Chapter 12
Carbon Sequestration

Leland J. Cseke, Stan D. Wullschleger, Avinash Sreedasyam, Geetika Trivedi, Peter E. Larsen, and Frank R. Collart

Abstract With the rising levels of atmospheric carbon dioxide (CO_2) threatening to alter global climate, carbon sequestration in plants has been proposed as a possible moderator or solution to the problem. This chapter examines the different mechanisms through which carbon sequestration can take place within the Earth's natural carbon cycle with special focus on events surrounding plant development. Unfortunately, endeavors that have purposefully and successfully altered plant traits to improve carbon sequestration are currently quite few. Consequently, we delve deeply into the specific biological processes that allow plants to capture, allocate, and store CO_2 long term in the form of both above-ground and below-ground biomass. Distinctions are made between the differing molecular mechanisms of C_3, C_4, and CAM plants, and we point out the importance of mycorrhizal and other soil community level interactions as an important reminder that healthy soils are required for the uptake of nutrients needed for efficient carbon sequestration. We suggest that, due the complexity of the biological interactions, modeling approaches designed to network multiple types of data input may provide

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L.J. Cseke (✉)
Department of Biological Science, 302L Shelby Center for Science and Technology,
The University of Alabama in Huntsville, Huntsville, AL 35899, USA
e-mail: csekel@uah.edu

S.D. Wullschleger
Environmental Sciences Division, Climate Change Science Institute (CCSI), Oak Ridge National Laboratory, Building 2040, Room E212, MS 6301, Oak Ridge, TN 37831, USA
e-mail: wullschlegsd@ornl.gov

A. Sreedasyam • G. Trivedi
Department of Biological Science, 302J Shelby Center for Science and Technology,
The University of Alabama in Huntsville, Huntsville, AL 35899, USA
e-mail: as0005@uah.edu; gt0001@uah.edu

P.E. Larsen • F.R. Collart
Biosciences Division, Argonne National Lab, 9700 South Cass Ave., Lemont, IL 60439, USA
e-mail: plarsen@anl.gov; fcollart@anl.gov

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the best means for generating more useful hypotheses that can target specific traits for improved carbon sequestration.

12.1 Introduction

Carbon sequestration in the most general sense refers to the storage of elemental carbon within a material or reservoir. Examples include man-made processes where carbon dioxide (CO_2) is captured from flue gases at industrial plants and stored permanently in underground reservoirs, such as deep saline aquifers or depleted gas fields. Natural mineralization over long periods of time or during volcanic activity can sequester carbon. Carbon derived from organic matter can be trapped through the process of pyrolysis, the high-temperature degradation of organic material. This can generate biochar or charcoal, which can then be deposited into landfills or made into useful industrial products (Warnock et al. 2007). However, the most common example of carbon biosequestration occurs during the processes of photosynthesis in plants, where atmospheric CO_2 is captured (or fixed) with the help of water and light energy and stored in the form of sugar molecules that are used for subsequent metabolism and growth. Consequently, such carbon sequestration transfers CO_2 from the atmosphere to the leaves, stems, roots, and even surrounding soils of trees, plants, and crops, thus storing the carbon long term away from the atmosphere in the form of biomass.

The Earth has a natural carbon cycle. Carbon circulates in an endless cycle between the Earth's atmosphere, the oceans, the plants, and the soil primarily through the processes of photosynthesis and respiration of living organisms, although abiotic factors such as volcanoes, forest fires, and other forms of disturbance or land use also play a major role (Fig. 12.1). The major components or reservoirs of carbon include the oceans, phytoplankton, terrestrial vegetation and soils, and the Earth's atmosphere. The term "carbon sinks" is also used to refer these natural forms of carbon reservoirs when carbon sequestration is greater than carbon released over some time period. There is, however, only a fixed amount of carbon in Earth's system, and it is sequestered in and exchanged among the various sinks over time periods ranging from days to millennia, depending on the sink being considered. This carbon cycle helps to regulate the amount of CO_2 present in our atmosphere and is therefore a major component of the Earth's climate system.

12.1.1 Why Is Carbon Sequestration Important?

Carbon dioxide is a natural component of the atmosphere that helps reflect the Earth's infrared radiation back to its own surface. This reflection causes heat to be retained in an effect similar to that of a greenhouse, hence the reason that CO_2 is referred to as a greenhouse gas. While greenhouse gases are currently classified by

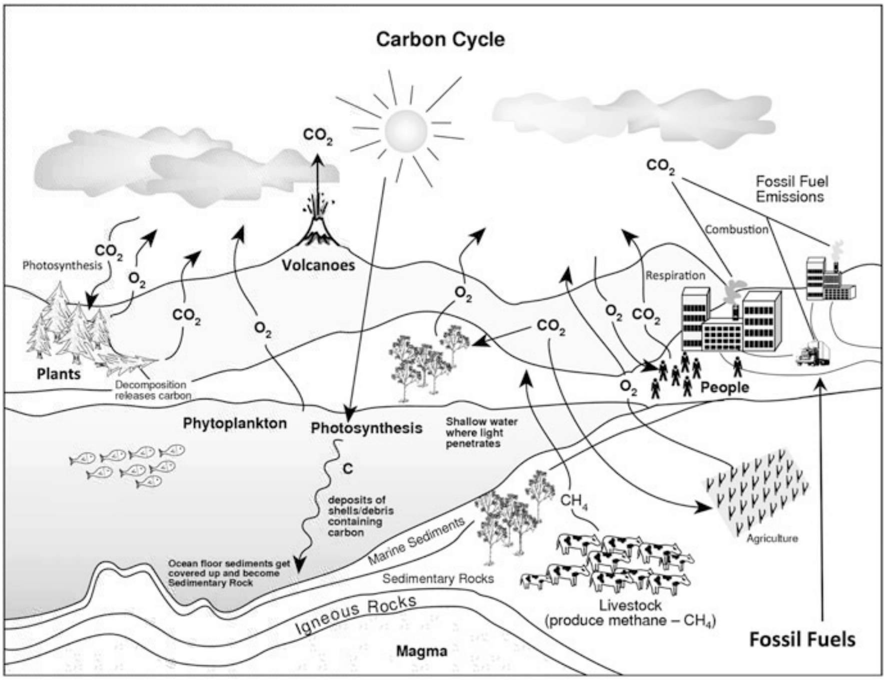

Fig. 12.1 An overview of factors that play an important role in the cycling of CO_2 in the Earth's atmosphere (modified from http://kids.earth.nasa.gov/guide/earth_glossary.pdf)

the US Environmental Protection Agency (EPA) as air pollutants (allowing regulation of emissions of such gases), the fact that CO_2 can act as a temperature buffer in our atmosphere has played a key role in the ability of life to develop and survive on this planet. Without CO_2, the planet would be inhospitable, with daily surface temperatures varying by hundreds of degrees. Because they capture the CO_2 that would otherwise rise up and trap heat in the atmosphere, trees and plants are important players in offsetting the effects of temperature fluctuation and global warming (Haynes 1995). In the process, plants and trees make use of the captured carbon to generate an abundance of products that humans have used throughout the recorded history. These products come in the form of food, shelter, clothing, wood for fire, tools for hunting, and more modern items like paper, pharmaceutical agents, and biofuels.

The balance of just the right amount of CO_2 in the atmosphere has been key to the stability of our climate. Over the millennium prior to the Industrial Revolution, atmospheric concentrations of CO_2 were relatively stable. This is because the major carbon fluxes between terrestrial vegetation, the atmosphere, and the oceans were generally in equilibrium. However, since the 1800s, the world's population has grown tremendously with a corresponding increase in the use of coal, oil, and natural gas. Average CO_2 concentrations in the atmosphere, which were approximately 280 parts per million (ppm) in preindustrial times, have risen to approximately 390 ppm in 2010 (Barnola et al. 1995; NRC 2010; Stott et al. 2000). Such an unprecedented rate of change has been attributed by many as a direct result of the corresponding increase in environmentally detrimental human activities.

The burning of fossil fuels and deforestation has introduced an additional flux into the natural carbon cycle (Fig. 12.1). Scientists have estimated that these two activities currently reintroduce almost eight billion metric tons of carbon to the atmosphere every year, and about 20 % of this is the result of land-use change such as tropical deforestation (IPCC Special Report on LULUCF 2000). Roughly half of these human-induced carbon emissions remain in the atmosphere for as long as a century or more, while the remainder is taken up in nearly equal portions by the oceans and land vegetation (http://www.epa.gov/sequestration/ccyle.html). As more fossil fuels are burned, the concentration of CO_2 in the atmosphere rises, and more heat is trapped through the greenhouse effect. Indeed, the average temperatures throughout much of the world have been increasing, and while many factors have an impact on the climate, most scientists agree that the current rise in average global temperatures is due to our extensive use of fossil fuels. Whether caused by humans or not, the reason that carbon sequestration is important is that it can help slow down atmospheric CO_2 buildup, thus tempering subsequent climactic changes.

12.1.2 Approaches to the Solution of Rising Carbon Dioxide Levels

Because CO_2 accumulates in the atmosphere before being removed by natural processes, slowing and ultimately reversing atmospheric CO_2 buildup will require deep reductions in CO_2 emissions. Environmentalists and scientists tend to agree that aggressive afforestation is the best natural means for minimizing the impact of rising CO_2 levels and their subsequent relation to global warming. Land management practices can be altered to sustainably increase live biomass and store more carbon. For example, sustainable forestry practices can increase the ability of forests to sequester atmospheric carbon while enhancing other ecosystem processes through the improvement of soil and water quality. Reforesting cleared or mined lands, conserving stands of large trees, and improving forest health through thinning and prescribed burning are just a few ways to increase forest carbon sequestration in the long run. Harvested trees can be used to produce long-lasting products, including high-quality building materials and furniture that can keep captured CO_2 from reentering the atmosphere via decomposition for many years.

Soil carbon sequestration is another potential tool for combating climate change because soils offer a large carbon sink. Plants facilitate the soil sequestration process by removing carbon dioxide from the atmosphere during photosynthesis and storing it as biomass. However, this biomass later decomposes to organic matter in the soil, and over many years that organic material can add up to a tremendous amount of stored carbon. It is estimated that the Earth's soils contain roughly three times as much carbon as all plant biomass, and researchers have suggested that, globally, soils have the potential to sequester ~24 % of the total emissions from fossil fuel combustion (approximately 1.9 GtC potential stored out of an 8 GtC emission). In addition, sequestration of carbon in the soil also promotes soil quality and health, which subsequently improves the health of the plants that contribute the biomass to begin with.

