Carbon Flows in Ecosystems—Ecosystem Processes

Plant Productivity, Partitioning, Respiration, Recalcitrance, Plant-Soil Interactions, and Carbon Biosequestration

Definitions of GPP, NPP, NEP, NBP

Gross primary productivity (GPP) is the annual photosynthetic carbon uptake of all leaves over an area of land. Integrated terrestrial GPP and oceanic CO_2 exchange account for the two largest fluxes of carbon between Earth and the atmosphere. GPP and its partitioning between plant autotrophic respiration (R_a) and net primary productivity (NPP) are key measures of the linkages among solar energy, atmospheric CO_2, and the terrestrial biosphere (see Fig. 3.1. Terrestrial Carbon Uptake and Storage, this page, and Fig. 3.2. Terrestrial Photosynthetic Carbon Cycle, p. 28). CO_2 uptake during photosynthesis is only temporary—R_a returns about half of the captured carbon to the atmosphere almost immediately. The rest is incorporated into biomass, comprising NPP—the total amount of organic matter created annually. Additional partitioning and processing distribute this organic matter to heterotrophs, and its subsequent assimilation and respiration eventually return most of the remaining carbon to the atmosphere. The organic carbon left after respiration by plants, heterotrophs, and decomposers is defined as net ecosystem productivity (NEP). The resultant aboveground biomass, woody plants, and soil organic matter (SOM) can persist for millennia.

Another measure of carbon flow within an ecosystem is net biome productivity (NBP)—the amount of organic matter in a biome minus carbon losses or gains from disturbances such as fire, disease, and human land use. Such disturbances can strongly influence an ecosystem's carbon flows and stocks. Over time, however, disturbance-induced losses and gains nearly balance out, with the remaining organic

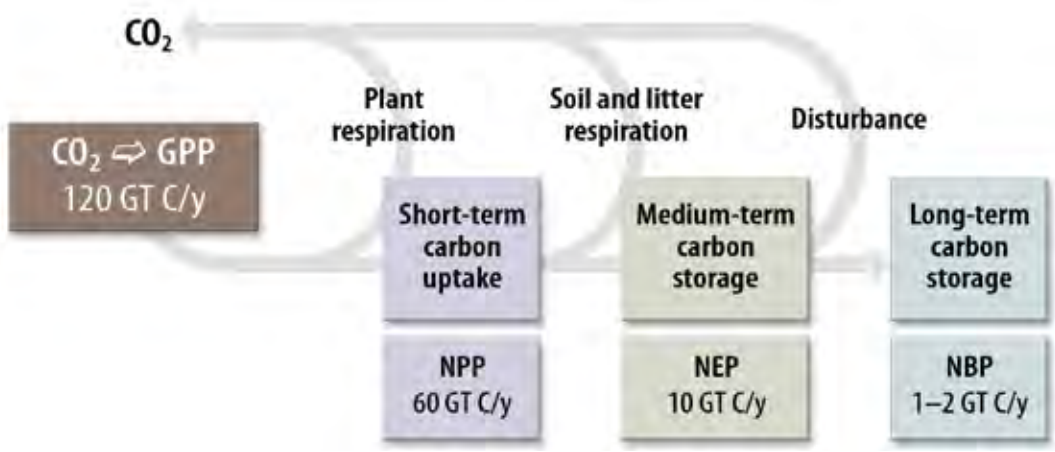


Fig. 3.1. Terrestrial Carbon Uptake and Storage. [Source: Figure adapted from International Geosphere-Biosphere Programme (IGBP) Terrestrial Carbon Working Group. 1998. "The Terrestrial Carbon Cycle: Implications for the Kyoto Protocol," *Science* **280**(5368), 1393–94. Reprinted with permission from AAAS.]

Key Research Questions

1. **What endogenous, environmental, and community factors affect GPP and its partitioning in ecosystems, especially to long-term carbon pools?**
2. **What are the consequences for long-term carbon storage under atmospheric and climatic change?**
3. **What environmental mechanisms can lead to enhanced biosequestration of carbon in soil organic matter?**

Box 3.1

Ecosystem Productivity Definitions

Gross Primary Productivity (GPP) – Total amount of CO_2 fixed by a plant in photosynthesis. The same term applies to biome, ecosystem, regional, and global scales.

Respiration (R) – Amount of CO_2 that is lost from an organism or system during metabolic activity. Respiration can be further divided into components that reflect CO_2 sources:

- $\mathbf{R_a}$ = Autotrophic respiration
- $\mathbf{R_{soil}}$ = Respiration (of new and old carbon) by plant roots and soil heterotrophs
- $\mathbf{R_h}$ = Respiration by heterotrophs
- $\mathbf{R_d}$ = Respiration by decomposers (microbes)

Net Primary Productivity (NPP) – Net amount of gross primary productivity remaining after including the costs of plant respiration. Therefore, $\mathbf{NPP = GPP - R_a}$.

Net Ecosystem Productivity (NEP) – Net amount of primary productivity remaining after including the costs of respiration by plants, heterotrophs, and decomposers. Therefore, $\mathbf{NEP = GPP - (R_a + R_h + R_d)}$. A measure of **NEP** is of great interest when determining the CO_2 balance between various ecosystems, even the entire Earth, and the atmosphere.

Net Biome Productivity (NBP) – Net ecosystem carbon balance that incorporates disturbance effects and represents a more complete and long-term understanding of ecosystem function.

matter (NBP) representing long-term carbon biosequestration (see Box 3.1, Ecosystem Productivity Definitions, this page).

Critical to long-term ecosystem productivity and carbon biosequestration are interactions between plants and microbial species (e.g., fungi and bacteria) in soils. Partitioning facilitates mutually beneficial exchanges between plants and microbes:

- Plants use water and nutrients derived by microbes from soil and air.
- Microbes, in return, benefit from photosynthate (photosynthetically fixed carbon) products from plants.

Such relationships include plant exchanges with soil microbial communities, as well as various endophytic and epiphytic interactions. These interactions are achieved by chemical signals exchanged among compatible plants, microbes, and fungal symbionts. This symbiotic chemical and molecular recognition also protects plants from pathogen infestation and microbes from plants' chemical defense systems. Such plant-microbe symbiosis is essential for each species' productivity and response to changes in climate and environmental factors (see section, Plant-Microbe Interactions and Their Impact on Carbon Cycling and Biosequestration, p. 40).

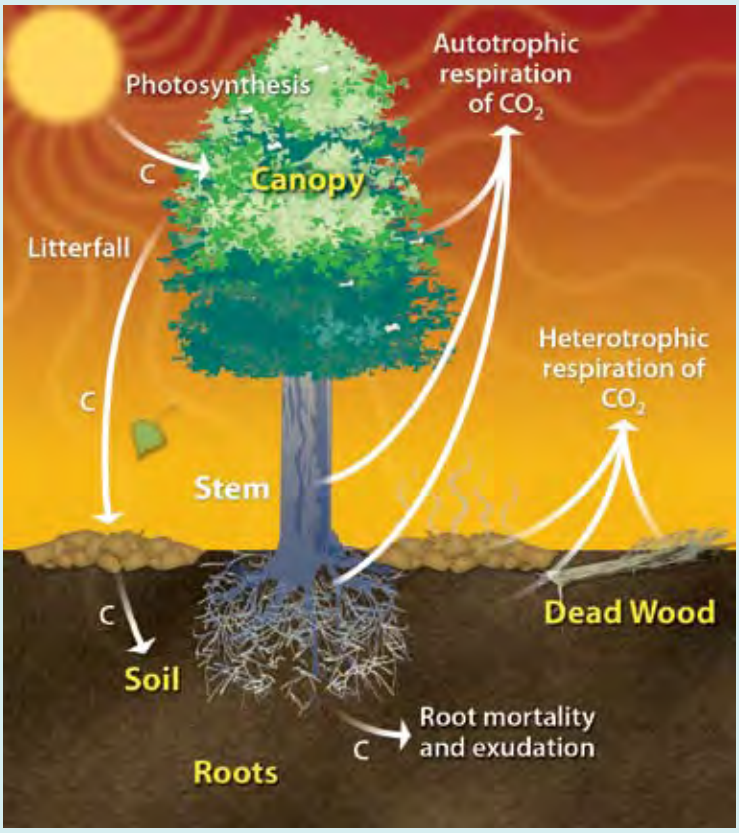


Fig. 3.2. Terrestrial Photosynthetic Carbon Cycle. [Source: Image adapted from and used courtesy of N. Scott and M. Ernst, Woods Hole Research Center, http://whrc.org.]

GPP Factors: Genomic Potential, Environmental and Biological Controls, Climate and Nutrients—Patterns and Consequences

The gross primary productivity of an ecosystem is determined by the interaction of its collective genome (i.e., plant traits + microbial capabilities) with (1) environmental characteristics [e.g., soil, altitude, length of day (latitude), and hydrology]; (2) climate variables (e.g., radiation, CO_2, temperature, precipitation amount and timing, ozone, length of seasons, and atmospheric deposition); (3) its developmental history; and (4) nutrient availability. Nutrients in turn are derived largely from physicochemical availability in soils, which is linked to water, soil chemistry, and atmospheric inputs. Nutrients such as Fe, P, S, Si, and Mg arise from soil mineralogy, which varies highly around the globe. Nutrient limitations have profound impacts on ecosystem productivity and ultimate carbon biosequestration. Thus, understanding connections among the cycling of carbon, nutrients, and water is critical. Equally important is the role of plant-microbe-soil interactions in determining nutrient and water availability. Capabilities for measuring and modeling this fully coupled plant-microbe-soil ecosystem in all its essential elements are critical to understanding and predicting ecosystem GPP, NPP, and the ratio of NPP to GPP, defined as carbon use efficiency (CUE; see also p. 38).

