Nitrogen-Cycling Communities in Organically Amended Versus Conventionally Managed Agricultural Soil

Lily Pereg and Mary McMillan

Abstract Productivity in agricultural ecosystems is often largely dependent on the input of nitrogen fertilizers such as urea, nitrate, and ammonia, despite the high financial costs and potential detrimental effects on the environment. Soil enrichment with organic matter, such as plant compost, straw, or manure, can enhance soil organic carbon and improve soil quality and increase aggregate stability, biological activity, and microbial diversity, including nitrogen cycles. The abundance of the *nifH* gene, thus nitrogen cycles, was found to be enhanced when organic fertilization was used and correlated mainly to the availability of organic carbon, potentially due to the presence of fuels required to run the energetically expensive nitrogen fixation process. On the other hand, readily available ammonia and nitrate, which are often associated with inorganic fertilization, tend to suppress the soil potential for nitrogen fixation. The impacts of fertilizers on denitrifying microbial communities are complex, due to the great diversity of the denitrifiers and the variation in their abundance in different environments. However, in general, the use of organic fertilizers increases denitrification potential and activity in soils when compared to inorganic fertilization. This is particularly important for increasing the abundance of those denitrifiers containing the *nosZ* genes and capable of the final step of the denitrification process, removing the potent greenhouse gas nitrous oxide by its conversion to dinitrogen. Bacterial and archaeal nitrifiers react differently to variations in soil conditions and to different fertilization management strategies. It is important to note that nitrogen fertilizers in access might turn into an ecosystem hazard where nitrifying microbes convert them to nitrate. This survey of the literature suggests that the addition of organic matter to agricultural soils, even where inorganic nitrogen fertilizers are used, enhances the soil potential for nitrogen cycling and soil sustainability.

Keywords Nitrogen cycle · Nitrogen fixation · Nitrification · Denitrification · Inorganic and organic nitrogen fertilizers · nifH · nirS/K · nosZ · amoA genes

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Abbreviations

amoA	Gene encoding the monooxygenase enzyme
amoA-arch	Archaeal *amoA* gene
amoA-B	Bacterial *amoA* gene
AOA	Ammonia-oxidizing archaea
AOB	Ammonia-oxidizing bacteria
ATP	Adenosine triphosphate
Av N	Available nitrogen (Kjeldahl method)
C	Carbon
CO_2	Carbon dioxide
DMPP	3,4-Dimethylpyrazole phosphate
IF	Inorganic fertilization
N	Nitrogen
N_2	Dinitrogen gas
N_2O	Nitrous oxide
NH_3	Ammonia
NH_4^+	Ammonium ion
nifH	Gene encoding the dinitrogenase reductase enzyme
nirK	Gene encoding the copper-containing nitrite reductase enzyme
NirK	Copper-containing nitrite reductase
nirS	Gene encoding the cytochrome cd1 nitrite reductase enzyme
NirS	Cytochrome cd1 nitrite reductase
NO	Nitric oxide
NO_2^-	Nitrite
NO_3^-	Nitrate

1 Nitrogen Fertilization and Microbial Communities in Agricultural Soils

Nitrogen (N) is a vital element for all forms of life including plants, with one to four percent of the living material, including proteins, DNA, and RNA, being composed of nitrogen (Woodmansee et al. 1978). Therefore, productivity in agricultural ecosystems is often largely dependent on the input of N fertilizers. The high demand for N in intensive cropping and other agricultural systems is generally met by the addition of fertilizers, such as urea, nitrate, and ammonia, to the soil. However, the effects that such agrochemicals have on soil function are largely unknown. In general, the availability and productivity of agricultural soil is under threat, due to greater urbanization and also intensified farming practices which utilize extensive irrigation, increasing amounts of agrochemicals, and heavy machinery (Berry 1978; Newman et al. 2015; Li et al. 2015). As these intensive agricultural practices result in land degradation, it is increasingly necessary to identify or develop sustainable cropping systems that result in large biomass yields and maintain or improve ecosystem services (Orr et al. 2015), such as soil N cycling.

In agricultural systems, N is taken out from the ecosystem when plants and/or animals are removed. Consequently, there is a need to constantly replace the N supply in these farming systems. This is achieved through the widespread use of N-containing fertilizers, despite the high financial costs and potential detrimental effects on the environment (Ladha et al. 2005; Spiertz 2010). Manufacturing N fertilizers from fossil fuels and transporting manures or other sewage-based fertilizers to agricultural areas are energetically expensive, especially when the amount of fuel required is considered. These processes also result in the release of CO_2 and contribute to the pollution of water bodies through the leaching of nitrate and generation of nitrous oxide, a potent greenhouse gas. These factors contribute to the growing range of environmental concerns around the intensive farming practices and the use of agrochemicals, such as soil compaction and erosion, overuse of land for cropping or grazing, reductions in soil organic matter, depletion of water supplies, and pollution of groundwater and surface waterways through agrochemical runoff (Hirsch and Mauchline 2015). Agrochemical runs off, and soil degradation can both contribute to eutrophication of aquatic habitats. Production practices that result in degraded soil quality may then drive increased use of irrigation and fertilization in an attempt to sustain soil productivity (Tilman et al. 2002; Zalidis et al. 2002). This can, in turn, cause further damage to the soil while also increasing the cost of crop production. While intensive arable farming and use of agrochemicals might negatively impact soil chemical, physical, and biological properties (Caravaca et al. 2002; Bellamy et al. 2005), the enrichment of soil with organic matter, such as plant compost, straw, or manure, can enhance soil organic carbon (SOC) and improve soil quality; increase aggregate stability, biological activity, and microbial diversity (Johnston et al. 2009; Morugán-Coronado et al. 2015; Pérez-Piqueres et al. 2006; García-Orenes et al. 2010, 2013, 2016; Prosdocimi et al. 2016); as well as reduce chemical input, increase plant productivity, and increase the sustainability of organically managed agroecosystems (Macci et al. 2013).