Carbon sequestration is unlikely to be a stand-alone solution to rising atmospheric carbon dioxide. Multiple complementary solutions will likely be needed to meet the challenge of stabilizing climate change, including more efficient energy use, alternative fuels, alternative transportation technology, electricity from non-CO_2-emitting sources, and improved agricultural and forestry practices combined with natural carbon sequestration. Much attention is also focused on the potential to develop plants and trees that have an enhanced ability to sequester carbon in the form of biomass. To appreciate how researchers are approaching the improvement of carbon sequestration, one must first understand the rather complex mechanisms that plants, including trees, use to capture and distribute carbon to their leaves, stems, roots, and surrounding soil. The remainder of this chapter focuses on these aspects, the traits that have been singled out to help improve carbon sequestration, and the novel approaches to the discovery of new traits involved in improved biomass production.

12.2 Photosynthesis and Carbon Capture

The recent rapid rise in atmospheric CO_2 will likely continue in the future and will affect climate and biogeochemical cycles. In today's world, most of the anthropogenic CO_2 emissions result from the combustion of fossil fuels for energy production. The increasing demand for energy, particularly in developing countries, underlies the projected increase in CO_2 emissions. Addressing this energy demand without increasing CO_2 emissions requires more than merely increasing the efficiency of energy production. Carbon sequestration, capturing and storing carbon emitted from the global energy system, will likely be a major tool for reducing atmospheric CO_2 emissions from fossil fuel usage and store it in the form of biomass. Photosynthesis has long been recognized as a means to sequester anthropogenic CO_2. It is the first in the sequence of reactions that constitute global carbon cycle. As such, photosynthesis provides the primary route for energy to enter into the global ecosystem, and carbon assimilation produces most of the global biomass.

Carbon sequestration occurs in crops, forests, and soils primarily through the natural process of photosynthesis. Atmospheric CO_2 is taken up through tiny openings in leaves, called stomata, and transferred to the chloroplasts where it is incorporated into sugars (primarily glucose, fructose, and sucrose) with oxygen produced as a byproduct. The carbon within the sugars is subsequently used along with other nutrients to generate the plant body (leaves, stems, roots, flower, and fruits), including the woody biomass of trees and leaves of promising bioenergy crops. Around 90 % of the dry weight of a given plant consists of carbon and oxygen obtained from the atmosphere via photosynthetic carbon assimilation within the leaves. A typical leaf is made up of an upper and lower epidermis, the mesophyll, the vascular bundles, and a varying number of stomata. The mesophyll cells have chloroplasts and are the prime site of photosynthesis. The upper and lower epidermal cells do not have chloroplasts and serve primarily as protection for the leaf. The stomata are pores that occur primarily in the lower epidermis and allow for gaseous exchange (i.e., CO_2, O_2, and H_2O) for photosynthesis and respiration. The vascular bundles or veins in a leaf are part of the plant's transport system, conducting water, nutrients, and photosynthetic products throughout the plant.

The process of carbon assimilation starts with a light-dependent reaction that converts solar energy into chemical energy. The energy of light captured by chlorophyll pigment molecules, present in thylakoid membranes of chloroplasts, is used to release high-energy electrons from molecules of H_2O. These electrons are used in a series of electron transfers to produce NADPH while at the same time generating a proton (H+) gradient across the thylakoid membranes. This electrochemical gradient is then used by ATP synthase to generate ATP. The NADPH and ATP formed by the light reactions enable the reduction of carbon dioxide through a universal pathway called the Calvin cycle, often termed the "dark reaction" due to the fact that it does not use photons of light. Not only does the Calvin cycle require ATP and NADPH generated in light reaction but many of the enzymes involved in carbon assimilation are active only in the light. The high energy conversion efficiency of the Calvin cycle (approximately 90 %) is derived from reactions that involve rearrangement of chemical energy rather than energy transduction. Two molecules of NADPH and three molecules of ATP are required to reduce each molecule of CO_2 to a sugar $[CH_2O]n$. The first step in this cycle is the addition of CO_2 to a five-carbon compound, ribulose 1,5-bisphosphate (RuBP). The six-carbon compound formed is split, giving two molecules of a three-carbon compound called 3-phosphoglycerate (PGA). Since the first stable organic compound formed contains three carbon atoms, these plants are called C_3 plants. C_3 plants include most temperate plants (excluding many grasses) and represent more than 95 % of all Earth's plants. Some of the most common examples of C_3 plants are wheat, rice, soybean, barley, tobacco, carrot, potato, tea, and coffee.

The process for assimilation of CO_2 and replenishment of RuBP in the Calvin cycle is mediated by 13 enzymes located in the chloroplast. Three of these enzymes, ribulose bisphosphate carboxylase/oxygenase (RuBisCO), sedoheptulose 1,7-bisphosphatase, and phosphoribulokinase, are unique to the Calvin cycle. RuBisCO is the key enzyme responsible for photosynthetic carbon assimilation catalyzing the carboxylation of RuBP to form two molecules of 3-PGA. As its name suggests, RuBisCO is a bifunctional enzyme capable of catalyzing two distinct reactions, acting both as a carboxylase and as an oxygenase. It uses the carboxylation substrate, RuBP, in a parallel reaction with O_2 to form one molecule of 3-PGA and a two-carbon product, 2-phosphoglycolate, which is of no immediate use in the Calvin cycle. Interestingly, this reaction is catalyzed on the same active site as carboxylase reaction; hence, CO_2 and O_2 are competitive substrates; O_2 inhibits carboxylase reaction and CO_2 inhibits oxygenase reaction. Each time RuBisCO catalyzes reaction of RuBP with O_2 instead of CO_2, the plant makes 50 % less 3-PGA, thus reducing the net gain in photosynthetic carbon. Not only does RuBisCO lack specificity for CO_2, but it has very low specific activity and for this reason is produced in massive quantity in leaf. It has been estimated that RuBisCO constitutes up to half of the soluble protein in the plant leaf (Ellis 1979). In C_3 plants almost 25 % of the nitrogen is invested in RuBisCO alone, which accounts for the considerable interest in the relationship of this enzyme to the nitrogen nutrition of plants (Leegood et al. 2000).

In the oxygenase reaction of RuBisCO, the 2-phosphoglycolate product enters the photorespiratory pathway, which eventually returns some 3-PGA to the Calvin cycle. In converting 2-phosphoglycolate back to 3-PGA, there is loss of carbon as CO_2 and consumption of ATP, but this pathway provides a mechanism to restore carbon to the Calvin cycle that would otherwise have been lost. Through photorespiratory pathway as much as 75 % of the carbon initially lost from the Calvin cycle is thus returned. Although there is inefficient use of carbon, energy, and reductant in photorespiration, the enzymatic pathway has been maintained throughout evolution. One of the most compelling arguments for this retention is the advantage of its scavenging role, i.e., returning carbon to the Calvin cycle that would otherwise have been lost. It has also been suggested that photorespiration helps plants to withstand environmental stress.

Some plants like corn, sugarcane, and many other tropical grasses have a special mechanism to overcome the tendency of RuBisCO to wastefully fix O_2 rather than CO_2 by photorespiration. These plants use a supplementary method of CO_2 uptake in which a 4-carbon compound, oxaloacetate (OAA), is formed in place of one of the 3-carbon compound of the Calvin cycle. Therefore, these plants are called C_4 plants. These plants initially bind CO_2 using an enzyme called phosphoenolpyruvate carboxylase (PEP carboxylase). This helps in a more efficient harvest of CO_2, allowing the plant to capture sufficient CO_2 without opening its stomates too often. C_4 plants utilize their specific leaf anatomy (kranz anatomy), where mesophyll and bundle sheath cells cooperate to fix CO_2. In C_4 photosynthesis, CO_2 is first incorporated into a 3-carbon compound, phosphoenolpyruvate (PEP), by PEP carboxylase, forming OAA in mesophyll cells which is then pumped to the bundle sheath cells. There, it releases the CO_2 for carbon fixation by RuBisCO. The decarboxylation reaction also produces three-carbon organic acids (C_3) that return to the mesophyll cells and regenerate as PEP in a reaction catalyzed by the enzyme pyruvate orthophosphate dikinase (PPDK). By concentrating CO_2 in the bundle sheath cells, C_4 plants promote the efficient operation of the Calvin cycle and minimize photorespiration. This CO_2 concentrating mechanism makes C_4 plants more nitrogen efficient such that RuBisCO constitutes only 10–15 % of leaf nitrogen (Sage et al. 1987). However, the C_4 photosynthetic pathway requires two more moles of ATP than C_3 pathway per mole of CO_2. This additional ATP requirement means that the C_4 pathway needs more light energy than the C_3 pathway for the assimilation of same quantity of CO_2. Therefore, most of C_4 plants are native to the tropics and warm temperate zones with high temperature and light intensity. They exhibit higher photosynthetic and growth rates under these conditions because of the efficient use of carbon, water, and nitrogen. C_4 plants are among some of the world's most productive crops and pasture, for example, maize (*Zea mays*), sugar cane (*Saccharum officinarum*), sorghum (*Sorghum bicolor*), amaranth (*Amaranthus* spp.), bermuda grass (*Cynodon dactylon*), blue grama (*Bouteloua gracilis*), and rhodes grass (*Chloris gayana*) are C_4 plants. Also some of the most dangerous and damaging weeds like crabgrass, nut grass, and barnyard are also C_4 species. Although C_4 plants represent only a small fraction of the world's plant species, which is only 3 % of the vascular plants, they contribute about 20 % to the global primary productivity because of highly productive C_4 grasslands (Ehleringer et al. 1997).

Other than C_3 and C_4 photosynthetic pathways, there is yet another strategy used by plants in arid regions to cope with extreme hot and dry environments like desert. Plants like cacti and pineapple that live in extremely hot, dry areas can only safely open their stomates at night when the weather is cool to avoid dehydration. These plants open their stomates at night to take in $CO_{2,}$ which is then incorporated into various organic compounds and stored in vacuoles. In the daytime, when the light reaction occurs and ATP is available, these plants extract CO_2 from the organic compounds for use in the Calvin cycle. These "CAM" plants (named for crassulacean acid metabolism after the plant family Crassulaceae where this process was first discovered) also initially attach CO_2 to PEP and form OAA similar to the process used in C_4 plants. However, CAM plants fix carbon at night and store the OAA in large vacuoles within the cell instead of fixing carbon during the day and pumping the OAA to other cells. In CAM photosynthesis there is temporal separation of two carboxylating enzymes, PEP carboxylase and RuBisCO. This allows CAM plants to avoid risk of dehydration and use the CO_2 for the Calvin cycle during the day, when it can be driven by the solar energy. Although primarily a means of surviving in arid conditions, CAM also enables plant to photosynthesize at low CO_2 because, like the C_4 pathway, it works by concentrating CO_2 for RuBisCO to assimilate into the Calvin cycle. There are many more species of CAM relative to C_4 species; however, only two CAM plants are of commercial importance, the pineapple (*Ananas comosus*) and the cactus (*Agave tequilana*). CAM plants are slow growing compared to C_4 and C_3 plants which have higher growth rates in their natural environment. The extra ATP requirement for CAM photosynthesis explains to some extent the slow growth rates relative to C_3 and C_4 plants. Also CAM plants undergo CAM idling, i.e., closing of stomata both day and night, which leads to complete retardation of growth in very arid environments. CAM species have not been exploited for carbon sequestration because of the diffusive (stomatal plus internal) constraints imposed by succulent CAM tissues on CO_2 supply to the cellular sites of carbon assimilation. However, areas of current and future research include elucidating the causes and consequences of CAM and providing a knowledge base that might inform and improve the potential of CAM plants for carbon sequestration and bioenergy production on marginal and degraded lands.