Plant-Trait Variation, NPP, and Carbon Biosequestration

Phenotypic-Trait Diversity within Communities

Recent research in natural communities suggests that greater genetic diversity among plant species enables increased, more stable, and more sustainable NPP and carbon storage (Hooper et al. 2005). Increased genetic diversity in turn yields greater variation in phenotypic traits among species [(Loreau et al. 2001); see Box 3.2, Phenotypic Traits, this page]. Phenotypic traits tend to be complex, influenced by both an organism's genome and its environment. Variations in these traits—resulting from increased genetic diversity—may be observed in a broader range of phenotypes (or a subset of particular traits) related to greater growth and resource acquisition and allocation compared with those of less diverse communities (Loreau et al. 2001; Cardinale et al. 2006). Enhanced NPP stability or sustainability results, therefore,

Phenotypic Traits

Box 3.2

Phenotypic traits are potentially important for NPP, carbon biosequestration, and competition (phenotypic trait = genome × environment).

- **Photosynthesis** (carbon assimilation, capacity).
- **Growth** (biomass accumulation, phenology) and **allocation** (carbon partitioning between biomass and respiration; biomass partitioning among leaf, stem, root, and seed).
- **Resource** (water, N, P, and other nutrients) **acquisition** [uptake, water use efficiency (WUE), and nutrient use efficiency (NUE)].
- **Root architecture** (shallow versus deep roots), **chemical composition** (e.g., lignin), and longevity.
- **Life history strategy** (annual, perennial) and **longevity**.
- **Stress tolerance** (susceptibility, thresholds).
- **Microbial partners** (endophytes, mycorrhizae).
- **Responses to environmental factors** (disturbance, elevated CO_2, climate change).
- **Leaf phenology** (deciduous, evergreen).

Box 3.3

Research on Identifying Environmental and Endogenous Predictors of Carbon Use Efficiency (NPP:GPP)

In the context described in the Technical Strategy chapter, the following approaches and methodologies are needed:

- Chronosequence measurements (independent NPP and GPP methods) to identify endogenous controls on CUE.
- Pulse-probe measurements using isotopic methods to quantify carbon residence time, CUE, and environmental signals for processes.
- Phenological measurements with remote and in situ methods—extended to link phenological events with environmental cues for genetic processes to provide predictive capabilities.
- Multisite analyses for rigorous assessment of the role of plant-trait variation, mechanisms underlying variation, and the extent to which variation in phenotypic traits plays a role in driving trajectories and rates of ecosystem response to future climate change. Studies should be large enough to accommodate relevant population and community dynamics, such as species turnover, competition, interactions with other trophic levels (e.g., microbes and predators), and immigration of new species. The duration of the experiments also should be long enough (decade or more) to capture these dynamics over ecologically relevant time scales (e.g., beyond the time scale of typical single-investigator experiments).
- Examination of how plant-trait variation, both within populations and among species, determines NPP, carbon biosequestration, and ecosystem responses to climate change could be particularly useful for identifying candidate species or mixtures of species for improved NPP, biosequestration, or sustainability of these functions in the face of future climate change.

from a broader range of phenotypic traits allowing diverse communities to more readily resist change or recover more rapidly amid shifting environmental conditions (Naeem and Li 1997; Hooper et al. 2005).

Phenotypic-Trait Diversity within Populations

Diversity of phenotypic traits within species populations also impacts community net primary productivity and carbon biosequestration (Wimp et al. 2005; Whitham et al. 2006). Diversity-enabled adaptation and survival mechanisms functioning at the species level likely operate within populations—that is, at the genetic level (Velland and Geber 2005)—and enhance NPP (Whitham et al. 2006), biosequestration, and the sustainability of both (Reusch et al. 2005; Hughes and Stachowicz 2005). Trait variation within populations may be particularly important for communities with few species or those dominated by a small number of organisms that are the biggest contributors to NPP, carbon biosequestration, or other ecosystem functions (Schweitzer et al. 2004; Reusch et al. 2005; Velland and Geber 2005; Whitham et al. 2006; Fridley, Grime, and Bilton 2007). In such cases, trait variation within populations, rather than among species, is likely to comprise the bulk of phenotypic diversity in a community.

While the importance of phenotypic-trait variation is widely acknowledged, few studies have comprehensively and quantitatively assessed such variation both within populations and among species in a single ecosystem or range of ecosystems. Even fewer studies have attempted to assess the implications of this variation for NPP and carbon biosequestration. The role of trait diversity amid environmental and climate variables must be evaluated thoroughly to better understand NPP and carbon biosequestration and identify potential mechanisms and species to sustain such processes under changing climate conditions. As components of plant traits, microbial symbionts must necessarily be considered in such evaluations.

Key Research Questions

1. **Which phenotypic trait or suites of traits are most important in determining NPP, carbon biosequestration, and stability of these processes over time? What are the relevant genomic markers for phenotype?**
2. **To what extent is phenotypic-trait variation within natural populations related to genetic or genomic variation?**
3. **To what extent does variation of traits (e.g., plasticity and genetic diversity) within populations or among species contribute to NPP, carbon biosequestration, and sustainability over time?**
4. **What is the relative importance of phenotypic-trait variation within populations (i.e., at the genetic level) versus among species in determining NPP, carbon biosequestration, and the sustainability of each over time?**
5. **What are the molecular controls on above- and belowground components of NPP? What are the biotic, abiotic, environmental, soil, and nutritional controls on GPP, NPP, and carbon biosequestration; how sensitive are these processes to climatic and edaphic conditions?**
6. **How are fundamental processes important to individual plant GPP and NPP integrated at the scale of plant populations and communities?**
7. **How might plant-plant competition, such as for limited soil resources, add complexity to an otherwise simple assessment of primary productivity?**
8. **How do atmospheric and climatic change alter plant-community composition through effects on successional processes, plant-plant competition, or differential sensitivity of different species to climate change?**
9. **How do changes in community composition interact with biogeochemical responses to alter carbon cycling?**

Some needed research activities on critical factors determining CUE are shown in Box 3.3, Research on Identifying Environmental and Endogenous Predictors of Carbon Use Efficiency (NPP:GPP), p. 30.

The Need to Understand Partitioning Mechanisms

Unlike photosynthesis, for which there is a robust mechanistic model, models of partitioning are empirical. Scientists know, for example, that partitioning is a complex interplay between genomic capability and ecosystem and environmental factors. It is influenced by nutrient and water availability and is an element in the carbon-nitrogen-water cycle linkage. Partitioning responds to resource-acquisition needs, such as in the formation of small roots. Fully understanding the mechanisms driving such processes and incorporating resulting data into terrestrial ecosystem models are critical.

Building useful models also requires partitioning information sufficient to represent processes both at the plant and ecosystem levels and at the molecular metabolic and regulatory levels. Detailed investigations of the mechanisms controlling partitioning at molecular and biochemical levels should provide a more robust modeling approach. Based on first principles, for example, predictions can be made concerning the relative amount of fine-root production versus wood production under various environmental conditions (e.g., nutrient limitations or additions and atmospheric and climatic change).

Few studies measure all components of the carbon budget. As a result, models lack dynamic, process-based descriptions of carbon flow through ecosystems on seasonal to interannual (and longer) time scales. To explore the consequences of changes in partitioning (e.g., the dependence of SOM pool stability on litter quality and

Box 3.4

Research on Plant Carbon-Allocation Patterns Influencing Short- and Long-Term Carbon Storage in Response to Climate and Disturbance

Terrestrial ecosystem models require partitioning information, yet few studies measure all components of the carbon budget to allow estimation of carbon allocation. Future research quantifying complete annual carbon budgets will contribute greatly to understanding partitioning. In quantifying complete budgets, each component must be determined by independent means to advance our understanding of carbon partitioning. Relevant components include mechanisms controlling the coupling of canopy and belowground processes; responses of root, rhizosphere, and heterotrophic respiration; partitioning to plant tissues in response to warming, elevated CO_2, and nutrient limitations or additions; and influence on plant-microbe symbioses in controlling partitioning and nutrient cycling. Research priorities follow.

