and adjacent rib osteomyelitis in human, has been renamed *Talaromyces piceae*. *Penicillium radicum*, which causes disseminated infection in dog, has been renamed *Talaromyces radicus*.

9.4.157 Peniophora [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Russulales; Family: Peniophoraceae; Genus: *Peniophora*. The *Peniophora* are plant pathogens that usually inhabit tree bark, they cause root and stem canker, and they have been associated with moldy hay. *Peniophora* has been found to cause lung infection of human although the fungal identification was not species specific.

9.4.158 Perenniporia [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Polyporales; Family: Polyporaceae; Genus: *Perenniporia*. The *Perenniporia* are plant pathogens, sometimes considered to be secondary plant pathogens, which produce a white rot of wood and form bracket or crust-like polypores. The genus *Perenniporia* has been identified as causal of a pulmonary fungal ball in human, but the fungal species was not determined.

9.4.159 Pestalotia [Infects: Reptiles]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Xylariales; Family: Amphisphaeriaceae; Genus: *Pestalotia*. The *Pestalotia* are saprophytic on dead and dying plant tissues, weakly parasitic of plants and infect plant wounds under moist conditions. Plant diseases caused by the *Pestalotia* include blighting of leaves, needles and twigs. *Pestalotia pezizoides* has been considered the generic type of *Pestalotia*, it was described from leaves and stems of wine grape *Vitis vinifera* collected in Italy, and presently is not known from either culture or DNA sequence. *Pestalotia pezizoides* currently is not an approved species name although this species name is historically

significant and still is used. I was unable to determine if this fungal species has been assigned a diffferent name. *Pestalotia pezizoides* has caused skin infections of snakes, including dermatitis in dusky pigmy rattlesnake *Sistrurus miliarius barbouri.*

9.4.160 Petriella [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Microascales; Family: Microascaceae; Genus: *Petriella*. The species *Petriella setifera* often is found in plant debris, seeds, dung, and soil, it infects soft roots, twigs and bark, plus it has been linked to decayed wood, compost, and animal manure. *Petriella setifera* has been isolated from persistent ulcerative cutaneous lesions of an Atlantic bottlenose dolphin *Tursiops truncatus* that was residing in an aquarium.

9.4.161 Phaeoacremonium [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Togniniales; Family: Togniniaceae; Genus: *Phaeoacremonium*. The *Phaeoacremonium*, perhaps most notably *Phaeoacremonium parasiticum*, are associated with wilt, decline and die-back diseases of woody plants. *Phaeoacremonium* diseases of human include subcutaneous infection, eumycetoma, fungemia, osteomyelitis, arthritis, and endocarditis. Among the species in this genus, *Phaeoacremonium alvesii*, *Phaeoacremonium amstelodamense*, *Phaeoacremonium griseorubrum*, *Phaeoacremonium inflatipes*, and *Phaeoacremonium krajdenii* produce mycetomas in human. *Phaeoacremonium parasiticum* has caused skin and soft tissue infection of human in association with *Paraconiothyrium cyclothyrioides*. *Phaeoacremonium rubrigenum*, *Phaeoacremonium sphinctrophorum*, *Phaeoacremonium tardicrescens*, and *Phaeoacremonium venezuelense* also infect human.

9.4.162 Phaeosclera [Infects: Mammals]

Phylum: Ascomycota; Class: (not assigned); Order: (not assigned); Family: (not assigned); Genus: *Phaeosclera*. The species *Phaeosclera dematioides* has been found on rocks and been isolated from the pith of lodgepole pine *Pinus contorta*, which is a genus of fire dependent pine tree. The species *Phaeosclera dematioides* is dematiaceous, causing phaeohyphomycosis in human and cattle. *Phaeosclera dematioides* can, when acting alone, attack not only the skin but also the mucous membranes. *Phaeosclera dematioides* additionally has been found to cause hyphomycosis as a mixed infection with members of the genus *Alternaria* (see the listing for *Alternaria*).

9.4.163 Phaeotrichoconis [Infects: Mammals]

Phylum: Ascomycota;Class: (not assigned); Order: (not assigned); Family: (not assigned); Genus: *Phaeotrichoconis*. The species *Phaeotrichoconis crotalariae* is a plant pathogen which, in Brazil, has been found growing as an endophyte in healthy leaves of Concord grape *Vitis labrusca*. *Phaeotrichoconis crotalariae* infects human to produce mycotic keratitis.

9.4.164 Phanerochaete [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Polyporales; Family: Phanerochaetaceae; Genus: *Phanerochaete*. The *Phanerochaete* are crust fungi of woody plants and cause white rot in tree bark. *Phanerochaete* are credited as being major agents of wood decomposition in temperate forests, where their ability to degrade lignin into carbon dioxide is achieved in part by lignin peroxidases and manganese peroxidases. These peroxidases are also able to mediate oxidation of organic pollutants. *Phanerochaete chrysosporium* causes granulomatous lung disease in human. *Phanerochaete stereoides* causes bronchopulmonary mycosis in human.

9.4.165 Phellinus [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Hymenochaetales; Family: Hymenochaetaceae; Genus: *Phellinus*. The *Phellinus* are plant pathogens that cause white rot decay including root rot in conifers, they are capable of causing considerable destruction in trees suffering from other stress. It has been reported that aboriginal Australians used *Phellinus* fruiting bodies medicinally. *Phellinus undulatus* causes soft tissue infection in human.

Note: *Phellinus tropicalis*, previously also named *Inonotus tropicalis*, is a pathogen of human and currently named *Tropicoporus tropicalis*.

9.4.166 Phialemoniopsis [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Xylariales; Family: (not assigned); Genus: *Phialemoniopsis*. The *Phialemoniopsis* include endophytic pathogens. Among the diseases which *Phialemoniopsis* cause in mammals are cutaneous infections of wounds and burns, and such infections can include the formation of subcutaneous nodules. *Phialemoniopsis* species also can produce internal infections

among which are peritonitis with fungus recoverable from the pleural fluid, osteomyelitis, and endovascular disease including endocarditis. The species *Phialemoniopsis curvata*, previously named *Phialemonium curvatum*, is broadly present in the environment, being found in air, soil, industrial water and sewage. *Phialemoniopsis curvata* causes in human: meningitis, arthritis, endocarditis affecting the heart valves, endophthalmitis, peritonitis, subcutaneous infection, and vascular infection. *Phialemoniopsis curvata* also causes pulmonary infection in dog. *Phialemoniopsis endophytica* is an endophytic fungus identified in smooth loofah *Luffa aegyptiaca*, and *Phialemoniopsis endophytica* along with *Exophiala jeanselmei* has caused concurrent skin infections of human. *Phialemoniopsis hongkongensis*, which causes subcutaneous phaeohyphomycotic nodules in human, may be the same species as *Exophiala hongkongensis* although both of these names currently are recognized (see the listing for genus *Exophiala*) and a natural ecology has not yet been defined for *Phialemoniopsis hongkongensis*. *Phialemoniopsis ocularis*, which previously was named *Sarcopodium oculorum*, infects humans by causing subcutaneous phaeohyphomycosis which can include nodules.

9.4.167 Phialemonium [Infects: Fish and Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: Cephalothecaceae; Genus: *Phialemonium*. The *Phialemonium* presumedly are primarily saprotrophic, they have been isolated from air, soil, industrial water, and sewage. *Phialemonium dimorphosporum* causes skin ulcers in striped mullet *Chelon tricuspidens*. *Phialemonium obovatum* has caused in human: infections arising secondary to burns, subcutaneous infection, endovascular infection, endocarditis, keratitis, peritonitis, and osteomyelitis. The ability of *Phialemonium obovatum* hyphae to invade blood vessels has contributed to these disease outcomes. *Phialemonium obovatum* also has produced osteomyelitis in dog.

Note: *Phialemonium curvatum* now is *Phialemoniopsis curvata*.

9.4.168 Phialophora [Infects: Mammals; Also Possibly Infects: Amphibians and Fish]

The *Phialophora* have been isolated from soil, wood, and wood pulp, they also are saprophytic of plants including apple *Malus domestica*. *Phialophora* have been reported to cause disease in amphibians but without speciic details. *Phialophora* also have been reported to cause systemic disease in Altantic salmon *Salmo salar* but the causative fungal species was not determined. The *Phialophora* have been

divided into two genera and I have numbered these two genera by the order that they are listed in the National Center for Biotechnology Information database.

Genus 1 [Infects: Mammals]
Phylum: Ascomycota; Class: Eurotiomycetes; Order: Chaetothyriales; Family: Herpotrichiellaceae; Genus: *Phialophora*. *Phialophora americana* causes subcutaneous cyst in human. *Phialophora bubakii* has caused subcutaneous abcess in human. *Phialophora cyanescens*, previously named *Cylindrocarpon cyanescens*, produces mycetomas in human. The species *Phialophora verrucosa* is isolated from soil and is a soft rotting fungus of wood including logs and bark. As a pathogen of mammals, *Phialophora verrucosa* has been found to cause long term infections of the skin and subcutaneous tissues including the formation of subcutaneous fungal nodules in cat, dog and human.

Genus 2 [not shown to infect vertebrates]
Phylum: Ascomycota; Class: Leotiomycetes; Order: (not assigned); Family: (not assigned); Genus: *Phialophora*.

Notes: *Phialophora europaea* now is *Cyphellophora europaea*. *Phialophora oxyspora* now is named *Cyphellophora oxyspora*. *Phialophora reptans* now is named *Cyphellophora reptans*. *Phialophora richardsiae* which causes bursitis in human and previously also was named *Pleurostomophora richardsiae*, now is *Pleurostoma richardsiae*.

9.4.169 Phoma [Infects: Fish and Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Didymellaceae; Genus: *Phoma*. The *Phoma* are plant pathogens. *Phoma herbarum* commonly is found growing saprophytically in soil. *Phoma herbarum* produces gibberellins when it grows endophytically, which means that this fungus is growth-promoting as an endophyte. *Phoma herbarum* grows saprophytically on dead plant tissues and has been isolated from necrotic areas of plants where it causes leaf spot and stem canker. *Phoma herbarum* has broad infectivity as a pathogen of plants, including as its hosts African oil palm *Elaeis guineensis*, hemp *Cannabis sativa*, hop (Family Cannabaceae), and pea *Pisum sativum*. *Phoma herbarum* is able to grow on natural lignin and synthetic lignin (dehydrogenation polymer, DHP) as its sole carbon source, and it grows on paper products which would include microbiology books. *Phoma herbarum* causes systemic infection in fish, characterized by mycelial invasion of the air bladder, affects upon the digestive tract including the production of gut obstruction, peritonitis, visceral necrosis and severe hemorrhaging, with observation of the kidney having been the organ most affected in natural infections. *Phoma herbarum* causes disease in various cultured salmonids including coho salmon *Oncorhynchus kisutch*, Chinook salmon *Oncorhynchus tshawytscha*, Sockeye salmon *Oncorhynchus nerka*, lake trout *Salvelinus namaycush* and rainbow

trout *Oncorhynchus mykiss gairdneri*, and also causes mycosis in gilthead seabream *Sparus aurata*. *Phoma herbarum* has been identified in human tissue samples, and those seem to have been samples from skin and lung.

Notes: *Phoma cava* which causes subcutaneous infection in human, previously was named *Pleurophoma cava* and now is *Neocucurbitaria cava*. *Phoma eupyrena* which infects human now is *Juxtiphoma eupyrena*. *Phoma exigua* which causes lung mass in human now is *Boeremia exigua*. *Phoma glomerata*, which infects goat and also the common chameleon *Chamaeleo chamaeleon*, previously was named *Peyronellaea glomerata* and now is *Didymella glomerata*. *Phoma heteroderae* which infects human now is *Didymella heteroderae*. *Phoma minutispora* which infects human now is *Westerdykella minutispora*. *Phoma oculi-hominis* which infects human now is *Stagonosporopsis oculi-hominis*. *Phoma sorghina* which infects human now is *Epicoccum sorghinum*.

9.4.170 Phomopsis [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Diaporthales; Family: Valsaceae; Genus: *Phomopsis*. The *Phomopsis* are plant pathogens. *Phomopsis phaseoli*, as a plant pathogen, causes twig blight, leaf blight, galls, and fruit rot. As a pathogen of human, *Phomopsis phaseoli* causes eumycetoma with osteomyelitis.

Note: *Phomopsis longicolla*, which causes cutaneous infections in human, now is named *Diaporthe longicolla*.