Overall carbon assimilation by C_3, C_4, and CAM photosynthesis each has distinct advantages and disadvantages in particular environment. C_3 plants are more abundant in temperate climates, C_4 plants in tropical climates, and CAM plants in arid regions with the distribution strongly influenced by water, nutrient, and light requirements. C_4 and CAM plants are more nitrogen and water efficient than C_3 plants. The CO_2 concentrating mechanisms of the C_4 and CAM pathways enable RuBisCO to function more efficiently than in C_3 plants, and therefore, these plants produce reduced amounts of RuBisCO protein relative to C_3 plants. C_4 plants also have the tendency to compete with C_3 plants in nitrogen-poor soils, where production of RuBisCO protein by leaves is limited by nitrogen availability. The C_4 and CAM pathways require more light energy than C_3 photosynthesis because of differences in ATP requirement due to additional reactions of the C_4 and CAM pathways. This gives C_4 and CAM plant an advantage in bright light and in warm temperatures.

12.2.1 Poplar as an Example of C_3 Plants

Poplar species, such as trembling aspen (*Populus tremuloides*), are the most widespread tree species throughout the world and have an important role in many different ecosystems. Poplar species have commercial utility for the production of wood products partly due to their ability to regenerate from their roots after cutting (Frey et al. 2003). Recent interest in terrestrial carbon sequestration is motivating efforts to explore opportunities for climate change mitigation and future energy resources. Since forests make a significant part of the global carbon cycle constant, efforts are being made to increase the sequestration of carbon in forests. Trees, through their growth process, act as a sink for atmospheric carbon. *Populus* (C_3 species) has emerged as a model tree system among all forest trees, which together comprise the largest fraction of the living terrestrial carbon reservoir (Dixon et al. 1994). The availability of extensive genetic resources like breeding populations, genetic maps, large expressed sequence tag (EST), and bacterial artificial chromosome (BAC) libraries and ease of transformation uniquely provide molecular tools and approaches to understand the mechanisms that control carbon allocation and partitioning (Cseke et al. 2007; Cheng and Tuskan 2009). Several studies on *Populus* have demonstrated that there is significant variation in biomass distribution among different clones and there exists a genetic basis for above-ground and below-ground dry mass distribution (Pregitzer et al. 1990; Heilman et al. 1994; Scarascia-Mugnozza et al. 1997; Dickson et al. 1998; Karim and Hawkins 1999; Cseke et al. 2009). Progeny from crosses within and between species has also shown considerable variation for the above- and below-ground distribution of biomass and tissue chemistry (Driebe et al. 2000; Schweitzer et al. 2004; Block et al. 2006; Fischer et al. 2006). Genomics and transcriptomics approaches are being used to identify potential genes that provide key control points for the flow and chemical transformations of carbon in roots. Particular focus is on genes that increase sink activity (greater root mass) and are involved in the synthesis of chemical forms of carbon that result in slower turnover rates of soil organic matter. Quantitative trait loci (QTL) analysis approach has been used to identify traits associated with soil carbon sequestration like productivity, biomass distribution to roots, and fine root/coarse root ratios and regions of the genome linked to these traits (Wullschleger et al. 2005). Current strategies are directed to modification of genes that have been shown to play significant role in carbon partitioning, including the invertase family, which controls sucrose catabolism. Past studies have indicated that sink strength has a dominant influence on source photosynthesis and carbon partitioning (Paul et al. 2001; McCormick et al. 2006). Sink strength is essentially regulated by sucrose metabolism channeling carbon into storage or structural components. Metabolic engineering has been employed to target the activity of enzymes of sucrose metabolism like sucrose synthase, invertase, and ADP glucose pyrophosphorylase to increase sink strength (Capell and Christou 2004; Roitsch and Gonzalez 2004; Bieniawska et al. 2007; Coleman et al. 2007; Smidansky et al. 2007). Future studies are likely to focus on employing combinations of advanced breeding techniques and targeted genetic manipulations to select or develop hybrid poplars with traits favoring the enhanced carbon sequestration and storage capacity in long-lived soil pools.

12.2.2 Miscanthus *as an Example of C_4 Grasses*

Perennial C_4 grasses have highly efficient C_4 photosynthesis that often yields more biomass than C_3 species. These grasses are promising candidates as energy crops as they have the potential for increased soil carbon sequestration. In addition, they have a low demand for nutrient inputs and higher biomass yields on relatively poor-quality land. An advantage of perennial C_4 grasses compared with trees is that they can be established more quickly and produce an annual harvest with low moisture content. Perennial crops accumulate and sequester carbon in the soil as well as produce combustible material that substitutes for fossil fuels. Different perennial grasses differ in their potential productivity, chemical and physical biomass properties, environmental demands, and crop management requirements (Lewandowski et al. 2003). Among the candidate perennial C_4 grasses, one of the most intensively investigated potential new energy crops is *Miscanthus* species native to subtropical and tropical regions of Asia. The rapid growth, low mineral content, and high biomass yield of *Miscanthus* make it one of the most preferred choices for bioenergy production. *M. sacchariflorus*, *M. sinensis*, *M. floridulus*, and *M. giganteus* have caught particular interest in recent years for biomass production (Deuter 2000). *M. giganteus* (Giant Miscanthus), the triploid hybrid species, shows superior characteristics, such as high biomass yield, and has been considered as the most promising *Miscanthus* species for bioenergy production (Lewandowski et al.

2000; Pyter et al. 2007). In the United States, Giant Miscanthus has been proposed for use in combined heat and power generation and it is also a leading candidate feedstock for the production of cellulosic ethanol (Heaton et al. 2004; Khanna et al. 2008). The occurrence of this hybrid shows promise for interspecific hybridization within *Miscanthus* because the genus has substantial genetic diversity within and between species.

Most research in the past decade was focused on enhancing the productivity and economic potential of *Miscanthus* species (Jones and Walsh 2001; Khanna et al. 2008). Essential to this process is the development of efficient tissue culture methods and transformation tools that will allow introduction of desirable traits into this. Although the biotechnological approaches for molecular breeding of these plants are still in immature stages, a number of important genetic resources such as genetic maps, cross-species markers, molecular markers, and physical and comparative maps have been developed (Jiang et al. 2012; Kim et al. 2012; Swaminathan et al. 2012). Recently, studies involving micropropagation and plant regeneration from embryogenic callus of *Miscanthus sinensis* have been reported (Głowacka and Jeżowski 2009a, b; Głowacka et al. 2010; Zhang et al. 2012). Progress has been made in the development of transformation techniques; particle bombardment and *Agrobacterium*-mediated transformation have been established using embryogenic callus of *M. sinensis* (Wang et al. 2011). Transgenic approach is being used to target genes for value-added traits, such as enhanced biomass and fermentation efficiency. The target traits that are important and will make large impacts in the molecular breeding include herbicide resistance, biotic and abiotic tolerance, low-fertilizer needs, high-efficiency photosynthesis for high productivity, promoted and synchronized flowering for hybridization, and organellar transformation for the effective accumulation of high-value chemicals and proteins.

12.3 Allocation of Carbon to Leaves, Stems, and Roots

Perhaps the best-known commodity produced by a tree's vascular system is "wood"—a valuable, renewable resource for lumber, paper, and energy production. What we call "wood" is actually a complex vascular tissue, formed by many cell types organized within an extracellular matrix composed primarily of cellulose and lignin (Plomion et al. 2001; Li et al. 2003). The secondary thickening that results from an active vascular cambium in stems and roots increases the girth of the plant and is especially important to the production of biomass and the ability of trees to store water and to transport carbon and nutrients. The vascular system of all terrestrial plants is key to assimilating the carbon that is fixed during photosynthesis because it provides a mechanism for carbon-laden sugars to move throughout the plant body to the location of growth and development. This makes the understanding of above- and below-ground secondary development one of the more important aspects of developing tree-based technologies to sequester CO_2.

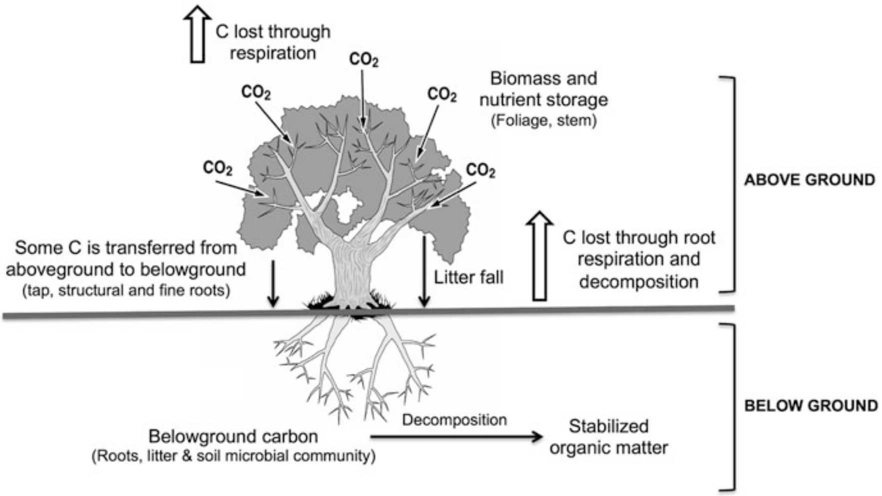

Fig. 12.2 Carbon sequestration—above- and below- ground. *Arrows* pointed *downward* indicate carbon capture. *Arrows* pointed *upward* indicate emissions of CO_2 into the atmosphere

12.3.1 Above-Ground Carbon Allocation

Carbon compounds produced through photosynthesis, along with minerals from the soil, are transported from "sources" (photosynthetically active leaves) to "sinks" to support plant growth, fruit development, and maintenance of the plant's permanent structure (branches, stems, and roots) (Fig. 12.2). In plants, sucrose is the end product of photosynthesis and is converted to a wide variety of storage compounds in different tissues such as seeds, shoots, roots, and woody parts. The process of storage of carbon in different parts of plant including reproductive sinks (fruit and seeds), temporary storage sinks (tubers), shoots, roots, and woody parts is termed as carbon allocation. The relative amount of carbon allocated in the various organs, which is also called as biomass allocation, is not fixed but may vary over time, across environments and among species. The question of how plants allocate carbon among different organs has long been a topic of ecological interest.