Soil microbial communities are an important component of soil. The structure and function of soil microbial communities vary depending on soil type, pH, temperature, plant cover and rotation, fertilization, tillage management, and water content (Bossio et al. 1998; Saleh-Lakha et al. 2005; Jangid et al. 2008; de Vries et al. 2013; Garbeva et al. 2008; Geisseler and Scow 2014; Quadros et al. 2012; Kibblewhite et al. 2008; Geisseler et al. 2010). Agricultural land management strategies can reduce the diversity and abundance of soil microorganisms and affect various soil properties (Caravaca et al. 2002). For example, decreased availability of water reduces soil organic C, altering the structure of soil microbial communities (Canarini et al. 2016; Bastida et al. 2017). It is widely acknowledged that water availability is essential for the maintenance of soil microbial communities. However, there is a lack of information on how different water management practices impact soil microbial communities (Bastida et al. 2008). In semiarid conditions, where water supplies are limited, the addition of organic matter may support the development of soil microbial communities and increase soil biodiversity (García-Orenes et al. 2010; Frenk et al. 2014; Wafula et al. 2015; Bastida et al. 2017). At the same time, maintaining crop cover on the soil surface, and minimizing soil tillage, can improve the soil physical structure, allowing improved water retention and maintenance of biological activity (Morugán-Coronado et al. 2015). Agrochemicals, including herbicides, fertilizers, fungicides, and insecticides, can affect the soil biota and the structure and function of soil microbial communities and therefore have a significant impact on soil quality (Imfeld and Vuilleumier 2012; Sofo et al. 2012). Soil microbial communities are very sensitive to changes in the soil, including physical disruption of the soil or changes in water or nutrient content. They can thus be considered as possible indicators of variations in the soil environment and of soil quality (Zornoza et al. 2009; Frenk et al. 2014).

An increased understanding of how agricultural management practices influence the structure of soil microbial communities and their overall impact on soil health is markedly important under semiarid conditions (García-Orenes et al. 2013). In semiarid areas, environmental constraints result in the overexploitation of land for food production, and these intensive farming practices result in a decline in soil structure, soil fertility, and a loss of organic matter (Caravaca et al. 2002). Sustainable farming practices, including the application of organic matter to enrich the soil, can enhance SOC and improve soil quality, as observed in arable agriculture following the addition of animal manures (Johnston et al. 2009). Soil organic amendments can also influence diverse soil microbial communities and enhance soil fertility. Organic nutrient sources have been suggested to be beneficial for both soil biodiversity and crop production, as they increase soil organic matter and potentially lead to improvements in a number of soil biological and fertility indicators, including soil aggregation, porosity, and water retention. In southern Spain, a decline in soil organic matter content and an associated loss of soil fertility have been observed as a result of intensive agriculture and the semiarid conditions (Caravaca et al. 2002). In contrast to conventional agriculture in this region, the use of organic amendments promotes the activity of soil microbial communities, increases microbial biodiversity, and improves soil properties. For example, application of oat straw to experimental plots on abandoned agricultural land led to soil restoration (García-Orenes et al. 2010, 2013). Morugán-Coronado et al. (2015) found that management practices including no-tillage, retention of vegetation cover, and application of manure improved soil conditions and increased soil organic matter and soil aggregate stability, leading to increased biological activity. The use of organic fertilization in grapevine production has been trialed over the last 10 years in semiarid regions in Spain. The use of organic amendments has resulted in improved soil quality indicators, including an increase in soil microbial diversity and enhanced soil biological activity, in comparison to traditional farming practices using chemical fertilization (García-Orenes et al. 2016). Earlier, Pérez-Piqueres et al. (2006) reported that the use of organic fertilizers, in the form of compost amendments, manure, and grapevine prunings, promoted an increase in soil microbial activity, enhancing the fertility and productivity of agricultural soils. Interestingly, Castañeda et al. (2015) found that organic rather than conventional management resulted in similar communities in grapevine soils to that of nearby forest in Chile, suggesting that the use of various types of organic amendments in agricultural soils would lead to sustaining soil biological and functional diversity and contribute to conservation of agricultural land. There has been a movement toward implementing organic fertilization systems to help improve soil properties, thereby increasing the productivity of agricultural land, and also allow for a reduction in agrochemical inputs, increasing the sustainability of such agroecosystems (Macci et al. 2013).