9.4.171 Pichia [Infects: Birds, Fish, and Mammals]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Pichiaceae; Genus: *Pichia*. The *Pichia* are found in soil as well as on fruits, in natural fermentations, and they are responsible for spoilage of pickled vegetables such as kimchi. *Pichia* cause disease of both invertebrate as well as vertebrate animal hosts. *Hansenula* is an obsolete synonym for *Pichia*. The species *Pichia kudriavzevii*, previously named *Candida krusei*, is found in sourdough. *Pichia kudriavzevii* is used in fermenting wine despite the fact of it being an opportunistic pathogen. *Pichia kudriavzevii*, along with *Geotrichum*, are involved in the fermentation of *Theobroma cacao* seeds, often called cocoa beans, for chocolate production. Those fungi naturally are present on the cacao seed pods although specifically selected fungal strains also may be used to impart desired flavor and aroma to the chocolate product which will be produced from the seeds. *Pichia kudriavzevii* can be isolated from the oropharynx and cloaca of birds, it causes crop mycosis in birds, and has caused necrotising ventriculitis due to combined infection with *Rhizopus microsporus* in an eclectus parrot *Eclectus roratus*. *Pichia kudriavzevii* has been found associated with

lesions in market fish. As an opportunistic pathogen of mammals, *Pichia kudriavzevii* causes bronchopneumonia and mastitis in cattle, and is found in cattle milk. *Pichia kudriavzevii* infections of human can be associated with high mortality and they include severe mucositis, invasive destruction of the intestinal wall, hepatosplenic candidiasis, pneumonia, renal cyst, typhlitis (infection of the cecum), plus infections of the aorta (aortitis), brain, bone marrow, esophagus, liver, lung, peritoneum, pharynx, rectum, and vagina. *Pichia kudriavzevii* also causes fungemia in human, including an outbreak of nosocomial fungemia that occurred in a neonatal intensive care unit. As if that list does not seem bad enough, *Pichia kudriavzevii* also causes skin infections in dog.

Notes: *Pichia burtonii*, which has been isolated from the lungs a a lizard, now is named *Hyphopichia burtonii*. *Pichia fluxuum*, which previously also has been named *Candida vini*, *Debaryomyces fluxorum*, and *Debaryomyces fluxuum*, now is named *Kregervanrija fluxuum*. *Pichia ohmeri*, which causes fungemia in human, has been renamed *Kodamaea ohmeri*.

9.4.172 Piedraia [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Capnodiales; Family: Piedraiaceae; Genus: *Piedraia*. The *Piedraia* have been found in soil. *Piedraia hortae* is found in soil of tropical and subtropical environments and affects human by causing a superficial disease commonly known as black piedra, which presents as nodules on the hair shaft, and most typically it appears on scalp hair. The term superficial means that the infection is restricted to the stratum corneum and it seems to cause no inflammation. Infection by *Piedraia hortae* mainly involves individuals who live in tropical areas, particularly South America, and use oily substances for hair care. Hair infection by *Piedraia hortae* may be done intentionally by humans for cosmetic purpose to darken the hair although *Piedraia hortae* can break the hair shafts. *Piedraia quintanilhae* has been isolated from chimpanzee *Pan troglodytes* in Central Africa, but not much else is know about this fungal species as a pathogen except for it being more common in chimpanzee than in human.

9.4.173 Pithomyces [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Astrosphaeriellaceae; Genus: *Pithomyces*. The *Pithomyces* are saprophytes common to soil and either dead or decaying plant materials including leaves and stems. *Pithomyces chartarum* is a pathogen of bread wheat *Triticum aestivum*, and it is found in pasture grass. Upon ingestion, the spores release sporidesmin into the gastrointestinal tract, with that toxin then causing blockage of the bile ducts and in turn injuring the liver. That sequence of effects results in photosensitivity for

exposed areas of the skin, produces sunlight induced irritation, and efforts by the animals to alleviate discomfort from sensitivity to sunlight results in rubbing that removes skin from facial areas. The resulting disease is termed facial eczema, which reportedly is more common in sheep and deer, with goats being less affected. *Pithomyces chartarum* additionally is infectious for mammals. *Pithomyces chartarum* causes necrotizing sinusitis in horse. In human, *Pithomyces chartarum* causes onychomycosis, skin and sinus infections, plus it has been isolated from the human respiratory tract by bronchoalveolar lavage, and isolated from muscular tissue. *Pithomyces sacchari* has been isolated from human lungs including by bronchoalveolar lavage, from sinuses, from nails, and also from cornea.

Note: *Pithomyces maydicus* now is named *Pseudopithomyces maydicus*.

9.4.174 Pleurophoma [Possibly Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Lentitheciaceae; Genus: *Pleurophoma*. The *Pleurophoma* are plant pathogens. *Pleurophoma* also are dematiaceous and this genus has been identified as causing phaeohyphomycotic cutaneous disease including disfiguring facial mycosis and osteomyelitis in human, although the fungal species was not determined. *Pleurophoma ossicola* has been isolated from bone but no specific factual information was found regarding that isolation.

Note: *Pleurophoma cava*, which causes subcutaneous infection in human and previously was named *Phoma cava*, now is *Neocucurbitaria cava*.

9.4.175 Pleurostoma [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Calosphaeriales; Family: Pleurostomataceae; Genus: *Pleurostoma*. The *Pleurostoma* are plant pathogens and found in rotting wood. *Pleurostoma ochraceum*, which previously was named *Pleurostomophora ochracea*, produces eumycetoma in human. *Pleurostoma repens*, previously named *Phialophora repens*, causes subcutaneous phaeohyphomycosis including nodule formation in human. *Pleurostoma richardsiae*, which used to be named both *Phialophora richardsiae* and *Pleurostomophora richardsiae*, grows on dead wood, occurs in graft unions of grapevine, and also causes trunk disease including decline and dieback symptoms of grapevine. *Pleurostoma richardsiae* is a dematiaceous fungus that causes bursitis and subcutaneous infection with granulomas in human, and is an uncommon cause of ocular infection including endogenous endophthalmitis associated with disseminated infection in human.

9.4.176 Polycytella [Infects: Mammals]

Phylum: Ascomycota; Class: (not assigned); Order: (not assigned); Family: (not assigned); Genus: *Polycytella*. *Polycytella hominis* produces eumycetoma in human. Based upon the nature of the disease which this fungal species produces in human, it logically could be presumed that *Polycytella hominis* is a saprophytic microbe eventually to be found in soil and plant material.

9.4.177 Porostereum [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Polyporales; Family: Phanerochaetaceae; Genus: *Porostereum*. *Porostereum spadiceum* is a crust fungus of rotting wood, it also is endophytic and produces gibberellins (Hamayun et al. 2017). *Porostereum spadiceum* causes bronchopulmonary mycosis in human.

9.4.178 Pseudallescheria [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Microascales; Family: Microascaceae; Genus: *Pseudallescheria*. The *Pseudallescheria* are found in field soil. *Pseudallescheria angusta* causes in human pulmonary mycetomas as a part of fibrocystic sarcoidosis, including hemoptysis (coughing up blood or blood-stained mucus from the larynx, trachea, and lungs).

Notes: *Pseudallescheria apiosperma*, which causes otitis externa in human, now is named *Scedosporium apiospermum*. *Pseudallescheria boydii*, which produces mycetomas and lung disease in human, now is *Scedosporium boydii*.

9.4.179 Pseudochaetosphaeronema [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: (not assigned); Genus: *Pseudochaetosphaeronema*. The *Pseudochaetosphaeronema* are endophytes. *Pseudochaetosphaeronema larense*, which previously was named *Chaetosphaeronema larense*, produces subcutaneous mycetomas in human. *Pseudochaetosphaeronema martinelli* causes subcutaneous mycosis in human.

9.4.180 Pseudogymnoascus [Infects: Mammals]

Phylum: Ascomycota; Class: Leotiomycetes; Order: (not assigned); Family: Pseudeurotiaceae; Genus: *Pseudogymnoascus*. The *Pseudogymnoascus* are found in soil, as well as being associated with covered, rotting wood. *Pseudogymnoascus destructans*, previously named *Geomyces destructans*, has become infamous by invading the skin of bats and causing ulcers in a syndrome termed white-nose disease. *Pseudogymnoascus pannorum*, previously named *Chrysosporium pannorum*, *Geomyces pannorum*, and *Sporotrichum pannorum*, is a soil organism that also has been found in caves and mines. *Pseudogymnoascus pannorum* degrades keratin based substrates, which is a characteristic that likely contributes to its pathogenicity in vertebrates. *Pseudogymnoascus pannorum* causes superficial infections of the skin and nails in numerous mammalian species including camel, cervids, dog, and human.

9.4.181 Pseudopithomyces [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Didymosphaeriaceae; Genus: *Pseudopithomyces*. The *Pseudopithomyces* are pathogens of plants and saprobic on plants. *Pseudopithomyces diversisporus* causes nail infections in human. *Pseudopithomyces maydicus*, previously named *Pithomyces maydicus*, is a plant pathogen and a plant saprobe which causes infections of human toenails and maxillary sinus.

9.4.182 Pseudozyma [Possibly Infects: Amphibians and Mammals]

Phylum: Basidiomycota; Class: Ustilaginomycetes; Order: Ustilaginales; Family: Ustilaginaceae; Genus: *Pseudozyma*. The *Pseudozyma* are found on wilting plant leaves. The genus *Pseudozyma* has been found to cause fungemia in human, but that isolated fungus was not identified at the species level, and well might have been *Pseudozyma aphidis* which taxonomically has been reassigned (see the note below). *Pseudozyma hubeiensis* has been found on plant leaves, and also has been found in the bile of anuran amphibian species *Duttaphrynus melanostictus* and *Fejervarya limnocharis*, but the possibility of a disease association with that presence in amphibian bile remains unclear.

Note: *Pseudozyma aphidis* now is *Moesziomyces aphidis*, it is an epiphyte which has caused fungemia with invasive fungal pneumonia in human.

9.4.183 Purpureocillium [Infects: Fish, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Ophiocordycipitaceae; Genus: *Purpureocillium*. The species *Purpureocillium lilacinum*, previously named *Paecilomyces lilacinus*, is a saprophyte that has been isolated from soil in numerous environmental zones, and it also has been isolated from estuarine sediments, sewage sludge, insects, root nematodes and nematode eggs. Indeed, *Purpureocillium lilacinum* is marketed for the control of plant-parasitic nematodes, because it attacks the eggs of root-knot nematodes *Meloidogyne*. *Purpureocillium lilacinum* causes wasting disease affecting both Mozambique tilapia *Oreochromis mossambicus* and Tilapia aurea *Melanochromis auratus*. *Purpureocillium lilacinum* causes in human: nodular cutaneous granulomas including tattoo-related skin infection, cavitary pulmonary disease, endocarditis, keratitis, and infections that can be mistaken for cellulitis. *Purpureocillium lilacinum* also infects eye, sinus, and lung in cat. *Purpureocillium lilacinum* additionally effects: ulcerative skin lesions, disseminated disease, diskospondylitis, and pneumonia in dog; pneumonia and keratitis in horse. *Purpureocillium lilacinum* has caused pneumonia in: gopher tortoise *Gopherus polyphemus*, yellow footed-tortoise *Chelonoidis denticulatus*, and green tree python *Morelia viridis*. *Purpureocillium lilacinum* additionally has caused: dermatomycosis in mata mata *Chelus fimbriata*; fungal shell erosions in pitted-shelled turtle (also called the Fly River turtle) *Carettochelys insculpta*; and white spot disease in Chinese soft-shelled turtle *Pelodiscus sinensis*.

9.4.184 Pyrenophora [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Pleosporaceae; Genus: *Pyrenophora*. *Pyrenophora biseptata*, previously named *Drechslera biseptata*, is present in soil and as a plant pathogen it causes leaf spots. *Pyrenophora biseptata* infects human, causing brain abcess in addition to colonizing both wounds and sinuses.

9.4.185 Ramichloridium [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Capnodiales; Family: Dissoconiaceae; Genus: *Ramichloridium*. *Ramichloridium schulzeri* seems to be the same fungal species as *Myrmecridium schulzeri* although both names currently are recognized (also see the listing for *Myrmecridium*). *Ramichloridium schulzeri* is a soil saprophyte also isolated from plant detritus, such as forest litter and rotting

wood. *Ramichloridium schulzeri* causes "Golden Tongue" in human, which is a syndrome that can include erosive lesions of the tongue.

Note: *Ramichloridium mackenziei* has been renamed *Rhinocladiella mackenziei*.

9.4.186 Rasamsonia [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Eurotiales; Family: Trichocomaceae; Genus: *Rasamsonia*. The species *Rasamsonia argillacea* is found in soil and air. The previous names of *Rasamsonia argillacea* include *Geosmithia argillacea*, and it often is listed as being an anamorph of *Talaromyces eburneus*. In human, *Rasamsonia argillacea* attacks the skin and can produce a disseminated systemic infection, causes invasive mycosis associated with human chronic granulomatous disease, colonizes the airway in patients with cystic fibrosis, and causes pulmonary as well as aortic graft infections. *Rasamsonia argillacea* also causes disseminated mycosis in dog. *Rasamsonia piperina* causes systemic infection in dog with pulmonary, pleural, splenic, hepatic and lymphoid involvement; in human, *Rasamsonia piperina* has caused a mixed mold chronic granulomatous disease with *Aspergillus nidulans* (previously named *Emericella nidulans*).