Fundamentally, the assimilation of carbon by leaves, and acquisition of mineral nutrients by roots, must be in balance with the utilization of carbon and mineral nutrients for plant growth. This functional balance at whole-plant level can be represented in terms of carbon produced by assimilation and carbon consumed in growth, enabling root–shoot responses to nutrients. In the end, the plant has to balance the carbon allocation to leaves, stems, roots, and other storage organs in a way that matches the physiological activities and functions performed by these organs. The source organ (leaves) maintain high concentration of carbon assimilates in the phloem at the points of loading. The transportation of carbon substrates at the points of loading (source) and unloading (sinks) occurs either by symplast or apoplast. The root, shoot, and cambial sink (utilization sinks) appear to possess symplastic connections to phloem, with no active phloem unloading. In this scenario substrate gradient is maintained between phloem and sinks cells, across plasmodesmata, by the utilization for growth and associated respiration. However, in reproductive sinks like fruit and seeds, carbon substrates are transported by apoplast from phloem to sink, possessing active phloem-unloading mechanism. This is the reason reproductive sinks have large sink strength compared to utilization sinks. In many crop plants this loading is through apoplast involving active transport, whereas in some woody plants less efficient symplastic loading mechanisms are utilized (Gamalei 1991; Gamalei et al. 1992). In past research focus has been on understanding the sink properties of seeds, fruits, and specialized storage organs that constitute yield of crop plants compared to shoot and cambial sinks.

Trees store a large amount of carbohydrates in the parenchymatous tissues of their wood and bark, mainly as starch. These stored carbohydrates play an important role in tree functioning; they can be used when current photosynthesis is not enough to meet the carbon needs for maintenance and growth. Carbohydrate storage in tree wood parenchyma has been considered as only a passive accumulation process, but this view is being challenged recently. It has been demonstrated that an increased C demand does not necessarily result in a depletion of carbohydrate concentration in wood (Silpi et al. 2007). It has been suggested that trees tend to adapt the level of stored carbohydrate to current metabolic demand, at the possible expense of other sinks (Silpi et al. 2007). In order to better understand carbon allocation among functional sinks—growth and secondary metabolites—long-term studies that enable the comparison of contrasting levels of assimilate availability are required.

Forest C allocation has drawn particular interest due to its responsiveness and potential effect on carbon sequestration and the global carbon balance. The differences in lifespan and decomposition rates among tree organs suggest that C allocation in trees strongly influences forest carbon cycling rates. Owing to the importance of forest C allocation, a number of contrasting approaches exist to model forest C allocation. There are five main categories of approaches to allocation modeling that have been identified, based on the key principles used to predict allocation: empirical, allometric scaling, functional balance, evolution based, and entropy based (Purves and Pacala 2008; Ostle et al. 2009; Ise et al. 2010; Franklin et al. 2012). Although there are guidelines for identifying approaches that are appropriate to predict allocation for different purposes, more research is required to further increase an understanding of allocation and how it can best be modeled.

12.3.2 Below-Ground Carbon Allocation

Soils are the primary carbon repository for three-fourths of the terrestrial carbon with 4.5 times more than the biotic pool (Lal 2004). The total quantity of below-ground carbon allocated by plants is enormous and is in the form of living plant roots and root exudates. Below-ground carbon allocation (BCA) allows flow of organic carbon to the soil from photosynthetically fixed CO_2 and makes a significant impact on the global carbon cycle (Fig. 12.2). It is estimated that terrestrial plants allocate nearly 60 Pg (petagram) of carbon below-ground out of the 120 Pg fixed through photosynthesis (Schimel et al. 1994; Grace and Rayment 2000). The large amount of BCA is essential for plants to obtain mineral nutrients and water in resource-limited terrestrial ecosystems. Furthermore, BCA regulates soil organic matter formation and influences bulk density, water-holding capacity, and cation-exchange capacity of soil. Overall, BCA is profoundly important to ecosystem carbon budgets playing a dominant role in whole ecosystem carbon exchange (Valentini et al. 2000; Giardina et al. 2005). Although the relevance of BCA to the functioning of forest ecosystems and global carbon budget is far recognized, controls on BCA are not completely coherent (Ryan et al. 1996; Giardina and Ryan 2002). The above-ground plant carbon allocation is, however, precisely captured in leaf-based physiological process models (Landsberg and Gower 1997), whereas below-ground processes are poorly captured in process models. The complexity of the global changes in environmental factors and above-ground and below-ground interactions compounded by the soil matrix further hinders efforts to validate below-ground models. Often the ecosystem models describing BCA response to global environmental changes rely on the assumption that the functioning and dynamics of above-ground processes sufficiently describe those of below-ground processes (Binkley and Menyailo 2005).

12.3.3 How CO_2 Is Assimilated into Roots, Soil, and Soil Communities

Perennial and herbaceous plants capture atmospheric CO_2 through photosynthesis and store large amounts of organic carbon in above-ground structures followed by its translocation below-ground into plant roots (Hinsinger et al. 2009). Soil carbon is also accumulated through deposition of canopy litter and via rhizodeposition. The flow of organic matter from roots into the soil, which includes shedding of root cap and cortical cells, fine roots formation, and root exudation of simple sugars, polysaccharides, organic acids, amino acids, and proteins, is called rhizodeposition (Pritchard 2011). The rooting process itself, however, is the primary means for most carbon entering below-ground soil carbon pool that act as a means for substantial long-term carbon sequestration. Overall, the root turnover, a metabolically expensive process, accounts for about 30 % of global terrestrial net plant productivity (NPP) (Jackson et al. 1997), while fine root exudates comprise extra 0.5–20 % of net ecosystem C assimilation (Farrar et al. 2003; Frank and Groffman 2009; Pritchard 2011). It has been predicted that plants with deeper roots contribute more towards increase in the global carbon sequestration, as loss of carbon due to microbial decomposition is mainly high in the upper soil strata (Pritchard 2011).

Good examples are perennial grasses such as *Miscanthus* and switchgrass that exhibit increased (5–25 %) soil organic carbon deposition through carbon sequestration in deep roots and reduced net CO_2 emissions (Anderson-Teixeira et al. 2009). In temperate and boreal forests, soil carbon accounts for four times more carbon stored in vegetation, and it is almost 33 % higher than the total carbon sequestered in tropical forests. On the other hand in grasslands, 98 % of total sequestered carbon is stored below-ground, which in total adds to 8 % of global soil carbon (Lal 2004; Jansson et al. 2010).

The coevolution of plant roots along with soil inhabitants like fungi and micro- and macrofauna has resulted in the plant carbon allocation strategies, root physiological behaviors, and root structural properties (Johnston et al. 2004). Association of plant roots with soil microbes like mycorrhizal fungi increases total plant carbohydrate budget by 10–20 % to support and maintain these fungal symbiotic partners (Johnson and Gehring 2007) in return for mineral nutrients and water. The plant organic carbon transfer to extraradical mycelia is considered a vital feature of soil carbon processes as fungal mycelia constitutes 20–30 % of soil microbial biomass and it is estimated to be 15 % of the total soil carbon in certain ecosystems (Leake et al. 2004). And the soil carbon transfer in the form of root exudates stimulates soil food web, resulting in greater microbial biomass and activity, which in turn increases soil organic nitrogen turnover C (Carney et al. 2007). Therefore, the association of fine roots with mycorrhizal symbionts and the necessity of plants to feed the soil community for improved nitrogen mineralization are responsible for significant amount of organic carbon deposition into the soil.

The impact of rapidly changing climate, particularly increase in the atmospheric CO_2 concentration, on ecosystem carbon cycling and below-ground carbon deposition has gained much attention in the recent past. Plants exposed to increased CO_2 concentrations respond with a significant increase in photosynthesis and growth. Often, carbon allocation was high in below-ground processes as compared to that of the above-ground processes. This uneven distribution of carbon contributes to increased root growth as well as the root-to-shoot ratio (Rogers et al. 1996; Pritchard and Rogers 2000). A substantial increase in the root production in grasses (Rillig and Allen 1999; Milchunas et al. 2005), coniferous trees (Prichard et al. 2008), and field crops (Wechsung et al. 1995; Pritchard et al. 2006) was demonstrated under elevated CO_2 concentration. However, the reports of decreased or unchanged below-ground processes such as root production in CO_2-enriched environments also exist but are not common (Arnone et al. 2000; Johnson et al. 2006; Brown et al. 2007). Furthermore, thorough understanding of the consequences of global climatic change on the functioning of ecosystem also requires consideration of relationship between plants and their below-ground microbial communities (Bardgett and Wardle 2010). Drigo et al. (2008) reported that under elevated CO_2 atmospheres along with the changes in root developmental patterns, plants released more organic carbon compounds into the rhizosphere, the portion of soil where microorganism-mediated processes are under the influence of the root system stimulating greater microbial biomass (Carney et al. 2007). And an increase in the CO_2 concentration by double the ambient level exhibited a 47 %

increase in the mycorrhizal abundance (Treseder 2004). In a different study, 34 % increase in the mass of ectomycorrhizal fungi and that of arbuscular mycorrhizal fungi showed an increase of 21 % in CO_2-enriched environments (Alberton et al. 2005), demonstrating that the effects of atmospheric CO_2 enrichment on mycorrhizal fungi are profound. Therefore, an increase in the atmospheric CO_2 concentration has considerable positive impact on the downward flow of carbon and thus its influence on soil food webs, i.e., the relationship between plant roots and other soil inhabitants (Drigo et al. 2010). Root carbon transfer to the soil consequently controls the impact of climate change on carbon cycling and climate mitigation by reducing atmospheric CO_2. Studies designed to explore approaches to increase plant productivity and biomass distribution to roots and soil communities that contribute to carbon cycle processes under field environments could help in understanding of mechanisms to enhance soil carbon sequestration.