- Fully quantify carbon transport and pools within trees at appropriate temporal scales in forests of various ages.
- Use automated instrumentation to track carbon transport to various plant organs and carbon metabolism via simple, continuous monitoring devices (e.g., attachments to tree stems).
- Quantify more-complete carbon budgets for live and dead pools and detect carbon metabolites in plant organs (e.g., to identify pools and transport rates).
- Use accompanying genomic and systems biology analyses to pinpoint partitioning mechanisms and environmental triggers.
- Test theoretical understanding from carbon-allocation studies using field experiments that include detailed analyses of NPP distribution in relation to environmental factors, species characteristics, and atmospheric and climatic change.
- Assess root-litter input and the vast heterogeneity of fine-root production and distribution in soil. Improve estimates of root-system turnover time (or longevity) in relation to species, soil type and depth, and aboveground growing conditions.
- Conduct advanced chemical analyses of soil organic matter. Such investigations include solid-state ^{13}C nuclear magnetic resonance analysis and application of a molecular-mixing model that can generate quantitative estimates of major terrestrial chemicals (e.g., lipids, proteins, carbohydrates, and lignin). Compound-specific isotope analysis of selected biopolymer components also can be used to evaluate the dynamics, sources, and stability of functionally meaningful soil carbon pools and their response to atmospheric CO_2 enrichment.
- Develop genomic analyses to quantitatively identify the species in a mixed-root sample. Use molecular approaches to better understand the causes of root mortality, metabolic changes occurring during root senescence, and whether nitrogen is resorbed into perennial roots when fine roots senesce.

placement), future research must quantify total annual carbon budgets and all their components. Doing so will further contribute to increased knowledge for developing process-based models of carbon partitioning (see Box 3.4, Research on Plant Carbon-Allocation Patterns Influencing Short- and Long-Term Carbon Storage in Response to Climate and Disturbance, this page).

Examples of Partitioning Variability

Two of DOE's Free-Air CO_2 Enrichment (FACE) experiments in forest stands illustrate the gap in understanding larger-scale ecosystem partitioning and its response to atmospheric and climatic change. Elevated CO_2 increased NPP in stands of both loblolly pine and sweetgum, but the additional carbon was preferentially partitioned—to wood in the pine stand and to fine roots in the sweetgum stand (Norby et al. 2004; DeLucia, Moore, and Norby 2005). Since turnover is

rapid in fine roots but not in wood, this difference in partitioning alters predictions of plant capacity to store additional carbon in biomass in response to elevated CO_2. On the other hand, increased production of fine roots, especially deep in the soil profile, promotes carbon storage in SOM pools (Iversen, Ledford, and Norby 2008). The relative amount of GPP partitioned to root growth and responding to environmental change also has implications for interactions with soil resources [e.g., nutrient and water uptake (Norby and Iversen 2006; Finzi et al. 2007)] and for the flux of carbon to mycorrhizae and rhizosphere microorganisms.

Partitioning variability also was observed in a FACE four-site comparison of NPP response to elevated CO_2, in which the percentage of NPP gain partitioned to wood ranged from 11% to 93%, with no discernible partitioning pattern among forest types (Norby et al. 2005). Current global productivity models vary nearly as much in the fraction of carbon stored in vegetation versus that in soil—from 35% to 85% (Dufresne et al. 2002).

Key Research Questions

1. **How do carbon allocation to biomass, carbon flux, and partitioning of GPP to plant components differ within and among plant functional types under a range of climatic conditions and following disturbances (e.g., harvests and fires)?**
2. **How do climate and disturbances influence carbon-allocation patterns after such events and over longer time frames, and how do they affect carbon storage in short- and long-term pools?**

Carbon Flows and Stocks in Soils

Examples of Plant Interactions with Soils and Microbial Communities: Developing a Genomic-Based Mechanistic Framework

Improving the Mechanistics of Soil Carbon Cycle Models

Carbon allocation and partitioning among plant organs (e.g., leaves, stems, and roots) and different fluxes within those organs (e.g., to respiration, storage compounds, defensive compounds, and structural components), and among different soil pools are not well understood and thus are not adequately represented in current models. To improve predictive capabilities and optimize productivity and carbon biosequestration, we must analyze the physiological and regulatory controls of finer-scale partitioning of carbon to different plant organs and metabolites, alteration of partitioning by atmospheric and climatic change, and the longer-term consequences for trophic cascades and carbon cycling in the soil.

Photosynthetically fixed carbon moves belowground via a number of pathways (including transfer to roots, degradation of plant litter, and exudation from roots to soil), in which it is subject to respiration. Belowground respiration (R_{soil}), measured as soil-surface CO_2 efflux, contributes a large fraction of CO_2 moving from terrestrial ecosystems to the atmosphere. R_{soil} comprises the respiratory fluxes of numerous organisms involved in many processes. However, it generally is separated into root respiration, which is part of autotrophic respiration (R_a), and heterotrophic respiration (R_h), which is the microbial respiration of both new and old carbon substrates (see Fig. 3.3. Conceptual Model of Components and Responses of CO_2 Efflux from Soil, p. 34).

Soil respiration is often a large source of uncertainty in modeling terrestrial carbon cycling and climate change predictions. The separation of R_{soil} into its component fluxes, R_a and R_h, in measurement campaigns is especially difficult—no extant techniques can unambiguously separate autotrophic from heterotrophic respiration or aerobic from anaerobic respiration. This separation is an important research priority because root respiration and microbial respiration respond differently to environmental signals, including those associated with atmospheric and climatic change. To improve quantification of processes contributing to soil CO_2 efflux, more detailed studies of the regulatory and physiological controls on root

Key Research Questions

1. **Can we identify and quantify mechanisms controlling autotrophic and heterotrophic components of soil CO_2 efflux using general principles applicable across ecosystems (not site-specific heuristic models)? Which mechanistic models best capture respiration responses to the environment?**
2. **How do climate and disturbance influence decomposition and autotrophic and heterotrophic components of soil CO_2 efflux? How does this relate to carbon storage and productivity?**

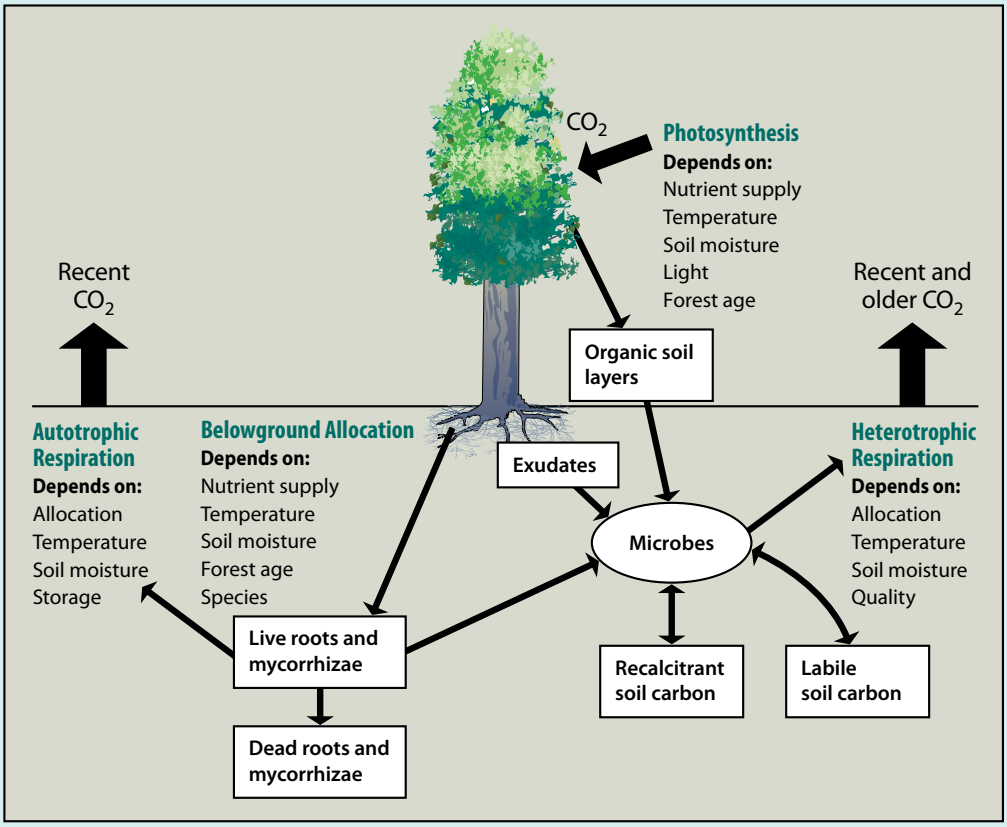


Fig. 3.3. Conceptual Model of Components and Responses of CO_2 Efflux from Soil. Both the autotrophic and heterotrophic components of soil respiration are strongly controlled by substrate availability—phloem transport of carbohydrate supply for root and mycorrhizal respiration and dead organic material for microbial respiration. [Figure adapted with kind permission from Springer Science and Business Media. From Ryan, M.G. and B.E. Law. 2005. "Interpreting, Measuring, and Modeling Soil Respiration," *Biogeochemistry* **73**(1), 3–27. doi: 10.1007/s10533-004-5167-7.]

and microbial respiration are needed. An important opportunity involves the use of molecular biology tools to provide better insight into the component mechanisms of R_{soil} (see Box 3.5, Soil Respiration Research, p. 35).