9.4.187 Rhinocladiella [Infects: Amphibians, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Chaetothyriales; Family: Herpotrichiellaceae; Genus: *Rhinocladiella*. The *Rhinocladiella* have been isolated from soil, plants, and decaying wood. *Rhinocladiella* has been reported to affect amphibians and reptiles by causing cutaneous and disseminated systemic infections, but without identification at the fungal species level. *Rhinocladiella aquaspersa* causes skin infections and chromomycosis in human. *Rhinocladiella atrovirens* causes mycetomas and infects the central nervous system in human. *Rhinocladiella basitona* causes phaeohyphomycosis in human. *Rhinocladiella mackenziei*, previously named *Ramichloridium mackenziei*, affects humans in ways that include cerebral phaemycotic abcesses, and central nervous system colonization that possibly is secondary to spread of the fungus through blood and lymph tissue. Both *Rhinocladiella phaeophora* and *Rhinocladiella similis* cause chromoblastomycosis in human, also called verrucous dermatitis, which is a long-term fungal infection of the skin and subcutaneous tissue (a chronic subcutaneous mycosis) noted for lesions on the chest and foot.

9.4.188 Rhizomucor [Infects: Birds, Fish, and Mammals]

Phylum: Mucoromycota; Class: Mucoromycetes; Order: Mucorales; Family: Lichtheimiaceae; Genus: *Rhizomucor*. The *Rhizomucor* are saprobes isolated from soil and decaying organic matter including compost, and have been isolated from corn meal. *Rhizomucor miehei* is used commercially to produce a microbial rennet to curdle milk for producing cheese. *Rhizomucor miehei* has caused disseminated infection in a human. The species *Rhizomucor pusillus* is a thermophilic fungus most commonly found in compost piles and also in mushroom compost. *Rhizomucor pusillus* has been isolated from the intestines, lung and liver of an African grey parrot *Psittacus erithacus erithacus* in a combined infection that included chlamydiosis. *Rhizomucor pusillus* causes necrosis of the head, skin and fins of cultured spotted snakehead *Channa punctata*. In mammals, *Rhizomucor pusillus* produces subcutaneous infections, sinus-orbital zygomycosis**,** and by itself also can cause abortion. *Rhizomucor pusillus* additionally has been found to cause abortion as a member of mixed infections that included members of the genus *Aspergillus* (see the listing for *Aspergillus*) affecting cat and cattle, and *Rhizomucor pusillus* has been isolated from cat brain. *Rhizomucor pusillus* also attacks human including causing rhinofacial infections, osteomyelitis, being found in sputum and bronchia of the lung, pleural infection, and fungemia.

Note: *Rhizomucor variabilis*, which is a pathogen of terrestrial slalmander eggs, has been renamed *Mucor irregularis*.

9.4.189 Rhizopus [Infects: Amphibians, Birds, Fish, Mammals, and Reptiles]

Phylum: Mucoromycota; Class: Mucoromycetes; Order: Mucorales; Family: Rhizopodaceae; Genus: *Rhizopus*. The *Rhizopus* have been isolated from soil and typically are saprobic, feeding on a variety of dead organic matter. *Rhizopus* also attack fruit and have been isolated from agricultural products. *Rhizopus* has been mentioned as causing disease in amphibians but without identification of the fungal species. *Rhizopus* species have caused skin erosions and ulcerations in sharp tooth catfish *Clarias gariepinus*, and additionally cause infections in Gibelion catla *Catla catla*, clown knifefish *Chitala chitala*, snakehead murrel *Channa striata*, Jayanti rohu *Labeo rohita*, and great snakehead *Channa marulius*. *Rhizopus* has caused the skin disease zygomycosis in dog, although the fungus was not identified to species level. The species *Rhizopus microsporus* is a plant pathogen known to infect corn, sunflowers *Helianthus*, and rice. *Rhizopus microsporus* interestingly contains as a bacterial endosymbiont *Paraburkholderia rhizoxinica*, which produces the antitumor drug rhizoxin. *Rhizopus microsporus* has caused disseminated infection in common canary *Serinus canaria*. *Rhizopus microsporus variant chinensis* has produced necrotising ventriculitis as a combined infection with *Candida krusei* (now named

Pichia kudriavzevii) in an eclectus parrot *Eclectus roratus*. The *Rhizopus* can cause infection in numerous mammalian species including immunocompromised, malnourished or severely burned people. As opportunistic pathogens of mammals, members of the genus *Rhizopus* cause both cutaneous as well as subcutaneous infections, and they can produce especially severe rhinocerebral infections when there is underlying diabetes. *Rhizopus microsporus* has a gastrointestinal presence and commonly is found in gastric ulcers of pig although a causal relationship with those ulcers remains uncertain. *Rhizopus microsporus* can independently cause abortion in mammals. The following variants of *Rhizopus microsporus* also cause disease in human: *Rhizopus microsporus var. chinensis*; *Rhizopus microsporus var. microsporus* which also has caused ulcers in the rumen and reticulum of cattle; *Rhizopus microsporus var. oligosporus*; and *Rhizopus microsporus var. rhizopodiformis*. *Rhizopus niveus* has been listed as affecting human but specific details about the associated disease were not provided. The species *Rhizopus oryzae* now includes the previously named *Rhizopus arrhizus*, it can degrade both plant and fungal polysaccharides. *Rhizopus oryzae* lives worldwide in dead organic matter plus it infects and causes a soft rot of carrot, pineapple *Ananas comosus* and mango *Mangifera indica*. The commercial uses of *Rhizopus oryzae* include fermentation of rice for saki, and as part of a fungal consortium *Rhizopus oryzae* also can ferment rice straw to produce ethanol. Under the previous name *Rhizopus arrhizus*, this species *Rhizopus oryzae* has caused pyogranulomatous obliterative laryngotracheitis in Atlantic spotted dolphin *Stenella frontalis*. *Rhizopus oryzae* acting alone has caused abortion in mammals, and it additionally has been found to cause abortion with members of the genus *Aspergillus* in mixed fungal infections (see the listing for *Aspergillus*). *Rhizopus oryzae* has caused systemic bovine zygomycosis in cattle, and experimentally *Rhizopus oryzae* has shown the ability to infect rabbit *Oryctolagus cuniculus*. *Rhizopus oryzae* has been isolated from resected bone as well as soft tissue specimens from a human case of sinonasal and palatal mucormycosis, and it also causes ventriculitis in human. *Rhizopus oryzae* causes fatal gastrointestinal infection in human, and also disseminated disease evidenced by soft tissue abcesses in human. *Rhizopus arrhizus variant tonkinensis*, which also is part of the species *Rhizopus oryzae*, causes rhinocerebral infections in humans. *Rhizopus oryzae* additionally causes skin pustules and pneumonic infection of garter snake *Thamnophis sirtalis*. *Rhizopus schipperae* and *Rhizopus stolonifer* have been listed as infecting human but specific details about the associated diseases were not provided.

9.4.190 Rhodotorula [Infects: Birds and Mammals; Also Possibly Infects: Amphibians and Fish]

Phylum: Basidiomycota; Class: Microbotryomycetes; Order: Sporidiobolales; Family: Sporidiobolaceae; Genus: *Rhodotorula*. The *Rhodotorula* are found as saprobes in moist environments, they have been isolated from soil, water, air, milk and fruit

juices. *Rhodotorula* typically produce superficial infections of the skin and cause secondary infections of skin lesions. *Rhodotorula* cause otitis externa, mastitis in mammals including cattle, and pneumonia in sheep. *Rhodotorula* can as well cause epididymitis and produce a septicemia which associatively includes infection of various internal organs among which are the lung and spleen. The *Rhodotorula* very notably cause liver abscesses and endocarditis. The genus *Rhodotorula* has been identified as causing mycosis of the gills and kidney in gilthead seabream *Sparus aurata* but that fungal identification was not species specific. The fact of that fungal identification not having been species specific, combined with the fact that at least one member of this genus has taxonomically been reassigned (see the note below regarding *Rhodotorula minuta*), results in my listing the *Rhodotorula* as possibly infecting fish. *Rhodotorula glutinis* causes dermatits in birds, granulomatous epididymitis in dog, and nail infections in human. *Rhodotorula mucilaginosa* is found naturally in rubber tree *Hevea brasiliensis* latex. *Rhodotorula mucilaginosa* has been found in bile of these anuran amphibians: *Duttaphrynus melanostictus*; *Hoplobatrachus rugulosus*; *Kaloula pulchra*; and *Sylvirana faber*; although the possibility of there having been a related disease association remains unclear. *Rhodotorula mucilaginosa* naturally is part of salmonid intestinal microbiota. *Rhodotorula mucilaginosa* causes dermatitis of birds including chickens and raptors, especially falcons *Falco*, producing lesions that may become hyperkeratotic. *Rhodotorula mucilaginosa* has caused bronchotracheitis in dog, skin infections in South American sea lion *Otaria byronia*, and additionally causes otitis in cattle. *Rhodotorula mucilaginosa* infections of human can result in keratitis, meningitis, peritonitis, onychomycosis, oral ulcers, dermatitis, arotic homograft endocarditis, lymphadenitis, and produce fungemia potentially associated with central venous catheters.

Note: *Rhodotorula minuta*, which affects humans and has been found in the bile of the anuran amphibian *Kaloula pulchra*, now is *Cystobasidium minutum*.

9.4.191 Rigidoporus [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Polyporales; Family: Meripilaceae; Genus: *Rigidoporus*. The *Rigidoporus* are plant pathogens, they invade through roots to cause white rot of roots and tubers, plus some also produce a fruiting body on deciduous trees. The species *Rigidoporus corticola* previously was named *Oxyporus corticola*, and as a plant pathogen it invades through roots, causes white rot, plus affects fruit of the genus *Prunus*. As an opportunistic pathogen of mammals, *Rigidoporus corticola* causes skin infections as well as lymphadenopathy and osteomyelitis in dog.

9.4.192 Rhytidhysteron [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Hysteriales; Family: Hysteriaceae; Genus: *Rhytidhysteron*. The *Rhytidhysteron* are endophytic. *Rhytidhysteron rufulum* is a fungal plant pathogen known to infect *Citrus*. *Rhytidhysteron rufulum* also is dematiaceous, and has been found to cause subcutaneous infections in human which can include the formation of nodules.

9.4.193 Roussoella [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Thyridariaceae; Genus: *Roussoella*. The *Roussoella* are plant pathogens. *Roussoella solani* causes keratomycosis (corneal infection) in human.

Note: *Roussoella percutanea* has been renamed *Parathyridaria percutanea*.

9.4.194 Saccharomyces [Infects: Mammals]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Saccharomycetaceae; Genus: *Saccharomyces*. The natural ecology of *Saccharomyces* may be an association with the bark and exudates of oak trees *Quercus*. *Saccharomyces bayanus*, *Saccharomyces cerevisiae* (baker's yeast), and *Saccharomyces pastorianus* affect human by causing invasive infections, notably fungemia.

9.4.195 Saksenaea [Infects: Mammals]

Phylum: Mucoromycota; Class: Mucoromycetes; Order: Mucorales; Family: Saksenaeaceae; Genus: *Saksenaea*. The *Saksenaea* are soil microorganisms and they are noted for producing severe necrotizing skin and soft tissue infections (necrotizing fasciitis) of human. *Saksenaea erythrospora* causes cutaneous infections following deep skin and soft tissue contamination with soil following either traumatic injury or surgery. *Saksenaea erythrospora* also has caused in utero infection of cattle resulting in fatal neonatal abomasitis and ulcerative dermatitis in a prematurely delivered calf. *Saksenaea loutrophoriformis* has been isolated from human eye. Both *Saksenaea oblongispora* and *Saksenaea trapezispora* cause cutaneous infections following trauma. The species *Saksenaea vasiformis* is found in tropical and subtropical soils, it causes subcutaneous infections that present as necrotizing fasciitis which can become severely invasive and disseminate to include yellow necrosis of skin, muscle, tendon, and fascia in human. *Saksenaea vasiformis*

also is known to invade burn wounds in human, and affects cattle including production of bovine cranial infections.

9.4.196 Saprochaete [Infects: Mammals]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Dipodascaceae; Genus: *Saprochaete*. *Saprochaete clavata*, which previously was named *Geotrichum clavatum*, naturally is found in soil, water and associated with plants. *Saprochaete clavata* is a commensal that has been isolated from the respiratory and gastrointestinal tracts of perhaps 30 percent of healthy humans. *Saprochaete clavata* also causes fungemia in human, and has caused disseminated invasive nosocomial infection in a hematological ward, although that infection was not necessarily associated with the hematological and oncological disorders. *Saprochaete clavata* additionally has a causal role in bovine mastitis and can be found in dairy products.