12.4 The Benefits of Mycorrhizal Interactions and Soil Communities

Plants constantly interact with a wide range of microbes in their environments. The beneficial microorganisms contribute to plant health by secreting plant hormones like auxins and cytokinins that positively affect plant growth and increase the availability of nitrogen, phosphorus, and other nutrients and provide protection against disease-causing microorganisms (Pritchard 2011). The majority of plants from terrestrial ecosystems form the mutually beneficial, long-term, symbiotic association between their roots and fungi to develop into functional structures called mycorrhizae. The functional basis of this relationship is the reciprocal transfer of nutrients and minerals, N and P, from the fungus to the plant and plant-derived carbohydrates to the fungus (Smith and Read 1997) (Fig. 12.3). Mycorrhizae are broadly categorized into two types, ectomycorrhizae (ECM) and arbuscular mycorrhizae (AM), particularly relying on the pattern of fungal colonization in plant roots (Bonfante and Genre 2010). AM fungi are widespread obligate biotrophs predominant in herbaceous plants. They form specialized organs called arbuscules within plant cells that aid in nutrient exchange with plant partners (Ferrol et al. 2002). Ectomycorrhizal fungi form symbiotic relationships with almost 90 % of the major forest trees belonging to temperate, boreal, and montane regions; thus, they dominate and can be said to have shaped the world's forests (Martin et al. 2008; Podila et al. 2009). The ECM fungal partner grows as a thick cover, termed the mantle, around the fine roots of the plant and can comprise up to 40 % of the colonized root biomass (Johansson et al. 2004). The ECM association increases the plant's fitness in many ways, beyond increasing the mineral N and P nutrition. It is known to increase the plant's water uptake efficiency and the plant's tolerance of various abiotic stresses, including salt, drought, and nutritional stress, allowing the plant to endure harsh climatic conditions (Larsen et al. 2011a). They

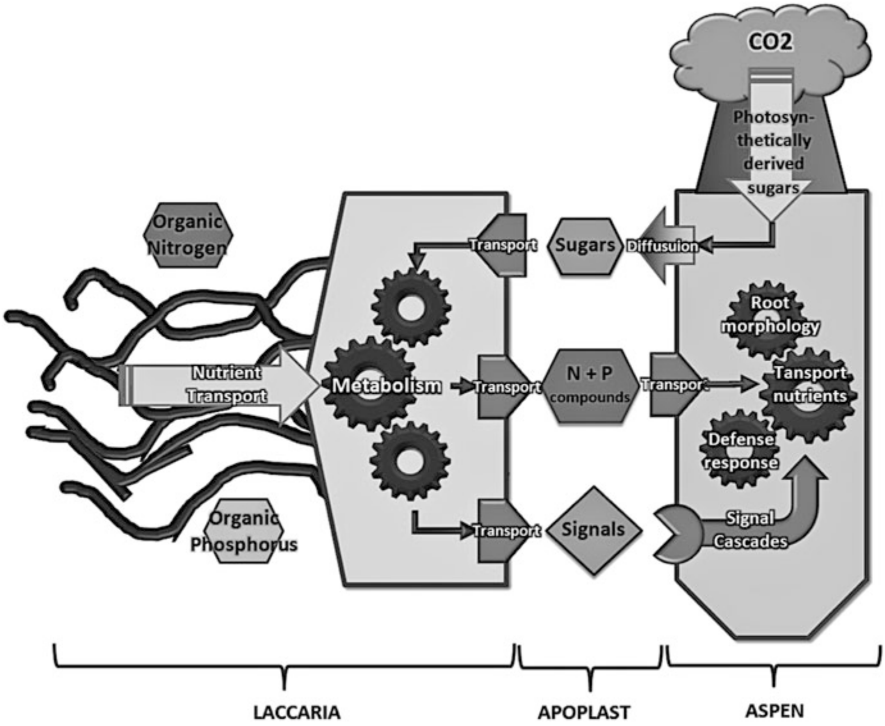

Fig. 12.3 Schematic depicting the transport of nutrients between interacting soil mycorrhizal fungi (such as *Laccaria bicolor*) and tree roots (such as those from *Populus tremuloides* or quaking aspen)

protect trees against heavy metal toxicity by reducing the translocation of heavy metals to the host (Shetty et al. 1994; Jentschke and Godbold 2000; Schützendübel and Polle 2002; Langenfeld-Heyser et al. 2007). ECM symbiosis, thus, improves overall tree health and acts as the key component for the stability of forest ecosystems that positively impacts the global environment. Additionally, the fungal mycelium associated with root tips forms carbohydrate sinks for the tree, and up to 18 times more photosynthates are relocated to the mycorrhizal structures relative to non-mycorrhizal roots, thus contributing to carbon sequestration (Cairney et al. 1989; Smith and Read 1997). Mycorrhizal fungi transform plant-derived carbon into trehalose. Several studies have indicated the possible role of trehalose in fungal interaction with certain bacteria (Izumi et al. 2006; Uroz et al. 2007). Other microbes such as rhizobia, phosphorus-solubilizing bacteria, and free-living N_2-fixing organisms are considered to exert strong influence on the plant physiology and growth, hence the total ecosystem productivity.

12.4.1 The Importance of Soil Nutrients to Carbon Sequestration

Plant growth and productivity greatly depends upon the availability of water, accessible mineral nutrients, temperature, and light intensity. An imbalance of mineral nutrients in the soil leads to nutritional stress, which compromises overall plant growth. Nevertheless, plants sense soil nutrient limitations and acclimate to available nutrients. They do that primarily by diverting their carbohydrate resources and altering patterns of carbon allocation and partitioning among different organs, allowing optimal growth in a specific soil type (Marschner 1995; Aerts and Chapin 2000; Glynn et al. 2007). According to optimal allocation theory (OA), in nutrient-rich soils plants direct greater proportion of biomass towards above-ground tissues as mainly light limits the growth processes (Poorter and Nagel 2000). On the contrary, OA predicts that plants allocate more biomass to roots in nutrient-limiting soils in order to acquire scarce minerals (Ibrahim et al. 1997; Shipley and Meziane 2002). Nitrogen and phosphorus are major essential plant nutrients needed in large quantities, and their deficiency limits plant growth significantly. Amending the soil with fertilizers is routinely used to provide these essential minerals to enhance plant growth. Though plants gain from fertilization in severe nutrient-limiting soil types, excessive fertilization can have negative influence on plant health and physiology (Herms and Mattson 1997). Fertilization can alter patterns of carbohydrate partitioning in roots and reduce the content and concentration of nonstructural carbohydrates (e.g., simple sugars, starch) and defense-related secondary metabolites resulting in predisposing seedlings to injury from drought and pathogens (Bennett and Wallsgrove 1994; Pearce 1996; Kleczewski et al. 2010, 2012), having significant impact on plant growth and therefore carbon sequestration.

12.4.2 Dependence of Plant Carbon Sequestration on the Access to Nutrients Such as Nitrogen and Phosphorus

Nitrogen is present in various forms, including ammonium, nitrate, amino acids, peptides, and other complex insoluble nitrogen compounds in the soil. Though nitrate and ammonium are preferred nitrogen sources for plants, they can also uptake nitrogen in the form of amino acids that are available in abundance in certain soils, but the complex organic nitrogen compounds are not accessible (Williams and Miller 2001). Low soil nitrogen availability limits leaf nitrogen concentration, which in turn affect carbon assimilation and plant growth (Field and Mooney 1986). Many studies have suggested that carbon sequestration increases with the increasing nitrogen supply (King et al. 2005; Magnani et al. 2007; LeBauer and Treseder 2008; Xia and Wan 2008). These conclusions were, however, based on the observed changes in above-ground biomass production that do not always reflect the below-ground carbon deposition (Giardina et al. 2004; Lichter et al. 2005). Nitrogen enrichment can alter carbon allocation to below-ground processes. Specifically, high input of nitrogen increases fine root mortality while decreasing the fine root production and longevity (Gower et al. 1992; Haynes and Gower 1995). In contrast, addition of nitrogen-free fertilizer was reported to extend the fine root longevity (Majdi and Kangas 1997). Furthermore, addition of nitrogen also alters microbial community composition and reduces microbial activity significantly (Treseder 2004, 2008).

Likewise, low phosphorus availability is also a major limiting factor for plant growth (Lynch and Deikman 1998). Plants grown under phosphorus deficiency exhibit retarded growth and their roots are thinner with higher specific root length leading to increased root-to-shoot ratio (Bougher et al. 1990; Nielsen et al. 1998). An increase in length and density of root hairs is associated with adaptation to phosphorus deficiency, which potentially contributes to phosphorus acquisition (Gahoonia et al. 1999; Bates and Lynch 2000). The observed increase in carbon allocation to below-ground structures under limiting phosphorus conditions is attributed to decreased plant productivity (Qiu and Israel 1992). Consequently, increased carbon allocation to roots is a primary constraint to total carbon sequestration under phosphorus-limiting conditions. Plants with better phosphorus uptake efficiency or with greater ability to utilize acquired phosphorus, therefore, perform better under low phosphorus availability (Nielsen et al. 1998). Mack et al. (2004) showed that in a natural ecosystem, large declines in total soil organic carbon occur with experimental addition of nitrogen when phosphorus was added simultaneously. Studies also indicated that changes in soil phosphorus availability could alter ecosystem responses to nitrogen deposition and deposition of phosphorus alone could also potentially modify soil organic carbon dynamics (Wassen et al. 2005; Cleveland and Townsend 2006). Additionally, Bradford et al. (2008) suggest that not only the availability of nitrogen and phosphorus influences soil organic carbon sink strengths but also the rate of their deposition will be the critical determinant of whether these macronutrients increase or decrease the long-term sequestration of plant carbon inputs to soils. Therefore, an emphasis on studies that assess responses to multifactor (nitrogen and phosphorus) resource manipulations is crucial to gain better understanding of global carbon budgets.

12.4.3 How the Health of Soil Bacteria and Fungi Influences Carbon Sequestration

The stability and productivity of terrestrial ecosystems greatly depend on soil quality, requiring the management of soil–plant systems and the sustainability of soil resources (Altieri 1994). Diverse chemical, physical, and biological factors and their interactions determine the soil quality. For proper management of soil–plant systems, understanding how the physicochemical and the biological or microbial components of soil function and interact is critical (Kennedy and Smith 1995). While many studies have investigated the physicochemical properties of soil quality (Parr et al. 1992), the biological components did not gain much attention. However, microbial activities are particularly relevant at the root–soil interface, the rhizosphere, where microorganisms, plant roots, and soil constituents interact (Werner 1998; Bowen and Rovira 1999).