Key Research Questions

1. **Will processes and patterns measured at the level of genomes and gene transcripts (and associated techniques) be predictive of organismal respiration (autotrophic, heterotrophic, aerobic, and anaerobic)? Can such predictions on an organismal level in turn be used to estimate ecosystem-scale respiration?**
2. **How do physical, chemical, and biological components in soil interact to alter the partitioning of plant organic matter between pathways that lead to mineralization to CO_2 versus biosequestration of carbon as recalcitrant soil organic matter?**

Microbial Processing of Plant Litter and Other Soil Organic Materials

Microbial metabolism of plant detritus and exudates contributes profoundly to the massive amounts of carbon stored in and released from soil, making it a significant component of the global carbon cycle. Heterotrophic communities in soil transform dead plant parts and microbial cells into soil organic matter (SOM), a heterogeneous array of molecules that can reside in terrestrial ecosystems for centuries and millennia. Along with their concomitant release of nutrients, microbially mediated processes are globally important for many reasons. First, Earth's soils contain twice as much carbon as the atmosphere, and two-thirds of the carbon globally stored on land resides in soil organic matter. Furthermore, respiration of microorganisms and plant roots in soil returns eight times more carbon to Earth's atmosphere than human combustion of fossil fuels (see Box 3.6, Processes for Heterotrophic Decomposition of Organic Matter, p. 35).

Understanding mechanisms of SOM formation across hierarchical levels of biological organization holds promise for revealing novel insight into long-term terrestrial storage of anthropogenic carbon. Scientists need to learn, for example, how microbial genes, enzymes, physiology, and community dynamics mediate biogeochemical processes.

Microbial formation of soil organic matter, because of its extreme complexity and the lack of relevant analytical tools and models, traditionally has been thought of as a "black box" into which dead plant and microbial matter and plant exudates flow.

Box 3.5

Soil Respiration Research

New methods are needed to quantify microbial metabolic rates and determine how they change seasonally and with shifts in microbial composition. Automated soil-chamber measurements should be coupled with automated soil-temperature and soil-moisture profiles at many locations within an ecosystem (e.g., wireless chambers and loggers) to reduce spatial and temporal uncertainty in estimates. Measuring autotrophic (R_a) and heterotrophic (R_h) respiration from the soil throughout the year to determine seasonal and annual fluxes of each may require a combination of automated soil-chamber measurements with trenching or other technology to distinguish the two. New methods for continuous monitoring of $^{13}CO_2$ in ecosystems may be especially valuable.

Molecular approaches for separating component fluxes of soil respiration would be based on the hypothesis that processes measured at the level of gene transcripts will be predictive of organismal respiration, which in turn could be used to estimate ecosystem-scale respiration. Quantitative real-time polymerase chain reaction (PCR) and microarray techniques can be used to assay portions of an ecosystem's transcriptome hypothesized to be indicative of autotrophic, heterotrophic, aerobic, and anaerobic respiratory activity. Developing a mechanistic, molecular basis that will contribute to a prognostic understanding of climate change effects on R_{soil} is one objective of such research. Another goal is to demonstrate whether information expressed at the genomic and metabolic levels for evolutionarily conserved and ubiquitous genes is sufficient for estimating the ecosystem function to which such genes are coupled. Developments necessary to accomplish these objectives include:

- Protocols for DNA and RNA extraction from plants and soils resulting in good-quantity and -quality DNA that is PCR amplifiable with appropriate primers.
- Efficient development of a primer set using metabolic enzymes.
- Identification of target enzymes with known sequences that fall in three distinct clusters representing the three organismal groups of interest—plants, bacteria, and fungi. There must be sufficient conserved and variable regions present in these enzyme sequences to design PCR primers allowing researchers to discriminate among the three organismal target groups.

Box 3.6

Processes for Heterotrophic Decomposition of Organic Matter

Heterotrophic organisms carry out respiration to obtain energy from the oxidation of organic matter. Electrons are donated in oxidation reactions and accepted in reduction reactions. Such processes are called redox reactions because they always must occur in pairs. Another means of metabolizing is fermentation.When organic plant residues are incorporated into soil, three general reactions occur:

- Carbon compounds are enzymatically oxidized to produce CO_2, water, energy, and decomposed biomass.
- Elements essential to plant nutrition, such as N, P, and S, are released or immobilized by a series of specific reactions relatively unique for each.
- Compounds highly resistant to microbial action are formed.

Aerobic and anaerobic respiration are distinguished by the type of electron acceptor available, as explained below.

- In **aerobic respiration,** microbes and plants use oxygen to metabolize organic compounds. Oxygen is the strongest electron acceptor and yields the most energy from oxidation.
- In **anaerobic respiration,** oxygen is absent, so soil microbes use different electron acceptors such as Fe^{3+}, Mn^{4+}, NO_3^-, SO_4^{2-}, or CO_2 to metabolize organic compounds. These secondary electron acceptors produce less energy from oxidation than oxygen. Their reduced oxidation states (e.g., NO_2^-) often are toxic to plants and soil microbes.
- In **fermentation,** both oxygen and secondary electron acceptors are absent, so soil microbes metabolize organic molecules into more-stable compounds. This process releases energy but less than that produced by either aerobic or anaerobic respiration.

Key Research Questions

1. **How does microbial community composition define or constrain a community's function in regard to soil carbon cycling processes? How do the microbial metagenome, transcriptome, and proteome respond to a changing environment?**
2. **Do compositionally distinct plant communities harbor compositionally and functionally distinct microbial communities in soil?**
3. **Does the biochemical composition of plant litter define pathways of microbial succession, ultimately directing SOM formation?**
4. **What are the chemical signals between plants and microbes that foster specific soil microbe–plant associations?**
5. **Do critical environmental variables influence the structure of soil microbial communities either phylogenetically or functionally?**
6. **Do the physical and chemical characteristics of soil have an equal or greater impact than plant litter quality for the structure and function of soil microbial communities?**

Our ability to simulate SOM dynamics in a wide range of terrestrial ecosystems has progressed by considering a community of microorganisms as a single catalytic unit. In addition, soil organic matter can be segregated into functional classes based on physical size, chemical solubility, or kinetic properties. This approach alone, however, does not capture or consider the underlying molecular mechanisms by which a phylogenetically and physiologically diverse microbial community interacts and competes for the biochemical energy locked in plant detritus and exudates. Neither does it fully reveal microorganisms' role in nutrient and water flows.

At a fundamental level, SOM formation is mediated by multiple classes of extracellular enzymes, including ligninases, cellulases, xylanases, chitinases, lipases, and proteases. These enzymes depolymerize lignin, carbohydrates (including cellulose and hemicellulose), chitin, lipids, and proteins—the organic compounds constituting the majority of plant detritus and exudates (see Fig. 3.4. Microbial Communities and Soil Carbon Cycling and Storage, p. 37). The presence of genes coding for these enzymes conveys physiological attributes that, along with morphological traits (e.g., single versus filamentous cells), shape the ability of particular soil microorganisms to compete for resources. Molecular approaches now offer unprecedented tools for unlocking the black box of SOM formation. These approaches will provide critical insight into SOM dynamics by revealing the genes coding for extracellular enzymes that mediate the biochemical process of plant-litter decay, identifying microbes in which those genes reside, and quantifying their expression in response to the environment. This insight in turn will advance scientific understanding of the competitive and symbiotic relationships dictating the composition of soil microbial communities that foster soil carbon storage and will allow prediction of such communties' response to climate change.

Refining our understanding of SOM formation and incorporating this new insight into next-generation models require integrating information across multiple levels of biological organization. Such integration will demand a new generation of scientific inquiry drawing on the expertise of molecular biologists, ecologists, organic chemists, and modelers. Challenges for these researchers are to (1) identify and understand, at a molecular level, the key processes mediating SOM formation; (2) understand interactions between plants and soil biota that foster, for example, symbiotic and other relationships and affect ecosystem structure and function; and (3) develop a conceptual framework that translates this understanding across all levels of biological organization to better anticipate the dynamics of the globally important carbon pool stored in soil and define strategies for optimum carbon biosequestration.

Using genomic, metagenomic, transcriptomic, and proteomic approaches (for plants and microbes), coupled with new metabolic-flux techniques, scientists are poised to develop an integrated understanding of SOM formation spanning the function of microbial genes to the global carbon cycle (see Box 3.7, Implications of Biological Hierarchy on the Global Carbon Cycle, p. 37).