Note: *Saprochaete capitata*, which previously also has been named *Blastoschizomyces capitatus*, *Dipodascus capitatus*, *Geotrichum capitatum*, and *Trichosporon capitatum*, has been renamed as *Magnusiomyces capitatus*.

9.4.197 Sarcinomyces [Infects: Fish]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Chaetothyriales; Family: (not assigned); Genus: *Sarcinomyces*. The *Sarcinomyces* are considered to be epiphytes. The only pathogen of vertebrates currently in this genus is *Sarcinomyces crustaceus*, which has been noted as causing gasophthalmus in black seabream *Spondyliosoma cantharus*. This fungal species seems not to have been reported as causing illness in other vertebrates.

9.4.198 Sarocladium [Infects: Fish and Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: (not assigned); Genus: *Sarocladium*. The *Sarocladium* are found in soil. The species *Sarocladium kiliense*, previously named *Acremonium kiliense*, is a ubiquitous cellulolytic soil saprophyte additionally found in plant debris, and it also is found in the gut of Saintonge termite *Reticulitermes santonensis*. The species *Sarocladium kiliense* produces keratitis and keratoconjunctivitis in dog, and in cattle this fungal species causes abortion. *Sarocladium kiliense* affects human by causing mycetoma and also produces fungemia that can include the lungs. *Sarocladium strictum* previously was named *Acremonium strictum*, it causes skin erosions and ulcerations

in sharp tooth catfish *Clarias gariepinus*. *Sarocladium strictum* affects human by causing localized, invasive and disseminated infections which can include cutaneous disease and peritonitis.

9.4.199 Scedosporium [Infects: Birds and Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Microascales; Family: Microascaceae; Genus: *Scedosporium*. The *Scedosporium* are soil fungi. The most common sites of *Scedosporium* infection are the lungs, sinuses, bones, joints, eyes, and brain. The illnesses associated with members of this genus also include infections of the skin and mucous membranes, nasal granuloma, osteomyelitis, discospondylitis (infection of the vertebral disks), and abortion. The range of outcomes from *Scedosporium* pulmonary invasion include sinopulmonary infections, extrapulmonary localized infections, and disseminated infections. Invasive *Scedosporium* infections following near-drowning accidents can include the central nervous system. *Scedosporium apiospermum*, previously named *Pseudallescheria apiosperma*, causes mycotic keratitis in chicken as noted in layer pullets. *Scedosporium apiospermum* causes in human: otitis externa and produces mycetomas, sinusitis, diabetic foot ulcers, mycotic keratitis and brain abcess. *Scedosporium apiospermum* causes in dog: rhinitis, nasal granuloma, and keratomycosis. *Scedosporium apiospermum* has caused systemic mycosis in a stranded northern elephant seal *Mirounga angustirostris*. *Scedosporium boydii*, previously named *Pseudallescheria boydii*, degrades hydrocarbons in crude oil–soaked soil. *Scedosporium boydii* has been found in the abdominal cavity of a dog, causes mastitis in goat and cattle, plus it produces granulomatous and eosinophilic rhinitis in cattle. In human, *Scedosporium boydii* produces mycetomas, brain abcess, and lung disease.

Note: *Scedosporium prolificans*, which also has been named Scedosporium inflatum, now is *Lomentospora prolificans*.

9.4.200 Schizangiella [Infects: Reptiles]

Phylum: Zoopagomycota; Class: Basidiobolomycetes; Order: Basidiobolales; Family: Basidiobolaceae; Genus: *Schizangiella*. The ecology of *Schizangiella* remains undetermined. However, I will presume that the genus *Schizangiella* may have some natural presence in soil because the Basidiobolaceae are mostly either arthropod pathogens or saprobes found in soil and litter. *Schizangiella serpentis* causes granulomas in snakes including Western rat snake *Pantherophis obsoletus*, previously named *Elaphe obsoleta*.

9.4.201 Schizophyllum [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Agaricales; Family: Schizophyllaceae; Genus: *Schizophyllum*. The species *Schizophyllum commune* decays wood, it is found growing in and on rotting wood and is a mushroom eaten by human populations. *Schizophyllum commune* causes human disease by infecting the skin and also producing nail infections. *Schizophyllum commune* additionally affects human by causing sinus infections (sinusitis), ulceration of the hard palate, osteomyelitis, and bronchopulmonary mycosis (bronchopneumonia, fungus ball in the lung). Pulmonary infections of human caused by *Schizophyllum commune* can disseminate to the brain resulting in cerebral abscess. *Schizophyllum commune* also has been isolated from cerebrospinal fluid of a human patient manifesting signs of atypical meningitis. There are repeated mentions of a case in which *Schizophyllum commune* had affected human by growing through the soft palate of a child's mouth and forming fruiting bodies in her sinuses, but that may be only anecdotal as I could not find actual publication of such an occurrence. *Schizophyllum commune* has been reported to cause osteomyelitis in dog. *Schizophyllum commune* also has infected a harbor seal *Phoca vitulina* to the extent that fungal hyphae were found in granulomatous lesions of its eyes, lung, heart, and lymph nodules. *Schizophyllum radiatum* naturally also rots wood, and it has been isolated from human respiratory tract clinical samples.

9.4.202 Scopulariopsis [Infects: Mammals and Reptiles]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Microascales; Family: Microascaceae; Genus: *Scopulariopsis*. The *Scopulariopsis* occur in soil and decaying organic matter. *Scopulariopsis asperula* causes onychymycosis in human. The species *Scopulariopsis brevicaulis* previously was named *Microascus brevicaulis*, it occurs in soil and decaying organic matter, and *Scopulariopsis brevicaulis* produces trimethylarsine if growing in the presence of inorganic arsenic. In mammals, *Scopulariopsis brevicaulis* causes keratitis, subcutaneous skin lesions which can include nodule formation, invasive sinusitis, endophthalmitis, and plumonary infections. These infections of mammals can disseminate to produce systemic disease including endocarditis and brain abscess. Among the mammalian species which *Scopulariopsis brevicaulis* infects are human, cat, cattle, goat, and horse. *Scopulariopsis brevicaulis* notably is an uncommon cause of ringworm in cattle. *Scopulariopsis brevicaulis* also infects the European bison *Bison bonasus*, and so presumably it additionally would infect the American bison *Bison bison*. *Scopulariopsis brevicaulis* has caused dermatitis in Common green iguana *Iguana iguana*. *Scopulariopsis brumptii* attacks human, including infection of the pleural space acquired from transplanted lungs, following the lung donor presumably having acquired that infection during prehospital placement of thoracostomy

tubes. *Scopulariopsis brumptii* also has produced in human a disseminated fungal infection involving the lungs, skin, pleural surface, heart, liver, kidneys, spleen, both sides of the diaphragm, colon, thymus and brain. *Scopulariopsis candida*, previously named *Microascus manginii*, has an ecology which includes recorded isolations from chicken litter, sand, soil, soybeans, sunflower seeds, and waste compost. *Scopulariopsis candida* has been isolated from superficial tissue of human, caused invasive sinonasal infection in human, and also was reported to be the cause of a disseminated infection in a leukemic human. *Scopulariopsis fusca* has been reported to infect human and it has been isolated from the inner tissue of the brown alga *Padina pavonica*. *Scopulariopsis koningii* also has been reported as infecting humans.

Notes: *Scopulariopsis acremonium* which causes sinusitis in human now is *Acaulium acremonium*. *Scopulariopsis gracilis* now is *Microascus gracilis*.

9.4.203 Scytalidium [Infects: Fish and Mammals]

Phylum: Ascomycota; Class: Leotiomycetes; Order: (not assigned); Family: (not assigned); Genus: *Scytalidium*. The *Scytalidium* have been isolated from soil and wood, and some are known plant pathogens. *Scytalidium cuboideum* previously has been named *Arthrographis cuboidea*, *Geotrichum microsporum*, and *Oospora cuboidea*, it has been isolated from human bronchial wash specimens, however the infectivity of *Scytalidium cuboideum* for human remains uncertain. *Scytalidium infestans* infects red seabream *Pagrus major* by producing lesions of the dermal and muscular layers with apparent granulomatous inflammation, and with the fungal hyphae possibly not penetrative into the internal organs. *Scytalidium lignicola* is a plant pathogen and it also may be antagonistic against some wood decay fungi. *Scytalidium lignicola* infects human by causing cutaneous and subcutaneous phaeohyphomycoses.

Note: *Scytalidium dimidiatum* now is *Neoscytalidium dimidiatum*.

9.4.204 Sepedonium [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Hypocreaceae; Genus: *Sepedonium*. The *Sepedonium* are saprophytic, naturally inhabit soil and plant material, and can parasitize the fruiting bodies of other fungi. *Sepedonium* have been identified as the fungal pathogen in several case studies of human pneumonia and intra-abdominal infection, but the causative *Sepedonium* fungi were not specified to species level.

9.4.205 Serpula [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Boletales; Family: Serpulaceae; Genus: *Serpula*. *Serpula lacrymans* causes brown rot and dry rot damage to timber. *Serpula lacrymans* naturally and typically is found in spruce and other conifers. Harvesting of those infected trees for constructing buildings transports the fungus across borders and brings the fungus indoors. *Serpula lacrymans* affects humans by causing hypersensitivity pneumonitis (which is not infectious), and infections which result in pulmonary fibrosis, sinusitis, bronchitis, and pneumonia.

Note: The name *Serpula* also refers to a genus of calcareous tube worms of the Class Polychaete.

9.4.206 Sporidiobolus [Infects: Fish and Mammals]

Phylum: Basidiomycota; Class: Microbotryomycetes; Order: Sporidiobolales; Family: Sporidiobolaceae; Genus: *Sporidiobolus*. The *Sporidiobolus* have been isolated from soil, plant material, and water. *Sporidiobolus salmonicolor* previously was named *Sporobolomyces salmonicolor*. *Sporidiobolus salmonicolor* has caused cutaneous discoloration and ascites in Chinook salmon *Oncorhynchus tshawytscha* fry and histologically been observed in lesions that included: aerocystitis (inflammation of the swim bladder), myositis, peritonitis, and dermatitis. In human, *Sporidiobolus salmonicolor* has caused skin infections, nasal polyp, endophthalmitis, lymphadenitis, pseudomeningitis, and been recovered from cerebrospinal fluid.

9.4.207 Sporobolomyces [Infects: Mammals]

The *Sporobolomyces* have been found in soil, associated with tree leaves, on rotting fruit and other plant materials. They also are associated with plant lesions caused by other plant parasites. *Sporobolomyces* produce a type of wet spore termed ballistospores that are forcibly discharged into the air during high humidity. The name *Sporobolomyces* now represents two genera. I have numbered these two genera by the order that they are listed in the National Center for Biotechnology Information database.

Genus 1 [Infects: Mammals]
Phylum: Basidiomycota; Class: Microbotryomycetes; Order: Sporidiobolales; Family: Sporidiobolaceae; Genus: *Sporobolomyces*. The species *Sporobolomyces roseus* frequently is associated with plants, it is a plant pathogen, and it also has invertebrate animal hosts including scale insects such as *Saissetia oleae*. *Sporobolomyces roseus* has been suggested as a biocontrol agent. In mammals, *Sporobolomyces roseus*

causes meningoencephalitis in dog, and in human *Sporobolomyces roseus* has caused meningitis and been found in the cerebrospinal fluid of an immunocompetent individual. *Sporobolomyces holsaticus*, now renamed *Sporobolomyces johnsonii*, causes infectious dermatitis in human.

Genus 2 [not shown to infect vertebrates]
Phylum: Basidiomycota; Class: Agaricostilbomycetes; Order: (not assigned); Family:(not assigned); Genus: *Sporobolomyces*.

Note: *Sporobolomyces* salmonicolor, which causes disease in fish, has been renamed *Sporidiobolus salmonicolor*.