Plant roots continuously produce and release diverse array of carbon-containing primary and secondary metabolites into the rhizosphere (Rovira 2005), which acts as a key factor for the enrichment of specific microbial populations in the rhizosphere. And the rhizosphere is important for improving soil quality and microorganism-driven carbon sequestration and nutrient cycling in terrestrial ecosystems. The soil microflora consisting of rhizobia, phosphorus-solubilizing bacteria, free-living nitrogen-fixing organisms, and mycorrhizal fungi exert great influence on plant health. Numerous abiotic and biotic factors influence the structural and functional diversity of the microbial community in the rhizosphere. However, in rhizosphere ecology not only the microbial and the physicochemical components interact, but different soil microflora also interact with each other (Berg and Smalla 2009).

Among the different organisms in soil microbial communities, mycorrhizal fungi are vital in regulating transfer of essential nutrients between the plants and the soil through widespread mycelial networks (Rooney et al. 2009). They also promote soil biological diversity through symbiotic interactions with other soil organisms. Some interactions between certain bacteria and mycorrhizal fungi are relevant to benefit plant fitness and sustainability of natural ecosystems (Jeffries and Barea 2001; Barea et al. 2002). For example, mycorrhiza helper bacteria (MHB) are associated with AM and ECM fungi and facilitate their root colonization capacity and suppress soil pathogens (Bending et al. 2006). Quoreshi and Khasa (2008) demonstrated the effectiveness of simultaneous inoculation of selected mycorrhizal fungal and bacterial species to poplar seedlings at the nursery stage with the perceived increase in plant nutrient status and mycorrhization. Therefore, the maintenance of diverse and active soil microbial communities is essential for improving soil quality (Kennedy and Smith 1995), carbon sequestration, ecosystem functioning, and nutrient cycling in natural ecosystems.

12.5 Approaches to Enhance Carbon Sequestration

One question that arises in discussions of carbon sequestration is how much carbon can forestry and agricultural practices actually sequester? To address such questions, it is important to remember that carbon sequestered in plants and soils can be released back to the atmosphere and there is a finite amount of carbon that can ultimately be sequestered within the sinks of Earth's carbon cycle. Carbon sequestration rates vary by plant species, soil type, regional climate, topography, and land management practices. Even with the advent of modern statistical sampling, computer modeling, and remote sensing, current estimates of carbon sequestration and emission sources vary over time despite the fact that they are more accurate and easier to generate than when rising CO_2 levels were first recognized.

In the USA, fairly well-established values for carbon sequestration rates are available for most tree species. Pine plantations in the Southeast can accumulate almost 100 metric tons of carbon per acre after 90 years, or roughly 1 metric ton of carbon per acre per year (Birdsey 1996). Interestingly, changes in forest management, such as lengthening the harvest-regeneration cycle, appear to result in less carbon sequestration on a per acre basis possibly because carbon accumulation in forests and their soils eventually reaches a saturation point, beyond which additional sequestration is no longer possible (Lal et al. 1999; West and Post 2002). This happens, for example, when trees reach maturity or when the organic matter in soils reaches equilibrium with the local plant life. Thus, the plant traits that are involved in carbon sequestration are complex and intimately connected with the surrounding ecology. A deep understanding of the molecular and cellular processes and how these processes interact is critical, which makes the targeting and manipulation of such traits all the more challenging.

Although the post-genomics era provides a unique opportunity to identify biochemical pathways and gene regulatory networks that underlie rate-limiting steps in carbon acquisition, transport, and fate, few studies have assessed the consequence of breeding for enhanced carbon uptake, allocation, or storage in perennial grass or woody crop systems. Investments in plant genomics could harness new approaches to increase biomass production and the distribution of that biomass to roots and recalcitrant pools of soil carbon in fast-growing trees and grasses grown in managed plantations (Jansson et al. 2010; Zhu et al. 2010; Garten et al. 2011). Research could focus on targeted improvements in light-use efficiency and photosynthesis (Long et al. 2006), in root growth and nutrient uptake (Hirel et al. 2007), and on overcoming constraints imposed on plant productivity by temperature and drought (Tuberosa and Salvi 2006). Genome-enabled increases in the production of plant biomass across a range of environments would, all else being equal, translate to enhanced input of carbon to soils via shoot and root litter, increasing the storage of carbon in terrestrial ecosystems. Microbial studies could target the interaction between plants and beneficial soil microorganisms as recently shown for important components of biogeochemical cycling in soils beneath *Miscanthus* (Mao et al. 2011) and for biomass production following inoculation of hybrid poplar with an endophytic, growth-promoting bacterium (Rogers et al. 2012). Gains in carbon sequestration might also be realized by understanding how genes and proteins that control the chemical composition of litter could impact the rates and magnitudes of carbon sequestration.

12.5.1 Genetic Manipulation to Improve Carbon Sequestration

12.5.1.1 Above-Ground Process

Attempts to increase the rates of photosynthesis through genetic engineering have focused on accomplishing this goal by increasing the total amount of RuBisCO in leaves (Suzuki et al. 2007). Contrary to original expectations, this research has been met with mixed success. Since RuBisCO is often rate-limiting for photosynthesis in plants, strategies have been employed to improve photosynthetic efficiency of plants by modifying RuBisCO (Spreitzer and Salvucci 2002). Additional research seeks to increase photosynthesis and plant productivity not by modifying the amount but by optimizing the distribution of resources between enzymes of carbon metabolism and/or by altering the kinetic properties of the RuBisCO enzyme itself. Theoretical analyses suggest that expressing RuBisCO as having either a higher specificity for CO_2 relative to O_2 or a higher maximum catalytic rate of carboxylation per active site could increase photosynthetic carbon gain by 25 % or more in C_3 plants (Zhu et al. 2004). Some have questioned whether substrate specificity of this enzyme can be improved (Tcherkez et al. 2006). Significant progress has been made in identifying natural variation in the catalytic properties of RuBisCO from different species. Also the development of the molecular tools for introduction of both novel and foreign RuBisCO genes into plants is in advance stages. The three-dimensional structure of RuBisCO has been determined for RuBisCO isolated from many organisms including tobacco, spinach, cyanobacterium (*Synechococcus*), purple bacterium (*Rhodospirillum rubrum*), and green sulfur bacterium (*Chlorobium tepidum*) (Schneider et al. 1990; Schreuder et al. 1993; Newman and Gutteridge 1993; Andersson 1996; Hanson and Tabita 2001).

One of the major challenges with the manipulation of RuBisCO in higher plants is that it is composed of eight large and eight small polypeptide subunits and that the genes for the small subunit are in the nuclear genome but those for the large subunit are encoded in the chloroplast genome (Chan and Wildman 1972; Kawashima and Wildman 1972; Smith and Ellis 1981). Therefore, the manipulation of the large subunit requires the gene (*rbc*L) to be introduced into the chloroplast genome. Though recent advances in chloroplast transformation have allowed experiments to be carried out to produce mutation and deletion in the *rbc*L gene, still there are very limited species for which chloroplast transformation has been successful. The other challenging issue has been the proper assembly of large and small subunits into the hexadecameric holoenzyme following the genetic manipulation, which requires sufficient expression, posttranslational modification, interaction with chaperonins, and interaction with RuBisCO activase (Gutteridge and Gatenby 1995).

One approach to increase RuBisCO efficiency is to produce hybrid enzymes, with the large subunit from one species and the small subunit from another species. One of the examples of hybrid RuBisCO is the replacement of the tobacco *rbc*L gene with the *rbc*L gene from sunflower by means of chloroplast transformation, which produced a catalytically active enzyme (Kanevski et al. 1999). Though the specificity factor of this hybrid enzyme was similar to that of wild-type tobacco, it had substantially reduced catalytic activity, achieving only 25 % of the carboxylation activity of either parent holoenzyme. One scenario is to introduce RuBisCO variants with naturally high specificity values, like the ones from the red alga *Galdieria partita* and purple photosynthetic bacterium *Rhodospirillum rubrum* into plants. This approach appears to hold promise in improving the photosynthetic efficiency of crop plants significantly; however, possible negative impacts have yet to be studied. Some progress has been made in this area including the replacement of the native *rbc*L of tobacco with *rbc*M from *Rhodospirillum rubrum* by chloroplast transformation (Andrews and Whitney 2003). A consistent finding from the above-mentioned studies and also from other genetic engineering experiments is that there is an inverse correlation between specificity and catalytic activity; with increased specificity for CO_2, the velocity of the carboxylase reaction decreases. This relationship has significantly affected the attempts to improve RuBisCO through genetic engineering, as each time the specificity is increased, the resulting modified enzyme has a reduced carboxylase activity. Another approach used is to increase RuBisCO content by overexpressing small subunit of RuBisCO gene, *rbc*S gene, but these have failed, often resulting in decreased RuBisCO content by cosuppression. Interestingly, increases in RuBisCO content have been observed in plants transformed with transgenes aimed at other targets (Pellny et al. 2002). Though considerable progress has been made in this area of research, but still a lot needs to be explored about the requirements for RuBisCO expression and its assembly in higher plants.

As an alternative, increased gains in carbon acquisition could be achieved by altering resource allocation to each of the enzymes involved in the Calvin cycle, photorespiratory metabolism, and sucrose and starch synthesis (Zhu et al. 2007). In this case, numerical simulations suggest that optimized allocation of resources to specific enzymes could greatly increase carbon gain without an increase in the total nitrogen invested in proteins involved in photosynthetic carbon metabolism. This process illustrates a potential win–win situation as even small gains in plant productivity distributed across a large land area could contribute to meaningful enhancements to carbon sequestration. Miguez et al. (2009) developed a process model to better understand the physiological controls on biomass production in *Miscanthus* × *giganteus* and concluded that harvestable yield (and presumably soil carbon storage) could be enhanced in this bioenergy crop through various mechanisms. Although none of these were explored in any detail, the approach illustrates how models and field studies could complement one another in support of breeding for traits of interest to carbon sequestration.

Since under optimum conditions of light and temperature, C_4 plants photosynthesize and grow at faster rates than C_3 plants, attempts have also been made to incorporate components of the C_4 pathway into C_3 species (Leegood 2002). Both conventional breeding and transgenic approaches have been used to achieve this goal (Haeusler et al. 2001). However, because of the independent inheritance of genes for morphological features, such as kranz anatomy, and of the C_4 pathway enzymes, breeding attempts have failed. Therefore, recent efforts are mainly focused on overexpressing the key C_4 cycle enzymes in C_3 plants, though there are pitfalls encountered when C_3 metabolism is perturbed by the overexpression of individual C_4 genes. Successful overexpression of C_4 PEP carboxylase and maize PEP carboxylase in C_3 plants like tobacco, rice, and potato has been reported (Izui et al. 1986; Ku et al. 1999; Hausler et al. 2002). One major issue with this approach is the differences in the regulation of C_4 cycle enzymes like PEP carboxylase in C_3 and C_4 plants. Similar to C_4 plants, PEP carboxylase in C_3 plants is subject to complex regulation by metabolites and covalent modification by reversible phosphorylation. In all of these experiments host plant regulatory mechanism, rather than the C_4 one, seemed to be operating in the transgenic plants. Therefore, though C_4 enzyme was overexpressed in C_3 plants, it was in an inactive state.