Biological Processes Underlying Carbon Metabolism in Soil

The functional metagenome in soil is raw genetic potential for directing metabolic processes that transform plant and microbial detritus into soil organic matter. Figure 3.4, p. 37, illustrates a framework for integrating genomic, transcriptomic, proteomic, and metabalomic information with biogeochemical process data

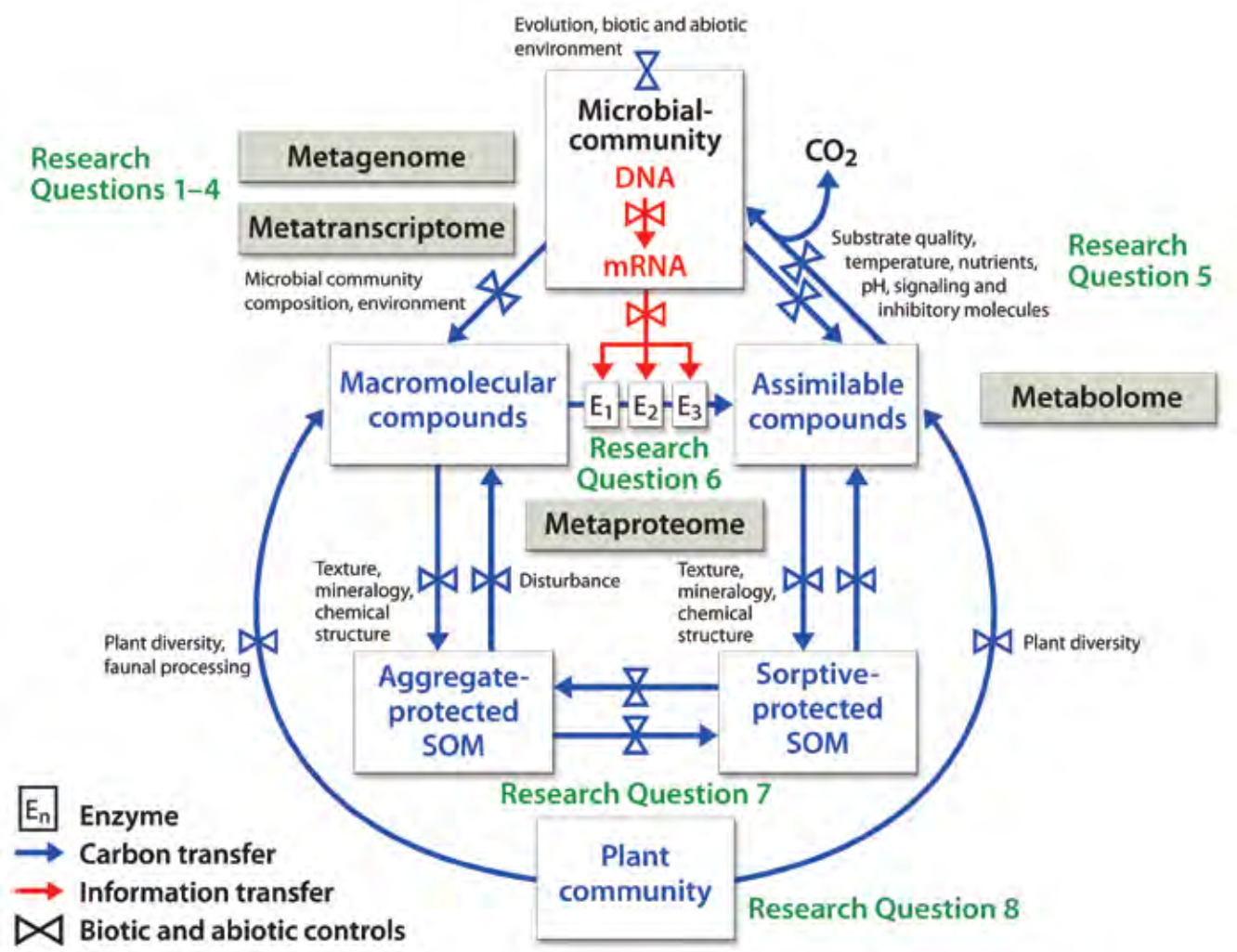


Fig. 3.4. Microbial Communities and Soil Carbon Cycling and Storage. This conceptual diagram provides a framework for integrating genomic, transcriptomic, proteomic, and metabalomic information with biogeochemical process data to better understand the relationship between microbial communities and soil carbon cycling and storage. Arrows represent the flow of information (in red) or carbon (in blue) among components. Advanced understanding of the microbe-carbon relationship requires a comprehensive molecular characterization of intact soil microbial communities, including descriptions of actively expressed genes (transcriptome) and genomic potential. This effort could be guided by stable-isotopic targeting of soil microbes important in mediating specific carbon transformations in soil. Synthesis of this descriptive molecular data and environmental drivers will help create models of the mechanistic basis of gross community carbon processing and enable prediction and simulation of microbial community carbon processing under changing environmental conditions (see Key Research Questions 1–8, p. 39, as noted on the figure).

Box 3.7

Implications of Biological Hierarchy on the Global Carbon Cycle

Genomic → Transcriptomic → Proteomic → Extracellular Enzyme Activities → Biochemical Processes → Biogeochemical Cycling → Ecosystem Function

1. Which aspects of soil carbon cycling can be advanced by applying the concept of biological hierarchy?
2. Which specific mechanisms or approaches will enable researchers to use this hierarchy for understanding and predicting patterns of soil carbon cycling, SOM formation, and, ultimately, ecosystem carbon storage?

to better understand the relationship between microbial communities and soil carbon cycling and storage. Within this framework, microbial functional groups are the primary unit of interest; metaomic approaches, coupled with stable-isotope and biogeochemical methodologies, are the most appropriate ways to evaluate microbial structure-function linkages influential in SOM formation (see this section's Key Research Questions 1–4, p. 39).

Contributing significantly to soil microbial communities' effect on carbon cycling are extracellular enzymes. Microbial communities, which are structured by interactions between evolutionary and environmental (biotic and abiotic) factors, produce and release such enzymes. These molecules break down plant- and microbe-derived compounds into small molecular–weight compounds (i.e., carbon substrates) that microbial cells can assimilate. High-frequency, high-resolution spatial information on the activities of specific extracellular enzymes is needed to fully understand carbon-stabilization mechanisms (see Key Research Question 5, p. 39).

Once assimilated into a microbial cell, carbon substrates are physiologically partitioned among biomass synthesis, energy generation, and other functions. Microbial carbon use efficiency (CUE) is the amount of new biomass produced per unit of assimilated carbon substrate and determines the amount of substrate released from the soil as CO_2. CUE is a key parameter in ecosystem-level SOM models. Understanding the factors controlling microbial CUE in soils—and using emerging technologies to do so—is critical (see Key Research Question 6, p. 39).

Also important are the physical and chemical factors affecting the longevity of organic material deposited in soil by microbial communities. Soil microorganisms produce and release both macromolecular and assimilable substrates (e.g., enzymes and extracellular polysaccharides) into soil through cell death and excretion. These substrates, derived from microbes and plants, are stabilized in soil through interactions with soil minerals and physical structures (i.e., aggregates). Research must define the relative importance of physical versus chemical stabilization mechanisms and the relationship between microbial community structure and carbon longevity in soil (see Key Research Question 7, p. 39). Furthermore, plants influence microbial community structure through a diverse array of substrates as well as growth, regulatory, and inhibitory compounds. Thus greater insight into these compounds (and other plant-community components) is needed (see Key Research Question 8, p. 39).

Microbial communities' evolutionary history also holds promise for elucidating the mechanisms of soil carbon storage. Such history is determined by complex feedbacks and interactions between microbial communities and their environment. Microbes can alter their environment, and the environment in turn can force a microbial community's evolution and gene expression. Phylogenetic analysis historically has been used to describe evolutionary processes in microbial communities. Thus an additional key research need involves understanding how microbial phylogeny might be used to provide insight for genomic analyses of the complex regulatory and metabolic processes controlling carbon storage in soils (see Key Research Question 9, p. 39).

Key Research Questions

1. **How dynamic are the soil metagenome, transcriptome, and proteome, and how are these linked with one another over time to influence SOM formation and storage?**
2. **How and to what extent do the microbial metagenome, transcriptome, and proteome respond to environmental change?**
3. **Can we characterize functional microbial groups that metabolize organic compounds in above- and belowground plant litter, microbial litter, and humic compounds in soil? For example, can we define microbial guilds participating in the degradation of lignin, cellulose, hemicellulose, proteins, chitin, peptidoglycan, and humic compounds? Achieving this requires a better understanding and characterization of the biochemical compounds in plant and microbial litter. Also necessary is improved knowledge of organic humic compounds to understand organisms that metabolize such material.**
4. **How do the physical and chemical environment and the temporal and spatial variabilities therein influence the activity of functional group members to express genes that mediate particular biochemical processes during the degradation of plant and microbial litter and humic compounds? That is, how does the environment interact with the functional metagenome to influence a biochemical process mediating SOM formation?**
5. **What are the activities and origins of extracellular enzymes? How do such enzymes modify their actions in response to the physical and chemical environment, and what are the consequences of this response to the biogeochemical cycling and storage of carbon in soil? The ability to measure in situ activities for diverse microbial enzymes that degrade plant and microbial litter and humic compounds in soil is critical.**
6. **How and why do growth efficiencies differ among microbial taxa in soil? A basic aspect of microbial physiology, growth efficiencies (unlike processes responsible for formation of new microbial cells and stabilized organic matter) control the return of carbon to the atmosphere. Our understanding of growth efficiencies, however, is limited to a small number of laboratory-grown bacteria and fungi. Fewer than 1% of soil microorganisms grow under laboratory conditions, thus little is known about in situ growth efficiencies, which may be modified by the substrate types metabolized and the interactions among organisms in soil.**
7. **How do physical surfaces interact (e.g., adsorption and aggregate formation) with the products of plant- and microbial-litter degradation to influence their longevity?**
8. **How do the composition and diversity of plant communities influence the composition and function of soil microbial communities? Moreover, as global change alters the distribution and dynamics of plant communities, how and when will microbial community composition and function respond? How do these relationships influence the cycling and storage of carbon in soil (see discussion on rhizobia versus other soil microbes in the following section)?**
9. **Does phylogeny inform us about the function of soil microbial communities? If so, can this information be extrapolated across communities and ecosystems?**
10. **Can we achieve better biochemical characterization of the compounds composing soil organic matter, especially for poorly defined classes such as humic compounds?**

Research to Evaluate Accuracy and Scalability of Microbial Processes Represented in Models Predicting Climate Change Impacts on Soil Carbon Storage

Essential to future research is determining whether conceptual and mechanistic carbon processes, from the genome to ecosystem level, can be used to inform and develop a new generation of models that more accurately predict the formation and dynamics of soil organic matter in ecosystems. Requirements for achieving this follow.