9.4.208 Sporothrix [Infects: Birds, Mammals and Reptiles]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Ophiostomatales; Family: Ophiostomataceae; Genus: *Sporothrix*. The *Sporothrix* are isolated from live as well as dead plants, the surrounding soil, and from peat moss *Sphagnum*. *Sporothrix* also infect invertebrate hosts. *Sporothrix brasiliensis* infects human, causing cutaneous disease that can disseminate, chronic meningitis and hydrocephalus. *Sporothrix globosa* causes in human skin lesions which can include localized lymphocutaneous complication. *Sporothrix luriei* causes pulmonary infection in human, and has produced necrohemorrhagic granulomatous lymphadenitis in a Pacific white-sided dolphin *Lagenorhynchus obliquidens*. *Sporothrix mexicana* causes subcutaneous nodules and ulcerations in human. The species *Sporothrix schenckii*, previously named *Sporotrichum schenckii*, is present in soil, it notably can be found on rose thorns, plus it also exists living upon as well as decomposing such plant materials as hay, cornstalks, sphagnum moss, and twigs. *Sporothrix schenckii* is associated with invertebrate animal hosts. *Sporothrix schenckii* also is broadly infective of birds and mammals including cat, dog, horse, cattle, camel, goat, sheep, pig, rodents, and human. The disease which *Sporothrix schenckii* causes in mammals can be minor in nature but progress dramatically, particularly in immunosupressed individuals, and is called sporotrichosis. In time, it may be determined that more than a single related species can cause the disease sporotrichosis, with at least *Sporothrix brasiliensis*, *Sporothrix globosa*, and *Sporothrix mexicana* being placed onto that list. Sporotrichosis results from implantation of fungal spores, typically is a cutaneous infection although it often may occur as an ocular mycosis, and those infections can be either subacute or chronic. Sporotrichosis has the older name Rose Handlers Disease due to its acquisition from thorn pricks and cuts on the skin. *Sporothrix schenckii* infections similarly have been acquired by tree nursery workers and also reforestation workers as a work-related disease. Infections of humans caused by *Sporothrix schenckii* additionally can be acquired from: close exposure to cats such as sleeping with cats; bites and scratches from other vertebrate animals including parrots, rats *Rattus*, snakes, squirrel (Family Sciuridae), and fish; scratches from the dorsal fin spines of fish *Tilapia*, and injury

from fire ant *Solenopsis* stings (please be aware that the name *Solenopsis* also applies to a genus of angiosperm belonging to the Family Campanulaceae). *Sporothrix schenckii* infections of humans also have been acquired as a contamination from intramuscular injections. *Sporothrix schenckii* infection classically begins as primary lesions at the site of cutaneous inoculation, the infection then can become subcutaneous, including the formation of fungal nodules and ulcerations. Typically, in human, those infections are noticed on the hands and arms, but they can arise on the stomach from scratches that occur when sleeping with cats, and on the lips from kissing infected cats. Humans can acquire the infection from cats without any evidence of trauma. *Sporothrix schenckii* similarly can infect the genital area including penis and testis. *Sporothrix schenckii* nodules can appear in the oral cavity, upper and lower respiratory tracts including the lungs, eyes and conjunctiva. Immunosuppression can lead to *Sporothrix schenckii* infections becoming multifocal and disseminating by becoming lymphocutaneous, resulting in systemic disease that includes meningitis. *Sporothrix schenckii* causes dermal mycosis with possible pustules in snake species including the dusky pigmy rattlesnake *Sistrurus miliarius barbouri*.

9.4.209 Stachybotrys [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Stachybotryaceae; Genus: *Stachybotrys*. The *Stachybotrys* are found in soil and cellulose-containing materials such as rotting grain, leaf debris, paper and wood products. *Stachybotrys* grows during the times when those substrate materials are soaking wet. *Stachybotrys chartarum* is the most notable species in this fungal genus. *Stachybotrys chartarum* can grow in the lungs as a infection of human, although the infectivity caused by *Stachybotrys chartarum* is overshadowed by its associated toxicosis. Growth of *Stachybotrys chartarum* requires a high moisture content and one of its more common habitats is cellulose-rich water damaged construction materials, with its original isolation having been from wallpaper collected at a home in Prague. *Stachybotrys chartarum* often is not harmful until it dries and the spores have gone airborne. Toxic effects associated with *Stachybotrys chartarum* result from the atranone and trichothecene compounds which it produces. The ensuing toxicoses include those from ingestion, inhalation, and dermal contact, which may be associated with moldy feed and moldy grain. In humans, horses and other animals the disease symptoms associated with *Stachybotrys chartarum* include irritation of the mouth, throat, and nose; shock; dermal necrosis; a decrease in leukocytes; internal hemorrhage; and nervous disorders including tremors. The effect of *Stachybotrys chartarum* can be fatal, including in large mammals such as horses. In cattle, *Stachybotrys chartarum* produces bovine hyperkeratosis and necrotic dermatitis, and also has been indicated to have association with mastitis and diarrhea which might be either infectious or allergic in nature. In chickens,

Stachybotrys chartarum can cause a moldy feed toxicosis termed "poultry hemorrhagic syndrome".

Note: *Stachybotrys echinata* presently is named *Memnoniella echinata*.

9.4.210 Stagonosporopsis [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Didymellaceae; Genus: *Stagonosporopsis*. *Stagonosporopsis oculi-hominis* is an endophytic pathogen of *Dendrobium huoshanense*, this fungal species previously was named *Phoma oculi-hominis* and causes corneal ulcer in human. *Stagonosporopsis oculi-hominis* was once considered a variant of *Stagonosporopsis dennisii* (prior name *Phoma dennisii*).

9.4.211 Staphylotrichum [Infects: Mammals]

Phylum: Ascomycota; Class: (not assigned); Order: (not assigned); Family: (not assigned); Genus: *Staphylotrichum*. The species *Staphylotrichum coccosporum* is a soil organism which causes subcutaneous infections in cat.

9.4.212 Stemphylium [Infects: Birds and Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Pleosporaceae; Genus: *Stemphylium*. The *Stemphylium* are plant pathogens which cause leaf blight and ray blight disease of the Family Asteraceae. *Stemphylium* also are found living saprophytically on decaying vegetation and in soil. *Stemphylium* has been isolated from the internal organs of poultry and from dead-in-shell embryos of poultry, but those fungal identifications were not species specific. Keratitis in human cornea has been caused by a combined infection of *Stemphylium, Acremonium*, and alpha-*Streptococcus*, but those causative microbes were not identified at the level of species. *Stemphylium botryosum* acting alone can cause keratitis in human.

9.4.213 Stenella [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Capnodiales; Family: Mycosphaerellaceae; Genus: *Stenella*. *Stenella araguata* is a common plant pathogen in some regions of South America, it is a dematiaceous fungus described as causing infection similar in appearance to tinea nigra. Tinea nigra is an uncommon

superficial dermatomycosis which produces patchy dark brown to black discoloration on the soles of the feet and the palms of the hand, usually caused by *Hortaea werneckii* (see the listing for *Hortaea*).

Note: Be aware when researching this fungus genus that *Stenella* also is the name for a genus of aquatic mammals in the Family Delphinidae.

9.4.214 Subramaniula [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: Chaetomiaceae; Genus: *Subramaniula*. The *Subramaniula* are found on plants. *Subramaniula asteroides* produces human eye and skin infections. *Subramaniula obscura* produces human toe infection.

9.4.215 Sydowia [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Dothideales; Family: Dothioraceae; Genus: *Sydowia*. The species *Sydowia polyspora* is an opportunistic endophytic pathogen on conifers and frequently is isolated from shrub trees. *Sydowia polyspora* infects human as evidenced by it having caused cutaneous phaeohyphomycosis, fatal disseminated infections including a case of fungal peritonitis in a patient who was undergoing continuous ambulatory peritoneal dialysis, and fatal pneumonia combined with fungemia following intense avian exposure in a human who had lived within an apartment accompanied by 130 uncaged birds. In order to learn more about *Sydowia polyspora*, I would suggest that you research under its previous names *Hormonema dematioides* and *Sclerophoma pityophila*.

9.4.216 Syncephalastrum [Infects: Mammals]

Phylum: Mucoromycota; Class: Mucoromycetes; Order: Mucorales; Family: Syncephalastraceae; Genus: *Syncephalastrum*. *Syncephalastrum racemosum* has been associated with degradation of rubberwood *Hevea brasiliensis* logs and leaf litter. *Syncephalastrum racemosum* is found on the skin of reptiles and has been isolated from Pacific white shrimp *Penaeus vannamei* although without a clear causal relationship for disease in either reptiles or shrimp. *Syncephalastrum racemosum* does affect human by causing nail infections (onychomycosis) and also by having caused invasive infection of the anterior abdominal wall and omentum following a penetrating adominal wound.

9.4.217 *Taeniolella* [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Kirschsteiniotheliales; Family: Kirschsteiniotheliaceae; Genus: *Taeniolella*. The *Taeniolella* are plant pathogens found on leaves and bark. *Taeniolella stilbospora* is non-lichenized and naturally lives either parasitically or saprophytically on wood and bark. *Taeniolella stilbospora* infects human, causing dark pigmented cutaneous lesions that can appear on the face.

Note: *Taeniolella boppi* has been renamed as *Cladophialophora boppii*.

9.4.218 *Tausonia* [Infects: Birds]

Phylum: Basidiomycota; Class: Tremellomycetes; Order: Cystofilobasidiales; Family: Mrakiaceae; Genus: *Tausonia*. *Tausonia pullulans* previously was named *Oidium pullulans*, and it is found in soil. *Tausonia pullulans* also affects plants by colonizing tree exudates including slime flux, and experimental mixed inoculation of *Populus nigra* clones showed that *Tausonia pullulans* reduced the plant root/shoot ratio (Mestre et al. 2017). *Tausonia pullulans* has been suspected of causing beak overgrowth in birds, specifically several blue tit *Cyanistes caeruleus*, of which the maxillary and mandibular beaks were severely overgrown and in occlusion. *Tausonia pullulans* also causes crop mycosis in poultry.

9.4.219 *Talaromyces* [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Eurotiales; Family: Trichocomaceae; Genus: *Talaromyces*. The *Talaromyces* typically are found in soil including a presence in rodent burrows. *Talaromyces* cause disseminated infections that can affect the lung and also cause abortion. *Talaromyces flavus* infects cattle. *Talaromyces helicus* infects dog. *Talaromyces marneffei*, previously named *Penicillium marneffei*, causes pneumonia in dog, and in human this fungal species causes systemic mycosis including the lung as especially noted in Southeast Asia. *Talaromyces purpureogenus* infects dog. *Talaromyces piceae*, which previously was named *Penicillium piceum*, causes pulmonary nodule and adjacent rib osteomyelitis in human. *Talaromyces radicus*, which causes disseminated infection in dog, previously was named *Penicillium radicum*.

9.4.220 Tetraploa [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Tetraplosphaeriaceae; Genus: *Tetraploa*. The *Tetraploa* are pathogenic for many kinds of plants and trees, with the *Tetraploa* being found just above the soil and also at the bases of leaves and stems. *Tetraploa* aristata infects human, causing keratitis and also subcutaneous cysts.

9.4.221 Thelebolus [Infects: Birds]

Phylum: Ascomycota; Class: Leotiomycetes; Order: Thelebolales; Family: Thelebolaceae; Genus: *Thelebolus*. The species *Thelebolus microsporus* is isolated from soil and dung in cold climates with an indication that 5 C is optimal for its growth and fruiting. *Thelebolus microsporus* causes fatal tracheal infections in skuas, it is known to infect both brown skua *Stercorarius lonnbergi* and South polar skua *Stercorarius maccormicki*.

9.4.222 Thermoascus [Infects: Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Eurotiales; Family: Thermoascaceae; Genus: *Thermoascus*. The *Thermoascus* are soil microbes. *Thermoascus aurantiacus* is a lignocellulolytic thermophilic fungus that has been examined extensively for its ability to secrete large amounts of thermostable enzymes usable in the depolymerization of cellulose and hemicellulose from plant biomass. *Thermoascus aurantiacus* is considered zoopathogenic but specific information about its pathogenic association was not freely available, viewing the article would have cost USD 24.00 per page and I considered viewing the article not to be worth that expense. *Thermoascus crustaceus*, previously named *Dactylomyces crustaceus* and also *Paecilomyces crustaceus*, has been found to infect human by causing peritonitis, the fungus had colonizing a peritoneal catheter used in peritoneal dialysis. I have seen references for *Thermoascus taitungiacus* producing peritonitis in humans, but I could not trace that fungal species name. *Thermoascus thermophilus* is a thermophilic soil fungus that also has been found in mushroom compost, and *Thermoascus thermophilus* causes abortion in cattle.

9.4.223 Thermomyces [Infects: Birds and Mammals]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Eurotiales; Family: Trichocomaceae; Genus: *Thermomyces*. The *Thermomyces* are noted for producing xylanase and lipase, they grow pathogenically on plant material and are found both in garden compost as well as sawdust. *Thermomyces* have been suggested for use in biomass deconstruction. Inclusion of this genus is done with specific reference to the species *Thermomyces lanuginosus*, which has been isolated from stacks of oil palm kernels. *Thermomyces lanuginosus* causes granulomatous pneumonia in White Stork chicks *Ciconia ciconia*. *Thermomyces lanuginosus* also infects human by causing ringworm (tinea corporis), endocarditis including that of prosthetic porcine aortic valves, and has caused arterial mycotic aneurysm.