This issue has been tackled by introducing modified PEP carboxylase enzyme that lacks phosphorylation sites into C_3 plants. These studies reported high activity of PEP carboxylase in transgenic plants, but the plants were stunted with increasing PEP carboxylase activity (Haeusler et al. 2001). This is because C_4 enzymes are also present in C_3 plants and overexpression of individual C_4 enzymes interferes with C_3 metabolism. Other than PEP carboxylase, pyruvate orthophosphate dikinase (PPDK) and phosphoenolpyruvate carboxykinase (PEPCK) have been targeted for transgenic approaches (Ishimaru et al. 1998; Suzuki et al. 2000). However, overproduction of PEP carboxylase, PEPCK, and PPDK, by whatever means, does not improve photosynthesis in C_3 plants. A moderate overexpression of PEP carboxylase combined with PPDK or PEPCK also had no significant effect on photosynthetic efficiency of the plants (Haeusler et al. 2001). A more current approach is to introduce groups of C_4 genes in C_3 plants but it remains to be seen whether this strategy will be any more successful. One of the major challenges of this approach is distribution of these enzymes so that they provide a CO_2-rich environment around RuBisCO. While most of the C_4 plants express PEP carboxylase and RuBisCO in different cells, the discovery of C_4 photosynthesis in single cells of *Borszczowia aralocaspica* and *Bienertia cycloptera* leaves indicates that kranz anatomy is not essential for C_4 photosynthesis (Sage 2002). This discovery might lead to simpler approaches toward engineering C_4 photosynthesis.

12.5.1.2 Below-Ground Process

In addition to targeting traits specific to CO_2 acquisition and uptake, carbon could be sequestered in soils if genome-enabled modification of leaf or root turnover times could be achieved. Driebe and Whitham (2000) collected senesced leaves from trees along a hybridization zone near the Weber River in Utah and showed that genotypic variation in foliar condensed tannin concentrations could largely explain variation in rates of litter decomposition for F_1 and backcross hybrids of cottonwood (*Populus* spp.). Although these authors did not make an explicit connection between rates of litter decomposition and soil carbon sequestration, the implications of their work clearly suggest that genotypic variation in traits related to litter quality and decomposition may be sufficient to impact carbon and nitrogen cycles in terrestrial ecosystems. Wullschleger et al. (2005) demonstrated in a large field study that significant phenotypic variation exists in poplar for a number of traits related to carbon storage including biomass production and the allocation of carbon to below-ground roots. Quantitative trait loci (QTL) analysis revealed that above-ground and below-ground patterns of biomass distribution were under strong genetic control and that such a genetic underpinning could be leveraged to potentially enhance carbon sequestration in managed bioenergy plantations (Bradshaw and Stettler 1995; Rae et al. 2009).

Although promising, few studies have demonstrated that long-term plantings of poplar will enhance soil carbon stocks; at best the results for stands ranging in age from 3 to 12 years are mixed (Hansen 1993; Grigal and Berguson 1998; Coleman et al. 2004; Sanchez et al. 2007; Satori et al. 2007; Gupta et al. 2009). Lignin biosynthesis has also been shown to be under strong genetic control and poplar species have been modified to possess reduced lignin content. Hancock et al. (2008) showed in short-term studies that aspen (*P. tremuloides*) expressing high syringyl/guaiacyl (S/G) lignin accumulated less total plant carbon and subsequently accumulated less plant-derived carbon in soil. Furthermore, Garten et al. (2011), using a modeling study, demonstrated that breeding for specific traits could enhance carbon sequestration in managed plantations by focusing on improvements to above-ground production, below-ground carbon allocation, and root decomposition.

12.6 Modeling Approaches to Select Likely Targets for Eco-engineering Plant Systems for Increased Carbon Sequestration

Controlling the flow of carbon through system to favor synthesis of materials recalcitrant to easy degradation carbon (e.g., lignin) over biomass that will more quickly return to the carbon cycle is the goal of eco-engineering for increased carbon sequestration. One significant carbon sink in the plant ecosystems is the community of root-associated microorganisms. Terrestrial plants process about 15 % of the total atmospheric carbon dioxide each year, drawing about 450 billion tons of carbon dioxide from the atmosphere (Beer et al. 2010). Depending on conditions and on ecosystems, between 20 % (Gamper et al. 2005) and as much as 40 % (Drigo et al. 2010) of that fixed atmospheric carbon is incorporated directly into the subsurface ecosystems, making subsurface microorganisms a sink for potentially billions of tons of atmospheric carbon annually. Modifying systems for increased carbon sequestration involves engineering at the ecosystem level, incorporating plants and their subsurface community, with various bacterial and fungal components of soil community structure. The metabolic mechanisms for carbon sequestration in these systems are already in place. The most profitable targets to engineer carbon sequestration are regulatory, not metabolic. The goal then of eco-engineering is to determine how does the community interact with its environment and other members of its community and how does that interaction direct carbon sequestration. This analysis will yield specific molecular targets such as transmembrane sensor proteins, regulatory elements, and intracellular signaling compounds. While the leaves are the most obvious surface that the plant exposes to the atmosphere and the woody tissues are the evident locations for carbon storage, to find the plant tissues most amenable to eco-engineering, we turn to the plant root. The "root-brain hypothesis" was first proposed by Charles Darwin well over a century ago, but recent advances in investigation techniques have begun to identify the specific molecular mechanisms of this interaction (Baluška et al. 2009). In the root-brain hypothesis, plants are recognized as organisms capable of active behavior in response to changes in their environment, and the mechanisms for regulation of plant behavior reside in the root apex.

The following is a selection of computational tools for identifying likely targets for eco-engineering.

12.6.1 Ecological Systems as Artificial Neural Networks

Artificial neural networks (ANNs) are nonlinear computational modeling tools comprised of a network of interrelated nodes and have direct applications to modeling certain biological interactions (Thivierge et al. 2012). Two recent approaches that use ANNs to describe ecological relationships and relevant to identifying targets for eco-engineering systems are microbial assemblage prediction (MAP) (Larsen et al. 2012) and expression interaction networks (EINs) (Larsen and Dai 2009).

MAP generates ANNs that represent microbial community structure in terms of mathematical equations that best explain the data and uses them to predict the relative abundance of taxa in time or space as functions of environmental conditions. These ANNs capture potentially causal relationships between the changing abundances of different taxa, although relationships between taxa could arise through taxon proxies for changes in environmental parameters. This approach requires data for population structures, such as 16s/18s sequence data or shotgun metagenomics, and environmental parameter data corresponding to the time and place from which the populations were samples. This approach can be used, for example, to model the subsurface microbial populations as they change in response to surface conditions or changes in plant phenotype/genotype.

Similar to MAP models, expression interaction network (EIN) construct networks of gene regulation interaction and models these reactions as an ANN. EIN can use networks of gene expression interactions to link environmental conditions to measured environmental phenotypes such as biomass or nutrient concentrations. The model constructed by EIN can be used to predict the measureable phenotypic response to previously unobserved or novel environmental conditions. Genes whose expression is identified to be associated with a measured phenotype in an EIN are excellent gene/protein candidates for targeted eco-engineering plant systems.

12.6.2 Sensory Components as Protein–Protein Interaction Complexes

To identify possible protein–protein interaction (PPI) networks that are likely to be associated with environmental sensing or community signaling, tools such as likelihood of interaction (LOI) (Larsen et al. 2007) or function restricted value neighborhood (FRV-N) (Larsen et al. 2010) are useful computational tools.

LOI uses collected transcriptomic data to identify likely PPI complexes. Transcriptomic data collected from multiple experimental conditions is preferred for this approach, and archived transcriptomic data for many systems is available in public repositories like Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/). LOI also requires a large set of previously identified PPI network and a set of relevant annotations for all proteins in the set of interactions. A number of such publicly available resources for experimentally observed PPIs are available for fungal, plant, or bacterial systems, such as the Saccharomyces Genome Database (http://www.yeastgenome.org/), the Arabidopsis Information Resource (http://www.arabidopsis.org/), and the Bacterial Protein Interaction Database (http://www.compsysbio.org/bacteriome/). The last input required by LOI is a discrete ontology for annotation of function assigned to predicted gene. The selected annotation needs to be uniform between genes in transcriptomic data and in databases of previously observed. Some possible sources of annotation include Gene Ontology (GO) annotation or descriptions of predicted subcellular location as in WoLF PSoRT (Horton et al. 2007). LOI used these inputs to identify pairs of function annotations enriched in previously observed PPI pairs and uses this information to propose likely PPI interactions from co-expression data in transcriptomic analysis. A significant advantage to LOI is its application of patterns identifies in model organism experimental data to organisms for which littler previous experimental data is available.

In addition to pairwise identification of pairwise PPIs as in LOI, the topology of entire PPI networks can be considered (Chen et al. 2006). A rank-based method function restricted value neighborhood (FRV-N) uses the network topology of previously experimentally observed PPI to impose biologically relevant network structures to the sets of PPIs predicted by methods like LOI.

As in LOI, FRV-N uses available large databases of known PPIs and a set of gene annotations. By identifying a characteristic neighborhood size associated with particular protein annotations, FRV-N applies a biologically relevant network topology to a set of predicted PPIs that provides increased accuracy to predicted PPI networks.

12.6.3 Metabolic Modeling of Systems

Predicted relative metabolic turnover (PRMT) is a method that infers metabolic data from metagenomic, transcriptomic, or proteomic data (Larsen et al. 2011b). Also, a database of known enzyme-mediated biochemical reactions, such as Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg/) (Ogata et al. 1999) or BioCyc (http://biocyc.org/), is required. To map detected gene or protein expression, an ontology describing the unique enzyme function of a gene product or detected protein, such as enzyme commission (EC) number (International Union of Biochemistry and Molecular Biology. Nomenclature Committee. and Webb 1992), is also needed. PRMT enables exploration of metabolite space inferred from the metagenome by associating changes in abundance of genes for enzyme activities with predicted changes in relative metabolic turnover for metabolites in a predicted metabolome. The complete output of PRMT analysis provides a numerical score for all thousands of predicted metabolites in a metabolome. An advantage of PRMT is its ability to make predictions for thousands of metabolites in complex systems from more easily obtainable sources of information like high-throughput sequencing data. The results of PRMT analysis can be used to direct more detailed biochemical analysis of systems for sets of metabolites predicted to be relevant to system interactions.