1. A new, community-wide focus on developing mechanistic, testable models of SOM dynamics at various spatial and temporal scales.
2. Development of a carbon-modeling framework accessible to the scientific community and having a clear mechanism by which new information and analyses are evaluated and incorporated. Such a framework requires:
   - Developing models that can both inform and be informed by experimental studies. Models serving as heuristic tools for the experimental community are essential.
   - Using multiscale models to provide a clear evaluation of the roles genomic-driven mechanisms play in the carbon cycle. Such an evaluation necessarily would include data on the varied extent of these mechanisms' influence.
   - Developing a suite of multiscale modeling tools that can be used collectively to create and evaluate scaling rules.
   - Narrowing the uncertainty in projections of future carbon dynamics by developing modeling tools able to assimilate complex data and concepts from soil microbial community research and testable with readily available information.

Plant-Microbe Interactions and Their Impact on Carbon Cycling and Biosequestration

In terrestrial ecosystems, plant-soil interactions control NPP and carbon biosequestration, but they can be difficult to predict and are a source of nonlinear behaviors and responses. Many of these interactions occur in the rhizosphere, the zone of soil adjacent to roots. The plant's root system exists in close association with rhizosphere bacteria, fungi, and archaea, and together their activities produce rhizosphere microenvironments having biological, chemical, and physical characteristics different from surrounding soil, including water potential, pH, salinity, density of mesofaunal grazers, concentration and action of viruses, physical compaction, and improved aggregation. The balance between consumption of fixed carbon by plant-root cells and associated microbes affects the rate of carbon turnover in soils. While it is well recognized that the flow of labile carbon from roots to microbes in the rhizosphere can significantly affect rates of SOM decomposition (and possibly formation), the mechanisms controlling such responses are less clear and thus require rigorous study.

Plant-soil interactions also foster critically important resource exchanges leading to plant growth and development changes that can affect productivity dramatically. To accomplish such exchanges, roots use chemical signals to communicate with microoganisms that in turn coordinate their action via signaling mechanisms. Complex "conversations" between roots and microbes can, among other important interactions, affect microbial mediation of nitrogen availability to plants (see Fig. 3.5. Nitrogen Cycle, p. 41, and Box 3.8, Plant-Microbe Symbioses for Nitrogen Fixation, p. 42).

While some microbial populations benefit plant growth, others have neutral and even harmful effects. Identifying metabolic requirements and environmental factors beneficial to microbes is thus important in devising optimal productivity and carbon biosequestration strategies.

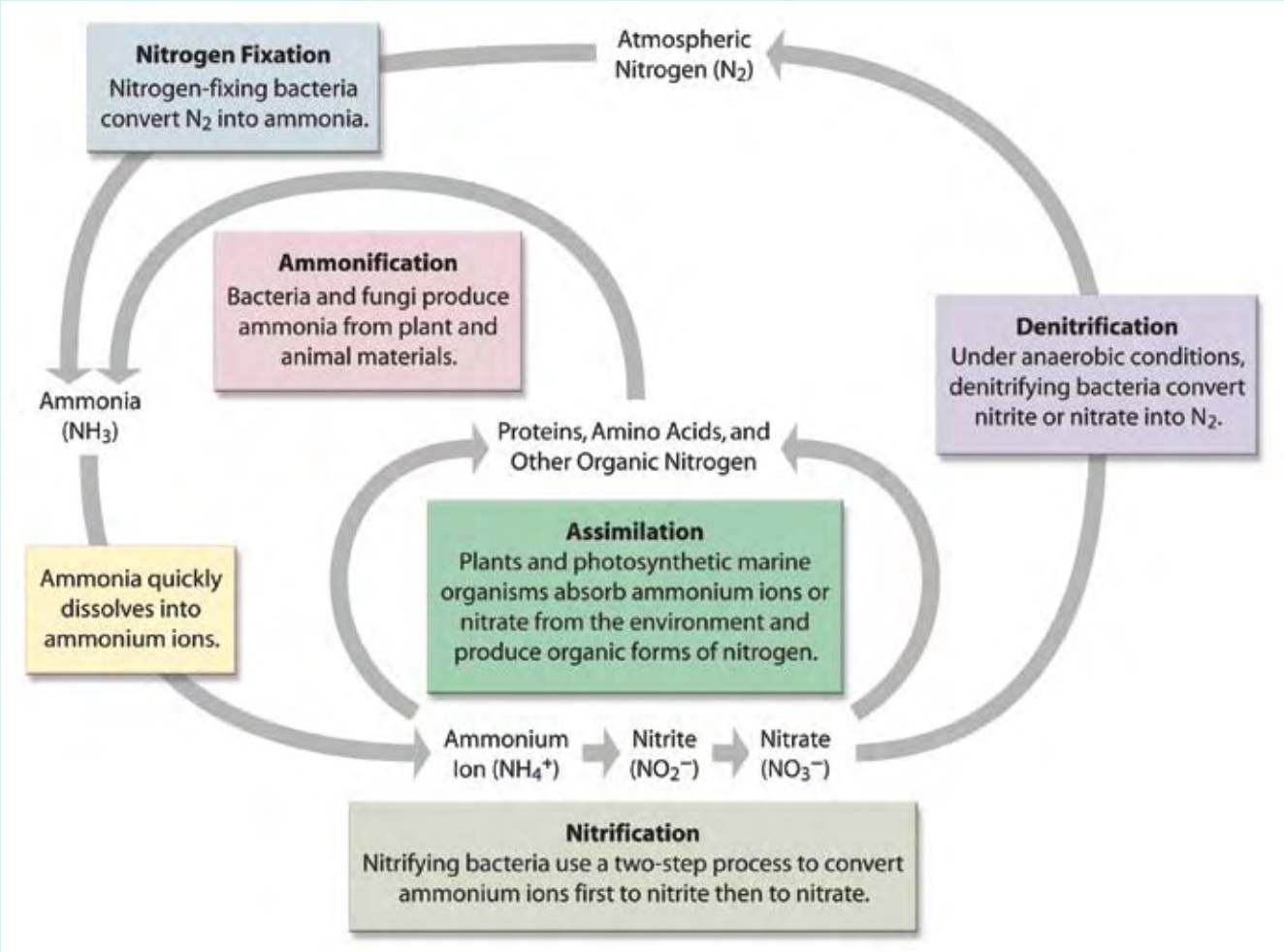


Fig. 3.5. Nitrogen Cycle.

Biological Nitrogen Fixation

Access to reduced nitrogen limits productivity of most of the world's agricultural and natural terrestrial ecosystems. Certain metabolic controls of carbon fixation and allocation are intimately linked to nitrogen bioavailability. Several key agricultural crops (e.g., corn and wheat) require nitrogen from fertilizers produced by the energy-intensive Haber-Bosch process. This industrial process for breaking the powerful triple bond between the pair of atoms in N_2 requires enormous amounts of energy to reach temperatures up to 500°C and pressures to 200 atmospheres and consumes significant quantities of fossil fuels such as methane. Increases in fixed carbon obtained by using nitrogen fertilizer thus are directly offset by the CO_2 released from the fossil fuels used to produce it.

Plants naturally receive reduced nitrogen from several key sources. One such source is nitrogen released from the decay of plant matter (see Fig. 3.5, this page, and Fig. 3.6. Soil Carbon and Nitrogen Dynamics: Heterotrophic Cascade in the Decomposition of Plants, p. 43).

In the second, the biosphere's primary source of nitrogen is atmospheric N_2 gas, which in biological systems is converted to reduced nitrogen (ammonia) by a process known as biological nitrogen fixation (BNF). This process is unique to prokaryotes (bacteria and archaea), forcing plants and other eukaryotes to depend entirely on prokaryotes and other external sources for reduced nitrogen and thus survival.