9.4.224 Thermothelomyces [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: Chaetomiaceae; Genus: *Thermothelomyces*. *Thermothelomyces thermophila*, previously named *Myceliophthora thermophila*, is a soil microorganism that affects humans by causing both pulmonary and disseminated infections including osteomyelitis. These infections often follow trauma to bones and cartilage resulting from farm injuries.

9.4.225 Thielavia [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Sordariales; Family: Chaetomiaceae; Genus: *Thielavia*. The *Thielavia* are found in soil plus they cause rot of stolons and roots. *Thielavia subthermophila* exists in desert soil and also is an endophyte associated with desert plants, in humans this fungal species causes ocular and brain infections. *Thielavia terrestris* previously was named *Acremonium alabamense*, it is a soil microbe that has produced cerebral infection in human.

9.4.226 Tranzscheliella [Infects: Mammals]

Phylum: Basidiomycota; Class: Ustilaginomycetes; Order: Ustilaginales; Family: Ustilaginaceae; Genus: *Tranzscheliella*. The *Tranzscheliella* cause stem smut in plants and typically are found as pathogens of grasses (Family Poaceae). *Tranzscheliella hypodytes*, previously named *Ustilago hypodites*, causes cutaneous infections of human.

9.4.227 *Trematosphaeria* [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Trematosphaeriaceae; Genus: *Trematosphaeria*. The *Trematosphaeria* cause disease of leaves, including attacking members of the genera *Eucalyptus* and *Corymbia* (please be aware that *Corymbia* also is the name for a genus of arthropods belonging to the Order Coleoptera, Family Cerambycidae). *Trematosphaeria grisea*, previously named *Madurella grisea*, is a soil fungus which also causes leaf blotch disease of plants including members of the genus *Eucalyptus*, and it seems geographically limited to tropical and subtropical regions. *Trematosphaeria grisea* produces mycetomas in cat, and typically subcutaneous mycetomas in human. In addition to being infectious, *Trematosphaeria grisea* produces cytotoxic naphthoquinones and naphthalenones.

9.4.228 *Trichoderma* [Infects: Amphibians, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Hypocreaceae; Genus: *Trichoderma*. The *Trichoderma* are a ubiquitous genus of filamentous fungi widely found in soil. *Trichoderma* cause rot of: grains including corn; *Citrus* fruit; sweet potatoes; tomatoes; paper; textiles; and damp wood. *Trichoderma* also grow on other fungi. In addition to being infectious, the *Trichoderma* produce trichothecene mycotoxins. *Trichoderma* species infect amphibians (notably toads), including causing dermatitis in Puerto Rican crested toad *Peltophryne lemur* but that causative fungus was not identified to the species level. *Trichoderma* also infect reptiles, and have been isolated from both Johnstone river crocodile *Crocodylus johnsoni*, and American alligator *Alligator mississippiensis*, although similarly with the fungal identifications not done to the level of species. *Trichoderma harzianum* is found in soil, it also is used as a fungicide applied by means of foliar application, used as a seed treatment, and similarly as a soil treatment for suppression of various other fungal pathogens affecting plants. *Trichoderma harzianum* infects human including the production of peritonitis, disseminated infections that can include brain abcess and mycotic aneurysm in the brain, and also has been found in the lung of human. *Trichoderma koningii* has been isolated from peritoneal fluid of human. *Trichoderma longibrachiatum* causes skin infections in human, and additionally in human it has caused infections of the brain, heart, lung, and stomach. Infections of human by *Trichoderma longibrachiatum* have included skin and also disseminated invasion in transplant recipients, peritonitis in patients with chronic renal failure undergoing continuous ambulatory peritoneal dialysis, lung infection in patients with chronic lung disease, chronic sinus infections, and it should be noticed that this fungal species can cause perirectal ulcer. *Trichoderma pseudokoningii* has been isolated

from peritoneal fluid in human. Interestingly, *Trichoderma viride*, which has caused human infections including keratitis, has been isolated from peritoneal fluid in human, and produced infection of a parahepatic hematoma in a human liver transplant recipient, has been used to enhance body weight and reduce mortality of Nile tilapia *Oreochromis niloticus*.

9.4.229 Trichophyton [Infects: Birds, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Arthrodermataceae; Genus: *Trichophyton*. The *Trichophyton* are soil organisms but many of them more naturally seem associated with the skin and keratin structures produced by animals. Members of the genus *Trichophyton* have wide host ranges, typically causing as infections dermatitis and rhinitis, and I am mentioning only some representative hosts for each of the *Trichophyton* species. *Trichophyton benhamiae*, previously named *Arthroderma benhamiae*, infects both dog and human, and interestingly the reservoir for *Trichophyton benhamiae* is the European hedgehog *Erinaceus europaeus*. *Trichophyton concentricum* infects human. *Trichophyton equinum* infects horse and human. *Trichophyton gourvilii* infects human. *Trichophyton mentagrophytes*, which previously was named *Arthroderma vanbreuseghemii*, infects rabbit, kangaroo, cat, cattle, guinea pig, coypu, horse, dog, sheep, goat, and in human it causes the skin infections of the body called tinea corporis, skin infections of the scalp called tinea capitis, and kerion which is an abcess most typically of the scalp. *Trichophyton rubrum* infects dog, horse, cattle, and human. *Trichophyton schoenleinii* causes tinea capitis which is a chronic inflammatory dermatophytic infection of human and *Trichophyton schoenleinii* is the usual cause of the same infection, also known as either favus or tinea favosa, in birds. *Trichophyton simii*, previously named *Arthroderma simii*, infects poultry, cattle, and human. *Trichophyton soudanense* and *Trichophyton tonsurans* infect human. *Trichophyton verrucosum* infects cattle, sheep, goat, kangaroo, horse, and human. *Trichophyton terrestre* has been associated with progressive digital necrosis of skink *Tiliqua scincoides*. *Trichophyton violaceum* infects human. *Trichophyton yaoundei* infects human.

Notes: *Trichophyton gallinae*, which infects poultry and a variety of mammals including human, previously was named *Microsporum gallinae* and now has been renamed *Lophophyton gallinae*. *Trichophyton redellii*, which infects bats, has been renamed *Arthroderma redellii*.

9.4.230 Trichosporon [Infects: Birds and Mammals]

Phylum: Basidiomycota; Class: Tremellomycetes; Order: Trichosporonales; Family: Trichosporonaceae; Genus: *Trichosporon*. The *Trichosporon* typically are isolated from soil but many also occur as natural constituents of mammalian skin and respiratory tract microbiota, they also affect invertebrate animal hosts. Members of the genus *Trichosporon* typically cause cutaneous infections and mastitis in mammals. They also can produce disseminated infections resulting in meningoencephalitis, bloodstream infections, pulmonary infections, and soft tissue infections. *Trichosporon asahii* causes dermatitis in birds and also infects both cattle as well as human. *Trichosporon asteroides* causes disseminated infections in human. *Trichosporon beigelii* infects cat and horse, plus in human this fungal species produces a range of disease that includes persistent disseminated infections, peritoneal shunt infection and peritonitis, chronic meningitis, stenosing esophagitis, endophthalmitis, arthritis, cholangitis and hepatitis. *Trichosporon faecale* affects human including skin infections that can become invasive and result in fungemia. *Trichosporon inkin* causes peritonitis and lung infections in human. *Trichosporon ovoides* causes nodules on human hair.

Notes: *Trichosporon cutaneum* which is notable for infecting amphibians, mammals and reptiles, now is *Cutaneotrichosporon cutaneum*. *Trichosporon montevideense* now is *Apiotrichum montevideense*. *Trichosporon capitatum*, which previously also has been named *Blastoschizomyces capitatus*, *Dipodascus capitatus*, *Geotrichum capitatum*, and *Saprochaete capitata*, now is named *Magnusiomyces capitatus*. *Trichosporon jirovecii* has been renamed *Cutaneotrichosporon jirovecii*. *Trichosporon mucoides* has been renamed *Cutaneotrichosporon mucoides*.

9.4.231 Tritirachium [Infects: Mammals]

Phylum: Basidiomycota; Class: Tritirachiomycetes; Order: Tritirachiales; Family: Tritirachiaceae; Genus: *Tritirachium*. The *Tritirachium* are widespread in decaying vegetation and in the soil, they are pathogens of invertebrates, they also are keratinophilic and that trait often is associated with their pathogenicity in mammals. *Tritirachium oryzae* causes scalp infections and onychomycosis in human. *Tritirachium roseum* causes corneal ulcers in human.

9.4.232 Tropicoporus [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Hymenochaetales; Family: Hymenochaetaceae; Genus: *Tropicoporus*. *Tropicoporus tropicalis* previously has been named *Inonotus tropicalis* and *Phellinus tropicalis*, it causes white rot of wood,

and this fungal species typically is found in warm humid regions. *Tropicoporus tropicalis* infects dog by causing pericardial effusion, myocarditis, and granulomatous mediastinal mass. In human, *Tropicoporus tropicalis* has caused cervical abscess and abscess in the subcutaneous tissues, sacral osteomyelitis, granulomatous mediastinal mass, pericardial effusion and myocarditis.

9.4.233 Truncatella [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Xylariales; Family: Bartaliniaceae; Genus: *Truncatella*. The species *Truncatella angustata* is typically associated with vascular plants as either an endophyte or a pathogen, it causes fruit rot particularly noted in olives, it affects the wood of roses, in grapevines it causes "grapevine trunk disease", and it also is a saprophyte. *Truncatella angustata* additionally is entomogenous, meaning that it grows either on or in the bodies of insects, and it produces ramulosin which inhibits both the germination of some plant seeds and the germination of some fungi. *Truncatella angustata* produces subcutaneous infections in human which likely follow accidental inoculation from rotten wood.

Note: There are two genera named *Truncatella*, one of those is this fungal genus, the other is a genus of gastropod mollusc.

9.4.234 Uncinocarpus [Possibly Infects: Birds]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Onygenales; Family: Onygenaceae; Genus: *Uncinocarpus*. The *Uncinocarpus* are found in soil. *Uncinocarpus queenslandicus* and *Uncinocarpus reesii* have been isolated as dermatophytes from chicken *Gallus gallus* and it has been suggested that these two fungal species can cause dermatophytic infections of poultry.

9.4.235 Ustilago [Infects: Mammals]

Phylum: Basidiomycota; Class: Ustilaginomycetes; Order: Ustilaginales; Family: Ustilaginaceae; Genus: *Ustilago*. The *Ustilago* produce smut disease of plants as a leaf infection and also smut galls. *Ustilago maydis* causes smut on maize *Zea mays subspecies mays* and teosinte. The name teosinte is used in common reference to several species and subspecies of *Zea*. *Ustilago maydis* forms galls on all aboveground parts of the *Zea* host species. Young smut galls are considered a delicacy for human consumption, and they are eaten in Mexico as the delicacy huitlacoche, typically consumed as filling in quesadillas and other tortilla-based foods, and in soups. *Ustilago maydis* causes fungal peritonitis in human.

9.4.236 *Vanrija* [Infects: Amphibians and Mammals]

Phylum: Basidiomycota; Class: Tremellomycetes; Order: Trichosporonales; Family: Trichosporonaceae; Genus: *Vanrija*. *Vanrija humicola* previously has been named *Asterotremella humicola*, *Candida humicola*, and *Cryptococcus humicola*, it is found in soil. *Vanrija humicola* reduces growth rates and can cause the death of tadpoles, referring here to the tailed larval stage of anuran amphibians (Order Anura). In human, *Vanrija humicola* causes ophthalmopathy, conjunctivitis, melanonychia, central nervous system infection, and infects burn wounds. *Vanrija humicola* has been found in cattle *Bos taurus* milk, suggesting that this fungal species causes sublinical mastitis in cattle.

9.4.237 *Veronaea* [Infects: Amphibians, Fish, Mammals, and Reptiles]

Phylum: Ascomycota; Class: Eurotiomycetes; Order: Chaetothyriales; Family: Herpotrichiellaceae; Genus: *Veronaea*. The species *Veronaea botryosa* is found in soil and associated with *Eucalyptus*. *Veronaea botryosa* infects amphibians, including having caused surface lesions on head, legs and back, plus internal systemic lesions on kidney, lung, liver, and spleen of Japanese toad *Bufo japonicus formosus*, Sambava tomato frog *Dyscophus guineti*, and green tree frog *Litoria caerulea*. In fish, *Veronaea botryosa* has infected cultured Siberian sturgeon *Acipenser baerii* to cause cutaneous erythema, nodular or cystic lesions that may be ulcerative of many organs including the skin and eye, plus additionally causing coelomic effusion and organomegaly. *Veronaea botryosa* similarly infects white sturgeon *Acipenser transmontanus*. In human, *Veronaea botryosa* produces nodules and superficial ulcerations plus disseminated infections. *Veronaea botryosa* infections of reptiles include obstructive tracheitis caused in Green sea turtle *Chelonia mydas*.