2 Microbial Functions in Agricultural Soils: The Nitrogen Cycle

Soil organisms undertake a large number of ecosystem services, such as nutrient cycling. Microorganisms carry out N cycling (Fig. 1), which is one of the most important soil functions (Fitter et al. 2005; Wallenstein and Vilgalys 2005; He et al. 2007). Several soil properties are related to mineralization and immobilization rates of N in soils, including microbial biomass, enzymatic activity, soil respiration rate, and microbial C and N contents (Alef et al. 1988; Hart et al. 1994; Tietema 1998; Barrett and Burke 2000; Bengtsson et al. 2003).

Although our atmosphere is composed of around 78% N (inorganic form), this N source is not available directly to plants and animals. The only organisms that can access this N_2 pool are bacteria and archaea that produce the enzyme nitrogenase. Nitrogenase-producing organisms can reduce the triple bond in atmospheric N_2 to ammonia, converting N into a source which can then be used by other organisms (Dixon and Kahn 2004; Wuebbles 2002). Thus, in nature, the major input of available N into the biosphere is through diazotrophic N fixation by prokaryotes

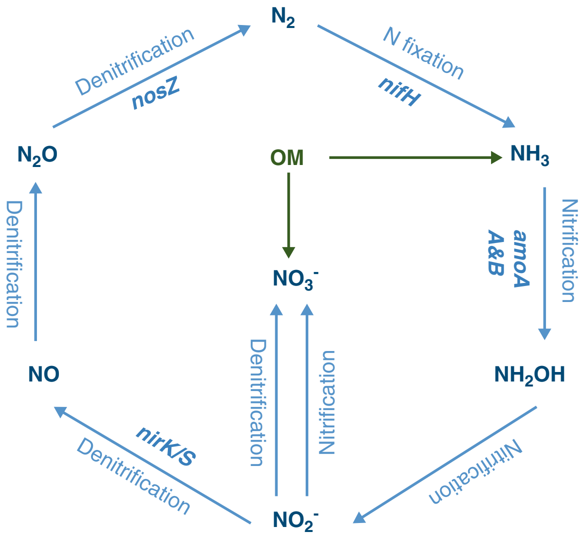

Fig. 1 Nitrogen cycling in the soil. The figure shows the main parts of the N-cycling pathway from N fixation, the main input of biologically available N into the biosphere, to the removal of fixed N from the biosphere. It also shows several genes encoding major enzymes in the N cycle (*nifH*, N fixation; *nirK*, *nirS*, *nosZ*, denitrification; *amoA*, nitrification). A, archaeal *amoA*; B, bacterial *amoA*; OM, organic matter

(Fig. 1). Nitrogenase is the key enzyme in N fixation. The N-reductase subunit of nitrogenase is encoded by the highly conserved gene *nifH* (Coelho et al. 2009). The abundance of the *nifH* gene in an environmental sample gives an indication of the potential for N fixation by bacteria (Coelho et al. 2009). Microbial assimilation of inorganic N is critical for improving soil retention of N (Vinten et al. 2002; Tahovská et al. 2013). Addition of N to the soil is often in forms unavailable for direct uptake by plants, including urea (Witte 2011) or organically bound N in other fertilizers. Therefore, microbial N cycling in soils is key in transforming these N sources into an accessible form of N for plants (Fitter et al. 2005; Wallenstein and Vilgalys 2005; He et al. 2010). In fertilized systems, the hydrolysis of urea, which results in the release of ammonia and carbon dioxide (CO_2), is a significant player in soil N balance. Urea hydrolysis is catalyzed by the bacterial enzyme urease, with the urease alpha subunit being encoded by *ureC* gene (Koper et al. 2004). The abundance of the *ureC* gene can therefore also be used as an indicator for the presence of N cyclers. Since a large portion of soil N is bound in organic matter in the form of proteins and other N-containing macromolecules, mineralization starting with proteolysis is an important step in the release of available N into the biosphere. Proteolysis is a rate-limiting step in soil N cycling. Protease-encoding microbial communities can be studied by quantifying genes encoding the *alkaline* (*apr*) and *neutral* (*npr*) *metallopeptidase*. Using gene abundance assessment, Lori et al. (2018) found that organically managed soils had a more stable N provisioning potential than conventional fertilized soils under drought scenarios, probably facilitated by a distinct and more adaptive proteolytic microbial community.

Nitrification and denitrification, involving ammonia oxidation and nitrate and nitrite reduction to N_2O and N_2, respectively, are major components of the soil N cycle (reviewed by Teixeira and Yergeau 2012). A variety of microbes decompose organic N into NH_4^+ (Zhou et al. 2012), and the preferred N form available for plants, namely, NO_3^-, is produced by nitrifying microorganisms, which oxidize NH_4^+ to NO_2^- and then NO_3^- (Horz et al. 2004; Fierer et al. 2012). Nitrification is an aerobic process which begins with the oxidation of ammonia to nitrite. Nitrite produced in this reaction is then further oxidized to nitrate by nitrite-oxidizing bacteria. The oxidation of ammonia is often the rate-limiting step and is catalyzed by the enzyme ammonia monooxygenase. The abundance of bacterial and archaeal *amoA* genes, which encode the alpha (A) subunit of ammonia monooxygenase, can be measured using molecular techniques and be used to estimate the soil's potential for nitrification. The relative contribution to nitrification by bacterial versus archaeal ammonia oxidizers in soils and other ecosystems is still under debate (reviewed by Teixeira and Yergeau 2012).