In the case of root community metabolome, significant carbon sequestration metabolism may not be discovered in root metabolism, but identification of the synthesis plant signaling compounds that regulate growth in more distant parts of the plant could be (e.g., auxin, gibberellin, or brassinosteroids). For example, in plants the hormone jasmonate regulates plant defense response, prioritizing defense over growth by blocking the gibberellin signaling (Yang et al. 2012). Sensor or signaling proteins in the plant root community might prove valuable targets for manipulating this interaction in plants, either by suppressing a defense response or by reducing jasmonate's ability to interfere with gibberellin-signaling cascade and promoting plant growth and carbon sequestration.

12.6.4 Assembling the Interactome

Only by understanding the interrelationships between plant and subsurface community, however, can one begin the process of rational eco-engineering of complex systems. Identifying the complete network of interactions between and within carbon-sequestering communities requires a "multi-omic" approach. While each of the above analytical tools can provide valuable information about interacting systems of themselves, no single investigative technique will uncover all the components of ecosystem interactions. The synthesis of the results of some or all of these tools into a single predictive model of regulatory and metabolic factors in carbon sequestration can be used to identify likely targets for manipulation and

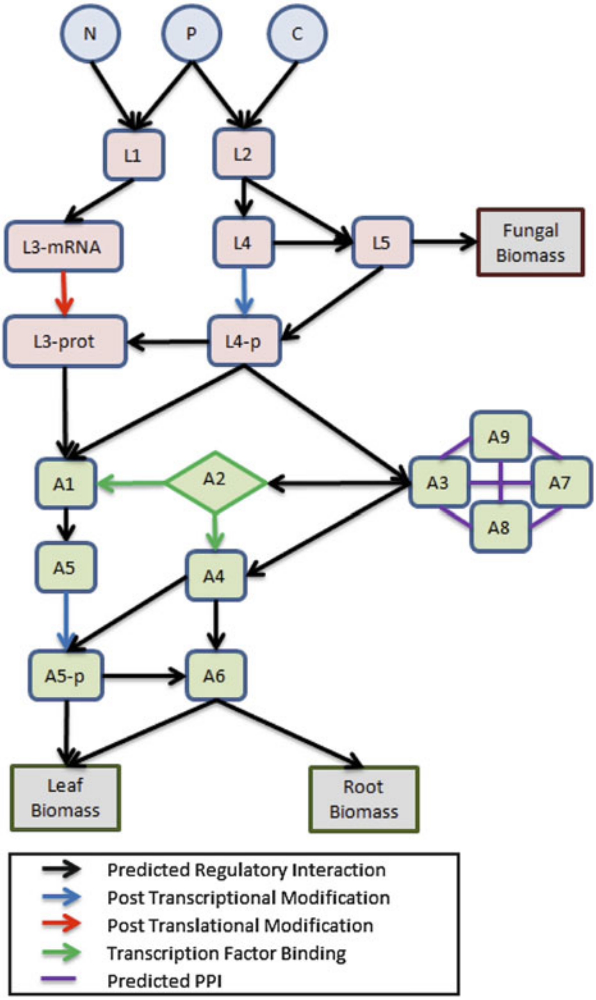

Fig. 12.4 Example of a system-scale mycorrhizal regulatory network. This simple, hypothetical interaction network provides an example of all possible interaction mechanisms that go into the regulation of mycorrhizal interactions as they relate to soil nitrogen and phosphorus levels using *Laccaria bicolor* and *Populus tremuloides* as partners. In this network, the topmost nodes represent nitrogen (N)-, phosphorus (P)-, and carbon (C)-containing components. *Pink* nodes L1–L4 represent *Laccaria* genes or proteins, and *green* nodes A1–A6 represent aspen genes and proteins. *Gray* nodes are relative measures of fungal, leaf, and root biomass. *Black-directed edges* are DBN-predicted regulatory interactions. *Blue-directed edges* indicate a posttranslational modification, identified by the difference between transcript and protein relative abundance. *Red-directed edge* is a posttranslational regulation indicated by proteomic analysis. *Green edges* are direct transcription factor binding interactions identified by ChIP-seq. *Purple edges* are predicted PPI interactions

generate testable hypotheses for modification of carbon storage. This set of interactions is not best interpreted as a simple linear path of sequential regulatory steps but as an interconnected network of biological processes that occur in parallel (Fig. 12.4).

When choosing targets for increased carbon sequestration, root-associated microbes are an appealing target for eco-engineering. Microbes are far more tractable than genetic engineering plants and trees. Also, while the prospect of replanting the landscape with modified or engineered plants is the daunting work of many years for plants to reach maturity and fix useful amounts of carbon, engineering the subsurface microbial population by the addition of selected or modified microorganisms offers an opportunity to eco-engineer existing plant ecosystems with a more rapid response.

12.7 Conclusion

Greenhouse gases are accumulating in the Earth's atmosphere at least in part due to human activities. Over time, this is causing surface air temperatures and subsurface ocean temperatures to rise, which in turn affects growing seasons, precipitation, and the frequency and severity of extreme weather events and forest fires. Humans have the unique ability to be aware of such potential threats, and more importantly we have the capacity to alter our environment and ward off pending hardship. Practices that aim to increase carbon sequestration generally enhance the quality of soil, water, air, and wildlife habitat. Tree planting that restores forest cover not only may sequester carbon but also improves habitat suitability for wildlife. Likewise, preserving threatened tropical forests would avoid losses in both stored carbon and biodiversity. Similarly, reducing soil erosion through tree planting or soil conservation measures not only sequesters carbon but also improves water quality by reducing nutrient runoff.

Combined with improved land and crop management practices, researchers have been developing a deep understanding of the molecular and cellular processes that orchestrate the conversion of carbon captured from the atmosphere into plant biomass that can sequester the carbon for many years. The plant traits behind these biological processes are complex and intimately connected with the surrounding ecology, which makes the targeting and manipulating of such traits very challenging. However, progress is being made.

Several paradigms that could potentially lead to breakthroughs might be pursued. The first builds from a focus on specific pathways for altering processes of interest to carbon sequestration including those linked to the photosynthetic uptake of CO_2 from the atmosphere, litter chemistry, and modification of leaf and root turnover. Some of the more promising research targets include improvements in light-use efficiency and photosynthesis (Long et al. 2006), improvements in overcoming the constraints imposed on plant productivity by temperature and drought (Tuberosa and Salvi 2006), and improvements in root growth and nutrient

Table 12.1 Summary of approaches used to enhance carbon sequestration

Approaches	Implementation	Goal achieved	References
Transgenic manipulation of candidate genes/mutagenesis			
(a) Rubisco	Done	Partial success	Kanevski et al. (1999), Spreitzer and Salvucci (2002), Andrews and Whitney (2003), Suzuki et al. (2007)
(b) Phosphoenolpyruvate carboxylase (PEP carboxylase)	Done	No	Izui et al. (1986), Haeusler et al. (2001)
(c) Pyruvate orthophosphate dikinase (PPDK)	Done	No	Ishimaru et al. (1998), Suzuki et al. (2000)
(d) Phosphoenolpyruvate carboxykinase (PEPCK)	Done	No	Suzuki et al. (2006)
Marker based			
Quantitative trait loci (QTL)	Done	Successful	Bradshaw and Stettler (1995), Wullschleger et al. (2005), Rae et al. (2009)
Breeding			
	Done	Successful	Deuter (2000), Driebe and Whitham (2000)
Modelling			
	Done	Partial success	Purves and Pacala (2008), Ostle et al. (2009), Ise et al. (2010), Garten et al. (2011)
Computational			
(a) Artificial neural networks (ANNs)	Potential	Promising	Larsen and Dai (2009), Thivierge et al. (2012)
(b) Protein–protein interaction (PPI) networks	Potential	Promising	Chen et al. (2006), Larsen et al. (2007, 2010)
(c) Metabolic modeling of systems	Potential	Promising	Larsen et al. (2011b)
(d) Assembling the interactome	Potential	Promising	

uptake (Hirel et al. 2007). The efficiency of the plants to access and uptake soil nutrients in particular is key to their ability to capture carbon and generate biomass. An improved understanding of such processes across a range of environments will improve the chances that manipulation of specific genes and proteins will translate to enhanced input of carbon to plant biomass and subsequently to soils via shoot and root litter.

The genetic and genomic resources are in place for the above studies, but the modification of traits for carbon sequestration is still in infancy (Table 12.1). In a second paradigm, focus is placed strictly on biomass production, knowing that increased biomass, through enhanced production of leaf and root litter, would lead to the storage of carbon away from the atmosphere as well as increases in carbon input to soils. Both of the above approaches would yield insights. However, it should be remembered that the process of carbon sequestration plays out over decades and verification for enhanced sequestration of carbon in soils can be exceedingly difficult.

In addition, other approaches could be used to focus on plant–microbe interactions and the role these play in shaping nutrient uptake, plant biomass, and soil carbon sequestration processes. When choosing targets for increased carbon sequestration, root-associated microbes, including bacteria and mycorrhizal fungi, are appealing targets for eco-engineering. Microbes are typically much more tractable than genetically engineering plants. Many tree species tend to have very long juvenile periods that inhibit breeding programs which are dependent on sexual maturity. Also, replanting the landscape with genetically modified plants is likely to be a daunting work, requiring many years for the plants to reach maturity and fix useful amounts of carbon. Thus, engineering the subsurface microbial population by addition of selected or modified microorganisms offers a promising alternative to alter existing plant ecosystems with a more rapid impact on stored carbon.

Investments in plant genomics, transcriptomics, proteomics, and metabolomics promise to identify and harness new approaches to increase biomass production. Modeling, based on the mining of such large datasets, is a largely untapped opportunity to translate our understanding of basic plant biochemistry and physiology into actions that might lead to enhanced carbon storage in plant biomass (i.e., wood) or soils. Garten et al. (2011) used this to some success in their model-based assessment of traits involved in carbon sequestration, but this need not be the only approach. Using new innovations in modeling, it is likely that we will identify novel traits that can be manipulated to improve carbon sequestration. Application of such modern system biology approaches and other advanced methodologies to improve fundamental understanding of soil microbial communities and their habitats is another approach that could further the potential for enhanced terrestrial sequestration. Such information could lead to new management practices and production of organic matter materials that optimize microbial activities for the transformation of residues specifically to enhance sequestration.

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