9.4.238 *Verticillium* [Infects: Fish, Mammals, and Reptiles]

The *Verticillium* seem to cause wilt disease by infecting the water-conducting xylem tissues in plants. The susceptible plant species include fruits and vegetables, ground covers, vines, and woody ornamentals. *Verticillium* also either live or survive in soil and rock as small, dark structures called microsclerotia. Unfortunately, the *Verticillium* associated with infections often are not identified at the fungal species level. *Verticillium* species have been noted to cause fungal infections in a number of fish species including Gibelion catla *Catla catla*, clown knifefish *Chitala chitala*, snakehead murrel *Channa striata*, Jayanti rohu *Labeo rohita*, and great snakehead *Channa marulius*. *Verticillium* infections of human include: skin infections that can

become subcutaneous and extend to nodule formation, keratitis, peritonitis and fungemia. *Verticillium* has been associated with cutaneous lesions in green anaconda *Eunectes murinus*. *Verticillium* is the name for three genera of Ascomycota. I have numbered these three genera by the order that they are listed in the National Center for Biotechnology Information database.

Genus 1 [infectivity range for vertebrates not specific]
Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: (not assigned); Genus: *Verticillium*.

Genus 2 [infectivity range for vertebrates not specific]
Phylum: Ascomycota; Class: Sordariomycetes; Order: Phyllachorales; Family: (not assigned); Genus: *Verticillium*.

Genus 3 [infectivity range for vertebrates not specific]
Phylum: Ascomycota; Class: Sordariomycetes; Order: Glomerellales; Family: Plectosphaerellaceae; Genus: *Verticillium*.

Note: *Verticillium lateritium*, which causes keratomycosis of human, has been renamed *Nectria inventa*.

9.4.239 Verruconis [Infects: Birds and Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Venturiales; Family: Sympoventuriaceae; Genus: *Verruconis*. The species *Verruconis gallopava* previously has been named *Dactylaria gallopava* and also *Ochroconis gallopava*, it is thermophilic and occurs in such naturally warm environments as hot springs and thermal soils. *Verruconis gallopava* also is found in poultry litter and other self-heating organic waste. *Verruconis gallopava* causes mycotic infections in birds, including fatal encephalitis in wild birds and also in farmed poultry (chickens and turkeys). In mammals, *Verruconis gallopava* produces subcutanious abcesses, necrotic lesions of the skin, pulmonary symptoms similar to allergic bronchitis, and fatal systemic infections that include such results as encephalitis (brain abcess) in cat and dog. *Verruconis gallopava* has caused in human: intraocular infections as well as cardiac and endovascular infections with dissemination especially in recipients of solid organ transplants such as liver, kidney, heart, and lung.

9.4.240 Volvariella [Infects: Mammals]

Phylum: Basidiomycota; Class: Agaricomycetes; Order: Agaricales; Family: Pluteaceae; Genus: *Volvariella*. *Volvariella volvacea* grows as a saprobe on rice straw. *Volvariella volvacea* is an edible mushroom at its immature stage, variously called either paddy straw mushroom or straw mushroom, and it is very commonly

used in Asian cooking. I have eaten straw mushrooms a great many times and I always bought them in canned form. I never suspected that live mushrooms of this species could cause a lethal invasive infection of human (Salit et al. 2010).

9.4.241 Wallemia [Infects: Mammals]

Phylum: Basidiomycota; Class: Wallemiomycetes; Order: Wallemiales; Family: Wallemiaceae; Genus: *Wallemia*. The *Wallemia* are described as being xerophilic and halophilic. *Wallemia sebi* is considered common in agricultural environments, it is a very xerophilic fungus that has been isolated from soil, air, hay, textiles, milk products, and food preserved by adding high levels of either sugar or salt. *Wallemia sebi* is a spoilage fungus of dried and salted fish which it makes look brown, and of other salty foods, as well as sweet foods such as jam. *Wallemia sebi* is very common in house dust and is thought to cause a hypersensitivity pneumonitis in human. There has been a suggestion of *Wallemia sebi* being associated with respiratory infections including bronchitis in human, and it causes subcutaneous phaeohyphomycosis in human.

9.4.242 Westerdykella [Infects: Mammals]

Phylum: Ascomycota; Class: Dothideomycetes; Order: Pleosporales; Family: Sporormiaceae; Genus: *Westerdykella*. The *Westerdykella* are found in a variety of natural environments including soil, and the Sporormiaceae in general are found in dung and rotting plant material. *Westerdykella dispersa* causes angioinvasive disease of human, and it also has caused human infection following severe burn. *Westerdykella minutispora*, which used to be named *Phoma minutispora*, causes skin lesions in human. The species *Westerdykella reniformis*, which has been isolated from orchard soil, typically attacks the skin and these infections have been known to disseminate into the kidney and intervertebral disks of dog.

9.4.243 Xenoacremonium [Infects: Mammals]

Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Nectriaceae; Genus: *Xenoacremonium*. *Xenoacremonium recifei*, previously named *Acremonium recifei*, is associated with plants. In human, *Xenoacremonium recifei* has caused post traumatic keratomycosis and also been isolated from mycetoma of the foot.

9.4.244 Yamadazyma [Infects: Mammals]

Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Debaryomycetaceae; Genus: *Yamadazyma*. The *Yamadazyma* can be found on rotting wood, tree bark, in ant nests, and living as endophytes in leaves. *[Candida] pseudoaaseri*, which causes catheter associated infections, fungemia and pneumonia in human, has been moved into this genus.

9.5 Fungal Genus and Species Names That No Longer Are on the "Active" List

These 54 genus names were deleted from the "Active" list! Most have been deleted because they no longer seem to contain species that are infectious for vertebrates. Some have been deleted because, even though often mentioned in the literature, they were not traceable on the internet. An exception to this list is *Tinea*. "Tinea" is a clinical term used to describe cutaneous fungal infections and the clinical terminology attributed to those various diseases unintentionally could be confused as fungal genus and species name. *Tinea* is not a fungal genus name. *Tinea* is the name for a genus of clothes-moths.

Absidia are mentioned as infecting amphibians, birds, mammals and reptiles, largely because of the species *Absidia corymbifera*. *Absidia corymbifera*, which previously also has been named *Mucor corymbifer* and *Mycocladus corymbifer*, now is *Lichtheimia corymbifera*. *Absidia hyalospora* now is *Lichtheimia hyalospora*.

Achorion gypseum, previously also named *Arthroderma gypseum*, *Closterosporia gypsea*, *Gymnoascus gypseus*, and *Microsporum gypseum*, now is *Nannizzia gypsea*.

Ajellomyces capsulatus now is the species *Histoplasma capsulatum*. *Ajellomyces crescens* now is *Emmonsia crescens*, which is beneficial for the memory of Chester Wilson Emmons and sadly seems to subtract something from our memory of Libero Ajello. Nevertheless, with pride we remember Libero Ajello who has been honored with the fungal Family name Ajellomycetaceae.

Amauroascus are mentioned as infecting poultry, however, *Amauroascus kuehnii* which is the noted dermatophyte of birds now is named *Auxarthron kuehnii*.

Asterotremella as a genus has been moved to the *Vanrija* and consequently *Asterotremella humicola*, which previously also has been named *Candida humicola* and *Cryptococcus humicola*, now is *Vanrija humicola*.

Biatriospora often are mentioned as infecting humans, although the renaming of *Biatriospora mackinnonii* as *Nigrograna mackinnonii* seems now to make the genus name *Biatriospora* irrelevant in mention as a human pathogen.

Blastoschizomyces are noted vertebrate pathogens because of *Blastoschizomyces capitatus*. The genus name *Blastoschizomyces* now has been redirected to *Dipodascus*. *Blastoschizomyces capitatus*, which infects humans and previously

also has been named *Dipodascus capitatus*, *Geotrichum capitatum*, *Saprochaete capitata*, and *Trichosporon capitatum*, has been renamed *Magnusiomyces capitatus*.

Closterosporia have been removed from the "Active" list because *Closterosporia gypsea*, previously also named *Achorion gypseum*, *Arthroderma gypseum*, *Gymnoascus gypseus*, and *Microsporum gypseum*, now is *Nannizzia gypsea*.

Coniothyrium have been mentioned as infecting humans. The taxonomic reassignment of *Coniothyrium fuckelii* as *Paraconiothyrium fuckelii* would seem to have eliminated the genus *Coniothyrium* from the list of fungal pathogens infective of mammals.

Cylindrocarpon has had three of its member species reassigned. *Cylindrocarpon* cyanescens, which produces mycetomas in humans, now is named *Phialophora cyanescens*. *Cylindrocarpon destructans*, which also produces mycetomas in humans and previously was named *Neonectria radicicola*, now is *Ilyonectria destructans*. *Cylindrocarpon lichenicola*, which causes cutaneous infections and mycetomas in human, now is *Fusarium lichenicola*.

Dactylaria fungi are mentioned as infecting poultry and humans. However, the responsible fungal species *Dactylaria gallopava* became *Ochroconis gallopava*, and now is *Verruconis gallopava*, such that the genus name *Dactylaria* seems no longer to belong on the "Active" list.

Dactylomyces is a genus name that no longer has official recognition. The fungal species *Dactylomyces crustaceus* previously also was named *Paecilomyces crustaceus* and now is named *Thermoascus crustaceus*.

Dipodascus are mentioned as causing mastitis and abortion in mammals including humans. However, *Dipodascus capitatus*, which previously also has been named *Blastoschizomyces capitatus*, *Geotrichum capitatum*, *Saprochaete capitata*, and *Trichosporon capitatum*, now is called *Magnusiomyces capitatus*.

Drechslera have been noted as pathogens of mammals and reptiles. However, that association has changed because *Drechslera biseptata* has been renamed *Pyrenophora biseptata*, *Drechslera rostrata* now is *Exserohilum rostratum*, and *Drechslera spicifera* currently is *Curvularia spicifera*.

Emericella as genus certainly still exists, but does so without its vertebrate pathogens. *Emericella echinulata* has been renamed *Aspergillus spinulosporus*, *Emericella nidulans* has been renamed *Aspergillus nidulans*, *Emericella quadrilineata* has been renamed *Aspergillus quadrilineatus*, and *Emericella rugulosa* has been renamed *Aspergillus rugulosus*.

Engyodontium album now is named *Parengyodontium album*. The species remaining in *Engyodontium* are entomopathogens, including *Engyodontium parvisporum* which notably is associated with insects hibernating in underground shelters.

Eurotium herbariorum has been renamed *Aspergillus glaucus*.

Geomyces destructans, the psychrophilic fungus that causes white-nose syndrome in hibernating bats, now is *Pseudogymnoascus destructans*. *Geomyces pannorum*, previously also named *Chrysosporium pannorum* and *Sporotrichum pannorum*, now is *Pseudogymnoascus pannorum*. And thusly, the *Geomyces* no longer belong on the "Active" list.

Geosmithia is a genus which causes cankers in plants and many of the species in this genus cause disease in arhropods. Its notable species which was pathogenicity for mammals, *Geosmithia argillacea*, now is *Rasamsonia argillacea*, thus *Geosmithia* seems no longer to contain pathogens that infect vertebrates.

Haplosporangium historically has been identified as infecting humans and other mammals, notably rodents, plus moles and shrews. However, because *Haplosporangium parvum*, which also has been named *Emmonsia parva*, now is *Blastomyces parvus*, the association between *Haplosporangium* and disease of mammals has been lost.

Hendersonia have been listed by others as effecting disease in humans and therefore likely also other mammals. However, *Hendersonia* does not seem to cause disease in vertebrates. The *Hendersonia* cause stem canker, twig blight, and scorch or sunscald particularly associated with the species *Hendersonia opuntiae*. Scorch or sunscald disease is particularly common and serious on prickly pear cactus *Opuntia*. The fungal species *Hendersonia* acicola has been noted to afflict pine needles and normally affects only needles already infected by some other organism.

Hormonema also have been listed by others as causing disease in humans. The fact that *Hormonema dematioides*, which previously also was called *Sclerophoma pityophila*, now is named *Sydowia polyspora*, suggests that the genus *Hormonema* may no longer belong on the "Active" list.

Inonotus tropicalis, which previously also was named *Phellinus tropicalis*, now is *Tropicoporus tropicalis*.

Lecythophora have been noted to cause disease in a wide range of mammals but they no longer seem to exist as an independent fungal genus. The *Lecythophora* member species instead now are included as part of the *Coniochaeta*. *Lecythophora cateniformis* has been renamed *Coniochaeta cateniformis*, *Lecythophora hoffmannii* has been renamed *Coniochaeta hoffmannii*. *Lecythophora mutabilis*, which interestingly has the ability to infect both fish and humans, now is *Coniochaeta mutabilis*.