The N cycle is considered to be completed when denitrifying microorganism reduces NO_3^- to NO, N_2O, and N_2, returning N to the atmosphere (Braker et al. 1998; Houlton and Bai 2009). Denitrification is a complex anaerobic process involving various enzymes that remove fixed N and convert it to other forms, including gaseous N compounds. N cycling is therefore also important from several other environmental perspectives, including controlling emission of the greenhouse gas nitrous oxide (N_2O). It has been estimated that approximately 5% of soil microbial biomass is denitrifying bacteria (Braker and Conrad 2011), which are functionally diverse and belong to over 60 genera (Philippot 2006). The rate-limiting step in denitrification is the reduction of nitrate (NO_2^-) to nitric oxide (NO), which distinguishes denitrifiers from other nitrate-reducing bacteria. This step is catalyzed by the enzyme nitrate reductase. Denitrifiers may contain one of two functionally and physiologically equivalent types of nitrite reductases: a Cu-containing enzyme (encoded by *nirK*) and a cytochrome cd1 (encoded by *nirS*) (Philippot 2006; Zumft 1997). The size of denitrifying microbial communities has been correlated with denitrification process rates (Throbäck et al. 2007; Hallin et al. 2009; Morales et al. 2010; Szukics et al. 2010; Petersen et al. 2012; Wu et al. 2012; Butterbach-Bahl et al. 2013). Another key reaction in the denitrification process is the reduction of N_2O to N_2. This reaction is catalyzed by the enzyme nitrous oxide reductase, encoded by the gene *nosZ*. The presence of the *nosZ*, a gene in the soil, may suggest that the microbial community present can reduce N_2O to N_2 and influence the balance of the two in the environment (Philippot 2006). Given that denitrifiers account for a significant proportion of soil microbial communities, the response of denitrifying microbes to agricultural management strategies is useful in assessing trends in soil functioning. Community composition has been shown to vary with the use of N and C fertilization (Hallin et al. 2009; Yin et al. 2014; Bastian et al. 2009), crop production practices and cropland use (Reeve et al. 2010; Bissett et al. 2011), as well as cover plant species (Bremer et al. 2007; Hai et al. 2009; Petersen et al. 2012). Our ability to identify agricultural practices that enhance immobilization and transformation of fertilizer N by soils requires an understanding of the impact agricultural management practices, such as the application of N, have on N-cycling microbes. Genes associated with the N cycle have been quantified and assessed in order to elucidate the effects of soil properties and management practices, such as soil geomorphology and land use (Colloff et al. 2008), pasture management (Wakelin et al. 2009), N fertilizers (Okano et al. 2004; Cavagnaro et al. 2008), and tillage (Cavagnaro et al. 2008), on the function of soil microbial communities. The main N-cycling gene targets for molecular analysis of soil N-cycling potential include the denitrification genes nirK, nirS, and nosZ, the nitrification amoA genes (in both bacteria and archaea), and the N fixation gene nifH (reviewed in Teixeira and Yergeau 2012).