Nitrogen-fixing prokaryotes can crack the N_2 chemical bond at room temperature and atmospheric pressure. Legume plants (e.g., soybean, peas, alfalfa, and clover) form symbiotic relationships with nitrogen-fixing soil bacteria (known as rhizobia) to use atmospheric N_2 directly as a nutrient source. This symbiosis uses energy of plant-derived photosynthate to produce ammonia from nitrogen gas (see Box 3.8, Plant-Microbe Symbioses for Nitrogen Fixation, this page). Although the role of bacteria in symbiotic BNF is relatively well understood, plant contributions to this relationship are decidedly less clear. Particularly lacking is an understanding of symbiotic BNF at the level of metabolic control and genetic factors affecting

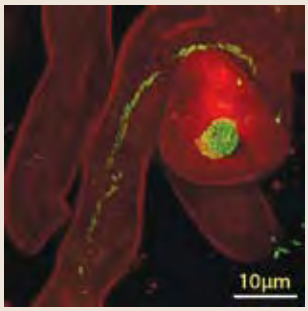
Box 3.8

Plant-Microbe Symbioses for Nitrogen Fixation

All life requires nitrogen—an essential component of proteins, nucleic acids, and numerous other organic compounds. Nitrogen is intimately linked to the carbon cycle because its biological availability can limit the extent and activity of primary production on land and in the oceans. Although nitrogen gas (N_2) makes up 78% of the atmosphere, only a limited number of prokaryotic microorganisms are capable of converting this gas into biologically usable ammonia through a process called nitrogen fixation. By carrying out most nitrogen fixation on Earth, these microbes act as gatekeepers of nitrogen into the biosphere.

Legume plants (e.g., soybean, peas, alfalfa, and clover) form symbiotic relationships with nitrogen-fixing microbes to use atmospheric N_2 directly as a nutrient source. Recent genomic and molecular insights into the symbiotic relationships between plants and microbes are discovering novel biological mechanisms for bringing nitrogen into the biosphere and revealing potential approaches to developing nonlegume crops that can fix nitrogen. Metabolic energy requirements for nitrogen fixation are high, using eight times more ATP—a molecular energy source in cells—than photosynthetic CO_2 fixation; thus legume crops tend to have lower yields than fertilized crops. Minimizing this trade-off between yield and nitrogen fixation will require a better understanding of the mechanisms controlling these agriculturally important symbioses.

The molecular conversations underlying plant-microbe collaborations in the root nodules of legumes are amazingly complex. Some key steps in the nitrogen-fixing symbiosis that establishes nodule formation in alfalfa root hairs are described below.



Legume Root Invasion. After an alfalfa root hair surrounds and internalizes a colony of *Sinorhizobium meliloti* bacteria, the bacteria (green) travel deep into the root through an infection thread. [Source: Limpens, E., et al. 2003. "LysM Domain Receptor Kinases Regulating Rhizobial Nod Factor–Induced Infection," *Science* **302**(5645), 630–33. Reprinted with permission from AAAS.]

1. The alfalfa root chemically attracts specific types of rhizobia bacteria in the surrounding soil by secreting a unique cocktail of bioflavonoids.
2. Rhizobia migrate toward the root and respond by secreting their own chemical messages called Nod factors.
3. The root-hair cells detect rhizobia's Nod factors. A spike in calcium concentration triggers changes in gene expression that initiate nodule development and changes in root-hair structure.
4. The root hair curls around and engulfs the rhizobia that penetrate the internal tissues of the root hair via a tunnel called an infection thread (see figure).
5. Deep inside the root hair, plant and bacterial cells divide repeatedly to form the nodule. Rhizobia can live freely in the soil but fix nitrogen only when housed within a root nodule. The rhizobia provide nitrogen in a form that plants can use; plants supply the bacteria with photosynthetically produced organic compounds.

nitrogen use efficiency. This efficiency, moreover, is strongly and negatively affected by abiotic factors, especially salinity and water stress. Identifying adaptive traits and mechanisms for efficient BNF in the presence of abiotic stress could therefore greatly advance efforts to manipulate ecosystems for increased productivity and to model their response to stress.

Increased understanding of BNF presents opportunities to potentially improve the process by using molecular breeding and transgenic approaches. Furthermore, recent advances in our knowledge of the early phase of legume-rhizobia interactions are beginning to make feasible the reconstruction of symbiotic development in nonlegume plants and manipulating BNF efficiency in legumes. Alternative strategies for BNF in nonlegumes also may develop from a better understanding of the ecology of endophytic bacteria (see section, The Plant Microbiome, p. 45), many of which have the capacity for BNF but whose current contribution to the plant nitrogen economy appears to be limited (see Box 3.9, Research on Plant-Soil-Microbe Interactions: Nutrient Limitations and Acquisition, p. 44).

Mycorrhizal Impact on Carbon Cycling and Biosequestration

Mycorrhizal associations between fungi and plants significantly influence the quantity, quality, and distribution of plant carbon delivered to soil. While nodules containing nitrogen-fixing bacteria are limited to only a few species of plants, mycorrhizae are relatively ubiquitous. In a mycorrhizal association, plant-produced carbohydrates are translocated to fungal partners. Plants in turn use the fungal mycelium's very large surface area and cell membrane chemistry to enhance absorption of water and mineral nutrients (especially phosphorus) from the soil.

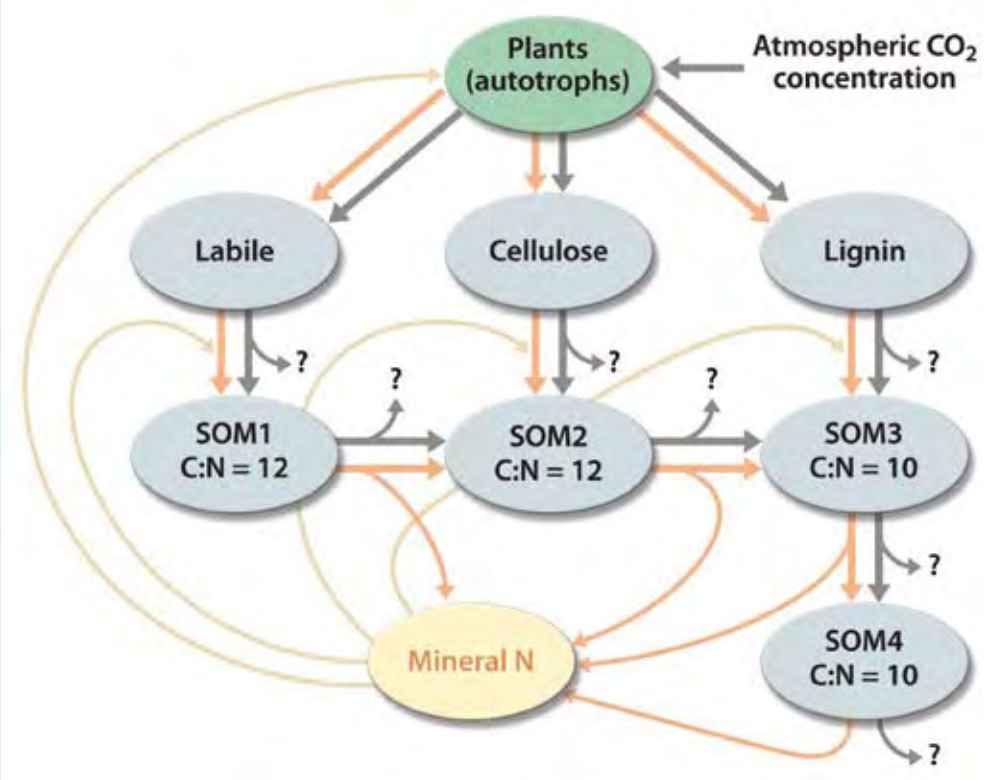


Fig. 3.6. Soil Carbon and Nitrogen Dynamics: Heterotrophic Cascade in the Decomposition of Plants. Heterotrophic decomposition of different plant material produces various pools of soil organic matter (SOM) and nitrogen. Better knowledge of the biochemical mechanisms and kinetic parameters associated with these processes is needed.

Box 3.9

Research on Plant-Soil-Microbe Interactions: Nutrient Limitations and Acquisition

Although the empirical effects of nutrient limitations on plant growth are well established, the biological and ecosystem mechanisms controlling such limitations are less understood. At the plant level, allocation patterns can shift with changes in nutrient status. To assess and predict the effect such shifts may have on carbon cycling, a better understanding is needed of the steady-state controls on allocation and how the process is affected by changing nutrient availability, CO_2 concentration, and climate.

Achieving this will require manipulative experiments and observatories that not only measure GPP and subsequent carbon fluxes but also nitrogen, other nutrients, and soil moisture. Also needed are widely deployable sensors that continuously measure nitrate- and ammonium-ion concentrations in soil and report such measurements in real time. Experimental and monitoring design should consider the following:

- Since nitrogen transformations in soil are microbially mediated, assessing soil microbiology using genomic tools provides a useful approach for measuring nitrogen availability to plants and identifying microbial molecular triggers and mechanisms for nutrient uptake.
- At the community level, different plant species have varying nutrient use efficiencies and allocation patterns, and as climate, CO_2, and nutrient availability changes, community structure also might shift, with important consequences for carbon-uptake potential.
- At the biome level, possible shifting dominance of entire vegetation communities (i.e., dynamic biogeography) in response to climate change could impact carbon cycling profoundly.
- At the global scale, rising CO_2 concentration potentially can affect nutrient availability (e.g., progressive nitrogen limitation), but various biomes, communities, and plants will respond differently to this forcing.

Mycorrhizae can affect plant tolerance of shifting climatic conditions such as changes in water availability. Furthermore, these plant-fungal associations can increase ecosystem NPP through their capacity to deliver potentially limiting nutrients to plants. On the other hand, as a major sink for photosynthate carbon, mycorrhizae also have the potential to decrease terrestrial NPP—they can absorb more than 30% of plant photosynthates. Recent data suggest some types of mycorrhizae produce extracellular enzymes involved in the breakdown of soil organic carbon; the implications of such activity are unknown.