Leptosphaeria have been noted to cause disease in humans. However, *Leptosphaeria senegalensis* has become *Falciformispora senegalensis*, and *Leptosphaeria tompkinsii* has become *Falciformispora tompkinsii*. The result of these taxonomic reassignments has been that the genus *Leptosphaeria* no longer seems to contain species that are pathogenic for animals. The remaining members of *Leptosphaeria* largely are noted for causing canker and fruit rot. *Leptosphaeria maculans* does in particular cause blackleg disease of the mustard family Brassicaceae.

Marasmius have been reported as causing bronchopulmonary mycosis in humans but I could not confirm pathogenicity of this fungal genus in vertebrates. *Marasmius palmivorus* causes oil palm bunch rot which perhaps most notably affects oil palm fruit, and also causes post-emergence damping off of coconut palm *Cocos nucifera* seedlings.

Microdochium nivale causes wheat blight and also has been reported to affect humans with unspecified symptoms. The human disease that has been attributed to *Microdochium nivale* may have represented confusion with *Microdochium dimerum*, which now is classified as *Fusarium dimerum* and has been found to cause in humans eye infections, endocarditis and disseminated infections.

Myceliophthora thermophila, which infects humans and therefore likely also would infect other mammals, now is *Thermothelomyces thermophila* and so the genus name *Myceliophthora* may no longer belong on the "Active" list.

Mycocladus corymbifer, which previously also has been named *Absidia corymbifera* and *Mucor corymbifer*, now is *Lichtheimia corymbifera*.

Myrothecium has loss its two species that are pathogenic for vertebrates with the reassignment of *Myrothecium roridum* to *Paramyrothecium roridum*, and *Myrothecium verrucaria* to *Paramyrothecium roridum*.

Neocosmospora vasinfecta, a pathogen of humans which causes plant wilt disease and root rot in peanuts, now is *Fusarium neocosmosporiellum*, and with that taxonomic reassignment, the fungal genus *Neocosmospora* seems no longer to belong on the list of fungal genera that are pathogenic for vertebrates.

Neonectria radicicola also had been named *Cylindrocarpon destructans*, and currently is named *Ilyonectria destructans*.

Neosartorya are soil organisms and this genus had been noted to infect humans, causing peritonitis and invasive gastrointestinal tract infections. However, *Neosartorya fischeri* now is *Aspergillus fischeri*, *Neosartorya hiratsukae* now is *Aspergillus hiratsukae*, *Neosartorya pseudofischeri* now is *Aspergillus thermomutatus*, and *Neosartorya udagawae* now is *Aspergillus udagawae*, and those taxonomic reassignments seem to have resulted in *Neosartorya* no longer containing species that are infectious for vertebrates.

Onychocola has lost its two vertebrate pathogens as follows: *Onychocola canadensis* has been renamed *Arachnomyces nodosetosus*, and *Onychocola kanei* has been renamed *Arachnomyces kanei*.

Oospora cuboidea, previously also named *Arthrographis cuboidea* and *Geotrichum microsporum*, now has the name *Scytalidium cuboideum*. *Oospora* no longer seems to exist as a genus name.

Oxyporus corticola is a plant pathogen that invades through roots to cause white rot which typically affects fruit of the genus *Prunus*. As an opportunistic pathogen of mammals, *Oxyporus corticola*, which has been reassigned the name *Rigidoporus*

corticola, causes skin infections as well as lymphadenopathy and osteomyelitis of dogs. Note that *Oxyporus* also is the name assigned to a genus of Coleoptera.

Periconia is a genus whose members often are found in soil, blackened and dead herbaceous stems, and leaf spots, typically affecting grasses, rushes, and sedges. As a rare human pathogen, this genus has been known to cause a case of mycotic keratitis but I could not trace its attributed species name *Periconia keratitis*.

Pestalotiopsis is a genus of plant pathogens that cause blight and interestingly, the plant pathogen *Pestalotiopsis microspora* is able to grow on the synthetic polymer polyurethane as its sole carbon source under both aerobic and anaerobic conditions, hence showing promise as a form of bioremediation for waste reduction, and *Pestalotiopsis pauciseta* produces taxol. As interesting as this fungal genus thus seems, its vertebrate pathogen *Pestalotiopsis clavispora* now is *Neopestalotiopsis clavispora*.

Peyronellaea is another genus that has lost its vertebrate pathogens. *Peyronellaea gardeniae*, which causes subcutaneous infections in humans, has been renamed *Didymella gardeniae*. *Peyronellaea glomerata*, which previously also was named *Phoma glomerata*, has been renamed and currently it is *Didymella glomerata*.

Phialosimplex has been reduced to a small genus with the transfer of its vertebrate pathogens into the large fungal genus *Aspergillus*. As such, *Phialosimplex caninus* now is *Aspergillus caninus*, *Phialosimplex chlamydosporus* is *Aspergillus chlamydosporus*, and *Phialosimplex sclerotialis* now is named *Aspergillus sclerotialis*.

Pityrosporum has had its members assigned to the genus *Malassezia*.

Pleurostomophora has had its members reassigned and the species once belonging to this genus now are part of *Pleurostoma*. This includes *Pleurostomophora richardsiae* which previously also was named *Phialophora richardsiae*, it causes bursitis in human and now is *Pleurostoma richardsiae*.

Pyrenochaeta has had its vertebrate pathogens reassigned and therefore this genus name probably should not be included in the "Active" list. *Pyrenochaeta mackinnonii* which produces mycetomas in humans now is named *Nigrograna mackinnonii*, *Pyrenochaeta romeroi* which also produces mycetomas in humans now is named *Medicopsis romeroi*, and *Pyrenochaeta unguis-hominis* which causes nail infections in humans now is named *Neocucurbitaria unguis-hominis*. *Pyrenochaeta* also has been found to cause keratitis in human, but without specification of the fungal species.

Sclerophoma pityophila, which previously also was named *Hormonema dematioides*, now is *Sydowia polyspora*.

Scolecobasidium are reported to infect mammals and reptiles, but taxonomic reassignment of two fungal species has taken this genus off the list of vertebrate pathogens. *Scolecobasidium constrictum* which infects humans now is *Ochroconis constricta*, and *Scolecobasidium humicola* which infects reptiles now is *Ochroconis humicola*.

Setosphaeria is a fungal genus name that now redirects to the genus *Exserohilum* and as such the species *Setosphaeria rostrata*, which geographically is found in the mobile surface layer of saharan desert soil, and in mammals causes cutaneous granulomas as well as corneal ulcers that can fulminantly disseminate resulting in meningitis, now is *Exserohilum rostratum*. Additional information on this fungal species can be found by researching under its other previous name *Drechslera rostrata*.

Sphaeropsis in a genus that typically causes tip blight or dieback, also stem-end rot, and calyx-end rot. *Sphaeropsis subglobosa*, which causes keratomycosis in humans, now has been renamed *Neodeightonia subglobosa* and thus *Sphaeropsis* seems to have lost its claim to pathogenicity for vertebrates.

Sporotrichum pannorum, which previously also has been named *Chrysosporium pannorum* and *Geomyces pannorum*, famously infects mammals and currently is named *Pseudogymnoascus pannorum*. *Sporotrichum schenckii* which infects reptiles, humans and also other mammals, now is *Sporothrix schenckii*.

Stemphylium are often found in soil, wood, decaying vegetation, an on leaves as a plant pathogen. They are disseminated as dry spores by the wind and their indoor growth is rare. As an allergen, they been known to cause Type I allergen symptoms. *Stemphylium mucorsporidium* has been reported to cause phaeohyphomycotic sinusitis in human, but I could not confirm identification of either that fungal species or the reported disease association.

Tinea is not a fungal genus name. *Tinea* is the name for a genus of clothes-moth belonging to the family Tineidae and inconsiderately clothes-moths have damaged some of my once very nice three-piece woolen suits. It is important to note that even though many fungi produce skin diseases called 'Tinea', and confusingly some of those infections also are termed ringworm, those infections which affect humans, other mammals, and birds neither are caused by fungi taxonomically named Tinea nor are the diseases caused by a worm. Tinea are generally superficial fungal infections (dermatophytosis). They include: tinea barbae (beard), tinea capitis (scalp), tinea corporis (body), tinea cruris (groin), tinea faciei (face), tinea imbricata (overlapping pattern), tinea incognito (disguised), tinea manuum (hand), tinea nigra (black), tinea pedis (foot), tinea unguium (nails), and tinea versicolor (various colors). The diagnosis of ringworm in chickens as a severe form of tinea capitis (scalp) often is called favus. There is no taxonomic genus named Favus.

Torula histolytica, which previously also has been named *Filobasidiella neoformans*, now is *Cryptococcus neoformans*.

Torulopsis as a genus name now redirects to *Candida*. *Torulopsis glabrata* was renamed *[Candida] glabrata*, and now is part of the genus *Nakaseomyces*. *Torulopsis haemulonii*, which causes deep cutaneous infections in humans and other mammals, was renamed *Candida haemulonii* and then divided into *[Candida] haemulonis* and *[Candida] duobushaemulonis* with both of those species now considered as belonging to the genus *Clavispora*.

Ulocladium is a ubiquitous species widely found in soil, dung, grasses, fibers, wood, and decaying plant material. It is normally disseminated as a dry spore in wind. Commonly found indoors on gypsum board, textiles including tapestries, jute carpet backing, paper, and paint, *Ulocladium* is noted to have a high water requirement for growth. *Ulocladium* spores have been reported to cause subcutaneous tissue infections in humans. However, *Ulocladium atrum* has been renamed *Alternaria atra*, *Ulocladium botrytis* has been renamed *Alternaria botrytis*, and *Ulocladium chartarum* has been renamed *Alternaria chartarum*. Those taxonomic reassignments have resulted in the genus *Ulocladium* no longer seeming to contain species that are pathogenic for vertebrates.

Xylohypha bantiana is a species typically found in soil and rotting plant material. The name of this species now has been changed to *Cladophialophora bantiana*.

9.6 Fungal Genera for Which I Could Not Locate Corroborating Infectious Disease Information

There are five fungal genera that are mentioned on various websites as being either present in soil or associated with plants, and similarly infectious for vertebrates, but for which I could not locate confirming information proving their infectivity for vertebrates. I am listing these here as a means of saving you some research time!

Agaricus **[not confirmed as infectious for vertebrates]**
Phylum: Basidiomycota; Class: Agaricomycetes; Order: Agaricales; Family: Agaricaceae; Genus: *Agaricus*. The *Agaricus* are grassland soil fungi which also live on compost. *Agaricus bisporus* commonly is eaten both in its immature brown or white colored stage, labelled by many names including "Button mushroom". In its mature brown stage, this is called a "Portobello mushroom". *Agaricus campestris* has been suggested as being infectious for human, but I could not confirm that suggestion.

Hypomyces **[not confirmed as infectious for vertebrates]**
Phylum: Ascomycota; Class: Sordariomycetes; Order: Hypocreales; Family: Hypocreaceae; Genus: *Hypomyces*. The *Hypomyces* are fungi that parasitize mushrooms. *Hypomyces chrysospermus*, previously named *Sepedonium chrysospermum*, has been suggested as being infectious for human, but I could not confirm that suggestion.

Lactarius **[not confirmed as infectious for vertebrates]**
Phylum: Basidiomycota; Class: Agaricomycetes; Order: Russulales; Family: Russulaceae; Genus: Lactarius. *Lactarius deliciosus* is an ectomycorrhizal fungi that grows in acidic soils beneath conifers. This species produces an edible mushroom. *Lactarius deliciosus* has been suggested as being infectious for human, but I could not confirm that suggestion.

Note: *Lactarius* also is the name for a genus of fish in the Family Lactariidae.

***Teunomyces* [not confirmed as infectious for vertebrates]**
Phylum: Ascomycota; Class: Saccharomycetes; Order: Saccharomycetales; Family: Debaryomycetaceae; Genus: *Teunomyces*. *Teunomyces kruisii* is found on the fruiting bodies of mushrooms and seems to be a symbiont of fungivorous beetles. *Teunomyces kruisii* has been suggested as being infectious for human, but I could not confirm that suggestion.

***Verticimonosporium* [not confirmed as pathogenic for vertebrates]**
Phylum: Ascomycota; Class: (not assigned); Order: (not assigned); Family: (not assigned); Genus: *Verticimonosporium*. The *Verticimonosporium* are soil microbes and they produce trichothecene mycotoxins, although they have been postulated as causing food associated toxicosis I was not able to confirm that as being true.

Compliance with Ethical Standards

Conflict of Interest Christon J. Hurst declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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