3 Nitrogen-Cycling Communities in Soils Under Various Fertilization Managements

3.1 Nitrogen Fixation

It may be anticipated that fertilizers containing N will have some impact on microbial communities involved in N cycling, as it may remove any selection pressure for the ability to carry out N_2 fixation. However, the Rothamsted Broadbalk experiment, which compared the effects of different N inputs over a 170-year period, showed no significant effect on the diversity of the nitrogenase gene *nifH*, despite observed changes in microbial community structure (Ogilvie et al. 2008). Nevertheless, other studies have reported differences. A study conducted in south-east Australia, which compared a number of different soil types and different land management practices, indicated that land use had no influence on *nifH* gene abundance. Instead, the primary factor influencing *nifH* abundance was the amount of microbial biomass carbon (Hayden et al. 2010). In other Australian studies, the use of organic mulch as a soil amendment increased the abundance of the *nifH* gene in cotton soils, and the application of manure helped to maintain the level of *nifH* before and after crop planting. In a Spanish study of grapevine soil, the abundance of the *nifH* gene was significantly increased under treatment with organic fertilizers than when inorganic fertilizer was used (Pereg et al. 2018). Morales et al. (2010) hypothesized that the *nifH* gene, indicating the potential for N fixation, would be found at higher levels in soils where leguminous plants were regularly grown. However, they found that the *nifH* gene was more abundant in soil samples collected from forested sites, or soils from sites used for successional planting, than it was in agricultural soils, including those regularly planted with soybeans. These findings have been attributed to the populations of free-living N fixers present in natural ecosystems. Pereg et al. (2018) also observed that organic treatments which included either leguminous plants or manure resulted in a similar abundance of the *nifH* gene, suggesting that factors other than leguminous plant cover may account for the increased potential for N fixation in organically managed systems when compared to traditional inorganic fertilization practices. Also, in agreement with Morales et al. (2010), Pereg et al. (2018) showed that the increased abundance of *nifH* under organic fertilization regimes correlated strongly with available N (Av N, Kjeldahl method) and total organic carbon (TOC) in grapevine soil. Pereg et al. (2018) suggested that the readily available nitrate and ammonia in inorganic fertilizer may have suppressed the abundance of N fixers, as indicated by the reduced abundance of *nifH* under inorganic fertilization compared with soil treated with slowly released organic N sources. Coelho et al. (2009) have also detected a reduction in free-living diazotroph communities in soil with increasing levels of inorganic N fertilizer. High levels of NH_3 and NO_3^- (available forms of N) found in inorganic fertilizers may repress the synthesis and/or catalytic activity of the enzyme nitrogenase (Bisseling et al. 1978), suppressing N fixation in soil treated with fertilizers and abolishing the competitive advantage that diazotrophs may have in environments poor in available N. The process of N fixation is energetically expensive and requires large amounts of adenosine triphosphate (ATP) and reducing equivalents. Therefore, readily available carbon (C) source is also essential for diazotrophs to fix N (Chan et al. 1994). Results from Pereg et al. (2018) indicated that organic amendments that release organic N and phosphorus gradually into the soil (García-Orenes et al. 2016) support the conservation of N fixer communities in grapevine soils. This is in agreement with earlier studies reporting that phosphorus fertilization stimulates N fixation in soils (Reed et al. 2007) probably due to the high energy requirements of the N fixation process. It should also be noted that other soil parameters will also influence the abundance and activity of N-cycling microbial communities. The seasonal dynamics of these N-cycling communities are also tightly coupled with seasonal changes in labile C and N pools, temperature, oxygen availability, soil compaction, and soil moisture (Meng et al. 2017; Butterbach-Bahl et al. 2013; Rasche et al. 2010; Menneer et al. 2004).

3.2 Nitrification

During nitrification, ammonia is converted to nitrite by ammonia oxidizers and then to nitrate by nitrite oxidizers. Ammonia-oxidizing archaea (AOA) are considered to be more abundant than ammonia-oxidizing bacteria (AOB) in the majority of soil types (Leininger et al. 2006). However, it is not yet clear what the relative contributions of these different groups are to soil nitrification and whether or not they can be considered to be functionally interchangeable (Jia and Conrad 2009; Xia et al. 2011). The AOA require less energy and less ammonia than AOB and are therefore likely to be more abundant than AOB in soils that are unfertilized and in natural soils such as forest soils, despite having lower rates of ammonia oxidation than their bacterial counterparts (Martens-Habbena et al. 2009; Tourna et al. 2011). However, AOA has also been found to be more abundant in a variety of arable and agricultural soils (Zhalnina et al. 2013). Acidic soils may also be more likely to support AOA over AOB, as at low pH there is a shift toward ammonium over ammonia, which limits substrate availability and growth of AOB (Hirsch and Mauchline 2015). Although AOA has been found to dominate in arable soils at neutral pH, it appears to be AOB that increase in numbers in response to the application of N. Nitrification increases as N availability increases, and it is assumed that AOB are responsible for these increases in nitrification (Hirsch and Mauchline 2015). Similarly, the size of AOB communities, but not AOA, has been shown to increase with increased N availability in farmed grasslands (Di et al. 2009) and in direct response to application of animal manure (Wakelin et al. 2013), while in contrast, AOA was shown to decline with increasing N in two different arable soils (Bates et al. 2010; Wessén et al. 2011). In acidic soils, however, it is AOA that show a greater response to the application of N fertilizers (Gubry-Rangin et al. 2010). This again reflects the availability of ammonia as a substrate and the different pH optima for the two groups: nitrification by AOB decreases at lower pH (below 7), while conversely, nitrification by AOA decreases as soil pH increases (Hirsch and Mauchline 2015). AOB fix C autotrophically, and ammonia is its only source of usable energy and reductants. AOB isolated from acidic soils are often ureolytic, containing the urease enzyme, and ureolytic AOB can grow at lower pH with urea as an ammonia and CO_2 source (summarized in Koper et al. 2004), two ecologically important traits. Koper et al. (2004) suggested that ureolytic AOB may have an advantage in soils receiving animal wastes or urea fertilizers. The structure of AO microbial communities is also influenced by pH: different groups of AOA and AOB have been found in arable soils maintained at a range of pH from 4.5 to 7.5 (Nicol et al. 2008). The structure and function of these microbial communities seems to be influenced more by the soil properties rather than by the land management strategies used (organic versus conventional), which may explain why significant heterogeneity has been observed in AOA and AOB abundance (Hirsch and Mauchline 2015).

In agreement with Wessén et al. (2010) and Zhang et al. (2010), a study by Pereg et al. (2018) comparing organic versus inorganic grape production indicated that *amoA* genes, involved in ammonium oxidation in both bacteria and archaea, were affected differently by the fertilization practices. Abundance of the bacterial *amoA* (*amoA*-B) gene was lowest in soils treated with organic fertilizer in the form of prunings plus manure (OPM) yet higher in soil treated with prunings and legume cover (OPL) and under inorganic fertilization (IF) (Pereg et al. 2018). IF (20 Kg ha^{-1} N annually) soil was treated with NH_4NO_3 containing NPK, whereas OPL (15 Kg ha^{-1} N annually) was not treated with inorganic fertilizers. Nevertheless, there was a greater abundance of *amoA*-B under both treatments than in OPM-treated (126 Kg ha^{-1} N annually) soil. While NH_3 is produced from degradation of the organic matter in fresh manure, a large proportion of it might be lost by direct conversion into NO_3^- by heterotrophic nitrifiers or by volatilization (Maeda et al. 2011 and references within). In contrast, archaeal *amoA* (*amoA*-arch) genes were evenly distributed under all treatments, OPM, OPL, and IF, in grapevine soil (Pereg et al. 2018). In contrast to Leininger et al. (2006), Di et al. (2009), Hai et al. (2009), and Pereg et al. (2018) found a higher abundance (approx. tenfold) of archaeal than bacterial *amoA* in the soil. Hai et al. (2009) found an even distribution of AOA, but not AOB, in tropical sorghum soils treated with manure or straw with or without urea and, similar to Santoro et al. (2008), concluded that the AOA populations are more stable than AOB populations. AOB and AOA were present in lower numbers in soils treated with ammonium sulfate, compared to other soil treatments (Hallin et al. 2009). Interestingly, treatment with sewage sludge negatively impacted on the size of the AOA communities, but not on the AOB communities. The results of this study confirmed that fertilization regimes could affect not just the abundance of ammonia oxidizers but also the AOB/AOA ratio (He et al. 2007). However, it is not yet clear exactly which conditions favor the dominance of one or the other of the two types of ammonia oxidizers, bacterial and archaeal. Zhang et al. (2017) observed significant effects of pig manure as a fertilizer on nitrification activity and ammonia oxidizer communities. Although in general AOA were obviously more abundant than AOB (hundreds of times greater), it seemed that the AOB community was more sensitive to the shifts in fertilization or soil heterogeneity. This study also found that other factors, such as C- and N-related soil nutrients and enzyme activities, were important factors in shaping AOA and AOB community structures (Hallin et al. 2009). Taken together, these studies also suggest that variations in environmental conditions affect bacterial communities more than their archaeal counterparts. Archaea were found to oxidize ammonia and assimilate C in an agricultural soil, with different groups carrying out either heterotrophic activity or autotrophic CO_2 fixation (Pratscher et al. 2011). Such heterogenicity in ammonia-oxidizing microbes could possibly explain why bacterial and archaeal *amoA* abundance was independent of TOC in grapevine soils as observed by Pereg et al. (2018) and in soils collected from Australian cotton fields.

To summarize, while nitrification takes place in most soils, in soils that are acidic or nutrient-poor, AOA may be responsible for most nitrification, while AOB contribute to a greater extent in neutral and fertilized soils. As a consequence, there may be a delay between the application of fertilizers to nutrient-poor soils and an increase in nitrification, until the AOB populations have the time to adjust and increase. AOA and AOB also show different responses to inhibitors of nitrification. For example, the inhibitor 3,4-dimethylpyrazole phosphate (DMPP) reduces size of AOB communities, but does not impact AOA (Kleineidam et al. 2011); similarly, high levels of sulfadiazine residues in pig manure inhibit growth of AOB to a much greater extent than they inhibit AOA (Schauss et al. 2009). N fertilizers in access might turn into an ecosystem hazard, where nitrifying microbes exist and convert available N to nitrate. Excess of nitrate might leach into, and cause eutrophication of, aquifers, groundwater, lakes, and estuaries (Vitousek et al. 1997; Galloway et al. 2008).

3.3 Denitrification

Denitrification is a useful ability for soil bacteria, allowing anaerobic respiration. Many of the denitrifiers identified are facultative anaerobes, switching to denitrification when soil becomes waterlogged and when organic matter and useable N are readily available. Fungi that contain a mitochondrial gene similar to the bacterial-type nitrite reductase gene can also be involved in denitrification (Kim et al. 2009). Some factors, such as reduced oxygen availability, and changes in nitrate concentration, that influence denitrification have been well-established. The addition of manures and other organic fertilizers to soil tend to increase the activity of denitrifiers, relative to the use of inorganic fertilizers, but the impacts on the structure of microbial denitrifier communities are less clear (Hallin et al. 2009; Philippot et al. 2007). Thompson et al. (2016) showed that the abundance of denitrifiers changed differently than the total bacterial community of soils, suggesting that denitrifier populations are regulated differently from the total bacterial community. However, a long-term Swedish study comparing different fertilizer inputs found that overall denitrification activity was correlated with the total bacterial biomass, with crop yields, and with levels of the *nosZ* gene, which encodes the enzyme nitrous oxide reductase (Hallin et al. 2009). In any case, the abundance, distribution, and diversity of denitrifying microbes appear to be influenced by both soil conditions and management practices, and their activity can be assessed by reference to *nir* gene abundances (Clark et al. 2012; Hallin et al. 2009).

The impacts of inorganic fertilizers on denitrifying microbial communities are complex, due to the great diversity of denitrifiers themselves and the variation in abundance in different environments (Hirsch and Mauchline 2015). However, in general, it has been demonstrated that the use of organic fertilizers increases denitrification activity in soils when compared to inorganic fertilization (Philippot et al. 2007). The Cu-containing enzyme nitrite reductase *nirK* has been reported at higher levels in arable soils and in soils that have been treated with increased N fertilizer inputs (Philippot et al. 2007), while the alternative, *nirS*, has been found to be more abundant in intensively grazed pasture soils with increased soil ammonia levels (Dandie et al. 2011). A study which compared various fertilization managements, including unfertilized bare fallow, unfertilized plots with crop, and plots with crop fertilized with calcium nitrate, ammonium sulfate, solid cattle manure, or sewage sludge, found that the abundance of nitrate reducers (*narG*) and denitrifiers (*nirS*, *nosZ*) were significantly lower in soils treated with sewage sludge than in the soils treated with manure. The positive effects expected by the high organic inputs may have been counteracted by the lower pH in the plots treated with sludge (pH 4.7) compared to the manure-treated plots (pH 6.0) as well as by the higher heavy metal content (Hallin et al. 2009; Bergkvist et al. 2003). Overall, the abundance of denitrifiers was one to two orders of magnitude lower in soils treated with ammonium sulfate, compared with the other treatments. This may also be explained by the lower pH of the soil in these plots compared to the other treatments (Hallin et al. 2009). Other studies have also described the impact of fertilization strategy on denitrifying communities. In Spanish grapevine soils, the abundance of genes involved in denitrification (*nirK*, *nirS*, and *nosZ*) was found to be greater under organic than under inorganic fertilization (Pereg et al. 2018). On the other hand, Zhang et al. (2013) reported different responses of the various denitrification genes (*nirS*, *nirK*, and *nosZ*) to different soil treatments. Clark et al. (2012) reported an overall similar behavior of *nosZ*, *nirK*, and *nirS*, in soil from wheat fields, but found that *nirS* was tenfold less common than the other genes. In contrast, Pereg et al. (2018) found that *nosZ* was approximately fivefold less abundant than *nirS* in the Spanish grapevine soil. Hallin et al. (2009) found nirS/nirK ratio in unfertilized bare fallow soils to be three to ten times higher than in soil from plots with crops. Since the reduction of nitrite by denitrifiers can be performed either by the cytochrome cd1 nitrite reductase (NirS) or by the copper-containing nitrite reductase (NirK), as denitrifying bacteria possess only one form of the enzyme, these findings indicate the habitat created by the presence or absence of plants will select for either NirS- or NirK-type microbes. They suggested that NirS and NirK, although being functionally equivalent, are not ecologically redundant.

The gene *nirS* has been found to dominate over *nirK* in various natural environments, particularly in aquatic environments (Bothe et al. 2000; Nogales et al. 2002; Prieme et al. 2002; Liu et al. 2003; Throbäck et al. 2004; Oakley et al. 2007; Deslippe et al. 2014), as well as in cultured denitrifiers (Zumft 1997), and Thompson et al. (2016) raised a concern about unspecific *nirK* amplification products in PCR assays. Therefore, some researchers shifted their focus from studying both genes to studies on *nirS* alone (Morales et al. 2010; Thompson et al. 2016). Nevertheless, *nirK* is abundant in aerobic, oxygen-rich environments (Desnues et al. 2007; Knapp et al. 2009), and an analysis of the *nirK* PCR primers developed by Henry et al. (2004) and optimized by Pereg et al. (2018) confirmed their suitability for specific *nirK* amplification from soil DNA. Therefore, Pereg et al. (2018) recommended these primers for use in studies estimating the abundance of the denitrifying gene in soil DNA.

Similar to Hallin et al. (2009), Pereg et al. (2018) showed that different N fertilization usage could impact on the size of denitrifying microbial communities.

An organic fertilizer, consisting of manure and plant residues with a relatively high C:N ratio (approx. 80), and six times higher total amount of slow-release N than that of inorganic fertilizer, increased denitrifier abundance in grapevine soils. In another study, such a high C:N ratio was found to correlate with decreased N_2O emissions (Huang 2004). The final step in the N cycle is the reduction of nitrous oxide, releasing N_2 back to the atmosphere. This reaction is catalyzed by nitrous oxide reductase, encoded by the *nosZ* gene, a gene present in the genomes of less than 70% of known denitrifying microbes (Jones et al. 2008). Even when present the gene is not always expressed, resulting in accumulation of N_2O, an important greenhouse gas. It has been reported recently that some microbial species only contain a functional *nosZ* gene (Jones et al. 2013; Sanford et al. 2012), even though they may not possess the other genes involved in the denitrification pathway. Indeed, the abundance of the *nosZ* was higher in the grapevine soils using this organic fertilizer, when compared to inorganic fertilization regimes (Pereg et al. 2018), suggesting a higher abundance of denitrifiers with the ability to reduce N_2O to N_2 and the potential to lower N_2O emissions (Miller et al. 2008). Agricultural management practices that encourage the growth of microorganisms with this functional *nosZ* gene could be important and potentially allow for a reduction in greenhouse gas emissions under conditions which make denitrification inevitable (Hirsch and Mauchline 2015).

4 The Relationship Between Management Strategies and N-Cycling Gene Abundance

One of the fundamental differences between inorganic fertilization and organic matter supplementation is that the first boosts the soil with short-lived nitrate and ammonia in relatively high concentrations, while the latter depends on the slower breakdown of organic matter and thus provides a gradual supply of N and C to the soil. This gradual release of available N and C improves various soil properties (García-Orenes et al. 2016) and supports the maintenance of diazotrophic and denitrifying communities (Pereg et al. 2018). The components of each soil treatment can, therefore, have a significant influence on soil microbial communities. Hartmann et al. (2015) found that systems not receiving manure harbored more dispersed and functionally versatile microbial communities containing oligotrophic organisms that possibly adapted to nutrient-poor environments. On the other hand, systems treated with organic fertilizers harbored specific microbial guilds that degrade complex organic compounds, for example, compost and manure (Hartmann et al. 2015). In a study of Spanish grapevine soils, Pereg et al. (2018) found a significantly high correlation between abundance of *nifH* and TOC, *nosZ*, or *nirS*. The correlation of TOC with *nifH* was particularly high, possibly due to the relatively low available N concentrations at any time in the soil, due to the slow release of available N from organic matter. In contrast to the findings of Pereg et al. (2018) (for grapevine soil, Spain) and (for cotton in Australia), Morales et al. (2010) found a weak negative correlation between the abundances of the *nifH* and *nirS* genes. The *nirS* gene abundance was not correlated to organic C levels and did not exhibit the same trend as *nosZ* (Morales et al. 2010). Hai et al. (2009) also found that there was an increased abundance of *nifH*, but not of *nirK*/*S*, in tropical agricultural soils treated with manure, when compared to untreated soil or soil treated with straw. Overall, N fixation and denitrification are opposite reactions. However, some soil microbes are capable of both N fixation and denitrification. For example, various rhizobia species contain *nifH* and *nirK* (Bedmar et al. 2005), while some N-fixing strains of the bacterium *Azospirillum brasilense* contain both *nifH* and cd1-type *nir* gene (Danneberg et al. 1986). Therefore, it is not surprising that the abundance of *nifH* and *nirS*/*nirK*/*nosZ* was found to be similar in grapevine soils under various conditions as shown in Pereg et al. (2018). While nitrification is aerobic and denitrification anaerobic, these processes can take place in different micro-niches in soil aggregates and thus run simultaneously where both contribute to soil production of nitrous oxide (Stevens et al. 1997).

Soil with a neutral or slightly basic pH is considered to be optimal for most diazotrophs (Belnap 2001), and soil pH has been considered as a major factor influencing microbial community structure in various studies worldwide (Fierer and Jackson 2006; Noll and Wellinger 2008; Wakelin et al. 2008; Griffiths et al. 2011; Zhalnina et al. 2015). However, often in agricultural managed ecosystems, farmers often manage their soils to achieve particular pH and other soil properties. Therefore, it is not surprising that studies of agricultural soils, such as that carried out in Pago Casa Gran, have shown there was no significant difference in the pH of soil under organic or inorganic fertilization (García-Orenes et al. 2016), so this factor cannot explain the differences observed in N cycler abundance (Pereg et al. 2018). Similar results were observed in a study of Australian cotton soil indicating that factors other than pH are major determinants of N-cycling microbial communities. High NH_4^+ availability and moderate pH favor nitrification in soils, whereas pH close to neutral, high availability of nitrate (electron acceptor), and labile, energy-rich C (Barnard et al. 2005) favor denitrification. There is obviously a great diversity in soil microbial communities across different soils, under different environmental and geographical locations. Moreover, diversity could also reflect temporal factors and technical differences, which may compromise comparisons between studies. When considering N fertilization strategies, higher N fixation than denitrification would be beneficial in order to conserve the newly fixed N in the biosphere. Where *nifH* and *nir*/*nosZ* genes are both abundant, it is the conditions in the soil that would determine which genes will be fully expressed. Methods such as in situ enzymatic activity, proteomics, and possibly transcriptomics could be employed to assess which conditions in the soil niche would activate particular processes at any given time and allow this to be taken into account when selecting land management strategies.

5 Conclusions

Despite the demonstrated negative environmental impacts, modern agricultural practices, with high levels of agrochemical, mechanization, and modern high-yielding crop varieties, such as cereal production, have increased the productivity of agricultural land and allowed the earth to sustain an increasingly large human population. Since 1700 the global population has increased by at least tenfold. It is currently over seven billion and predicted to grow up to around nine billion people by the year 2050 (Hirsch and Mauchline 2015). This continual population growth places increasing pressure on land for food production. However, we also see aggravated climate change in response to increased industrialization. Food security and the need to feed a growing population must, therefore, be balanced against the negative environmental impacts of intensive crop production (Hirsch and Mauchline 2015). Utilizing N in agricultural systems in ways that consider and help manage microbial communities involved in the N cycle, notably using organic matter supplementation, will play an essential role in ensuring sustainability.

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