Mycorrhizae are commonly divided into ectomycorrhizas and endomycorrhizas. The two groups are differentiated by the activity of fungal hyphae—branching, filamentous cells that collectively constitute the mycelium. Hyphae of ectomycorrhizal fungi do not penetrate individual cells within plant roots. Hyphae of endomycorrhizal fungi, however, penetrate plant cell walls and invaginate cell membranes. These invaginations increase the contact surface area between a hypha and the cell cytoplasm, facilitating nutrient release into the plant (see Fig. 3.7a–b. Distribution of Micronutrients in Plant Roots and Associated Fungal Hyphae, p. 45).

In addition to nitrogen, phosphate availability greatly affects plant productivity. Although some soils are inherently phosphate poor, many more have the nutrient but in forms inaccessible to plants. In agricultural systems, phosphate is supplied in fertilizer, increasing plant growth and thus biomass accumulation. However, the primary source of such fertilizer—rock phosphate—is diminishing rapidly.

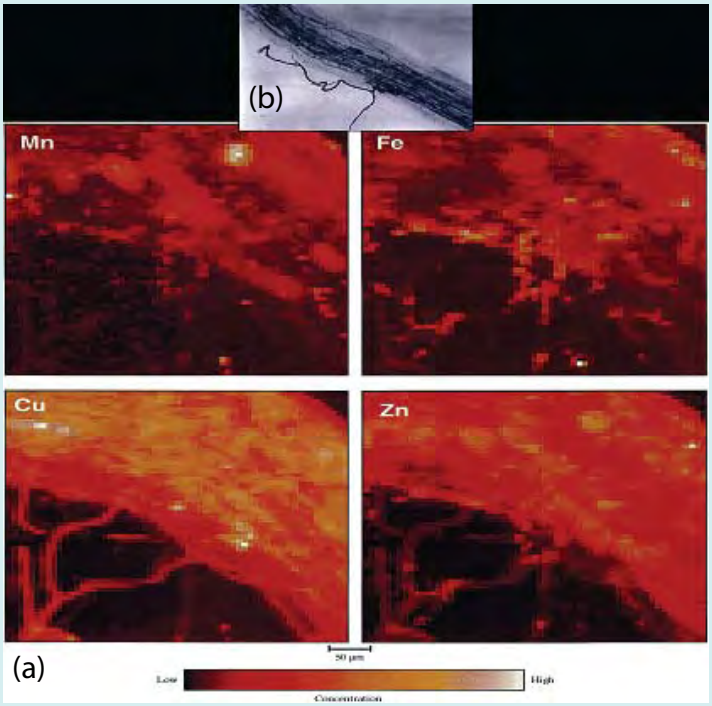


Fig. 3.7a–b. Distribution of Micronutrients in Plant Roots and Associated Fungal Hyphae. (a) Selected element-specific XRF images of a hydrated *P. lanceolata* root infected by the mycorrhizal fungus *G. mosseae*. The concentration ranges for the individual images are 0.03 to 0.87 mg cm^{-2} for Mn, 0.11 to 27.9 mg cm^{-2} for Fe, 0.03 to 1.55 mg cm^{-2} for Cu, and 0.04 to 4.98 mg cm^{-2} for Zn. The concentrations have not been corrected for small changes in the X-ray beam intensity or for attenuation of the X-ray fluorescence by the sample and may be accurate only to within a factor of two. **(b)** Confocal reference image of the X-ray images shown in (a). [Source: Yun, W., et al. 1998. "X-Ray Imaging and Microspectroscopy of Plants and Fungi," *Journal of Synchrotron Radiation* **5**, 1390–95. http://dx.doi.org/10.1107/S0909049598007225.]

Absent artificial application of phosphate, most plants derive the nutrient from mycorrhizae. Analogous to legume-rhizobia interactions, plants supply energy and carbon to fungi in the form of photosynthate in exchange for phosphate, other nutrients, and water scavenged from soil by fungi.

Increased understanding of the molecular mechanisms and ecological factors influencing the ubiquity and efficiency of mycorrhizal symbioses could yield great benefits to agricultural systems, especially as rock phosphate supplies dwindle. Likewise, deeper insight into the function of these symbioses in natural ecosystems will enable prediction of mycorrhizae response to climate change and shifts in plant species diversity. Such predictions in turn will guide strategies to prevent potential forced disruptions in mycorrhizae efficiency.

The Plant Microbiome

Plant surfaces and internal passages are colonized by a diverse array of microorganisms, many of which benefit their hosts. Mutualistic inhabitants of this plant microbiome, consisting of endophytic, epiphytic, and rhizospheric microorganisms, can influence plant metabolism, strengthen resistance to abiotic and biotic stress, enhance plant growth, increase access to limiting nutrients, and compete with or antagonize potential pathogens. Interestingly, there is evidence for specificity in many plant-microbe interactions, suggesting both strong selective pressure and competition within the microbiome. For example, chemical recognition factors allow plants to screen and recruit particularly useful bacteria from among the diverse microbial community. The factors underlying such specificity and selection are only now beginning to be revealed (see Box 3.8, Plant-Microbe Symbioses for Nitrogen Fixation, p. 42).

Due to the potential importance of these interactions to plant primary productivity, research must strive to understand the nature and function of the

Key Research Challenges

1. **Defining the structure and diversity of the plant microbiome, including differences among plant species.**
2. **Understanding the ecology of individual plant and microbial species and consortia and identifying features distinguishing beneficial microbial communities from detrimental ones.**
3. **Understanding the mechanisms by which microbial communities influence plant performance.**
4. **Characterizing specific adaptations underlying endophytic and epiphytic microbial functions.**
5. **Elucidating the mechanisms used by plants and the degree to which they influence the composition and properties of co-resident microbial populations to gain deeper insight into symbiotic mechanisms (e.g., plant avoidance of pathogen infestation and microbial avoidance of plant chemical defenses).**

plant-associated microbiome. Such knowledge could inform strategies to control beneficial plant-microbe associations, potentially providing an indirect but important means to alter plant productivity. For example, measurements by Rohde et al. (2007) of the sequenced tags that were differentially expressed at various developmental stages of dormant poplar buds identified 141 poplar-derived genes, 122 bacteria-derived genes, and 142 transcript-derived fragments (called TDFs) of currently unknown origin. This proportion suggests a close association of poplar with a bacterial community during the development process.

Mechanisms of Carbon Transfer from Plants to Soils

Mesofauna

The movement of organic matter from plant to soil systems is a key component of the terrestrial carbon cycle. Influencing this transfer of material are multiple factors outside plant and microbial communities, including mesofauna and the hydrologic cycle. Changes in the distribution of mesofauna (e.g., earthworms) directly correlate with shifts in organic matter stocks (Drake and Horn 2007; Bohlen 2006; Daane, Molina, and Sadowsky 1997) and thus likely with regional carbon fluxes. Such correlations emphasize the importance of these organisms in carbon flow through ecosystems (see sidebar, The Role of Earthworms in Processing Soil Organic Carbon, p. 47).

Hydrologic Cycle

Another component profoundly influencing terrestrial carbon flow is the hydrologic cycle. Delivery of soluble carbon to subsurface environments via the hydrologic cycle is particularly critical. Soluble-carbon fluxes impact long-term carbon stabilization through sorption. Such fluxes also affect microbial carbon dynamics through the horizontal and vertical movement of carbon. Projected shifts in the hydrologic cycle resulting from climate change will in turn alter terrestrial carbon cycling and stabilization. Thus understanding the mechanisms of carbon transfer, especially as it is coupled to the hydrologic cycle, is critical to projecting the future cycling of carbon.

Key Research Questions

1. **What are the abiotic and biotic factors and interactions that determine the availability of nutrients?**
2. **How are the carbon, nutrient, and water cycles linked to determine ecosystems' productivity, carbon biosequestration, and responses to climate change?**

Senescence

Senescence is a highly orchestrated developmental end stage in the life cycle of plants or their substructures. The onset of senescence is controlled by signaling cascades that initiate changes in gene expression and the synthesis of new proteins (Hopkins et al. 2007). Roots in particular provide an obvious path for moving carbon and energy from plants to soils. Thus, root senescence and eventual mortality significantly impact the carbon cycle in terrestrial ecosystems (Matamala et al. 2003). However, accurate estimates of root life cycles elude researchers, and significant uncertainties surround the influence of root senescence on rates of soil carbon biosequestration. In addition, the uncertainties in estimating these factors prevent accurate quantification of NPP and belowground carbon allocation in a range of

The Role of Earthworms in Processing Soil Organic Carbon

The lowly earthworm carries out a multitude of sophisticated functions in the biochemistry of SOM formation. Earthworms can process large amounts of litter and soil in many productive ecosystems, and their activity often is associated with faster nutrient turnover rates. Interactions with soil microorganisms mediate earthworms' effects on nutrient cycling and organic-matter turnover. As prokaryotic microbes transit the gut of the earthworm, a mobile anoxic microzone is created, having high concentrations of organic substrates. These substrates stimulate a subset of denitrifying and fermentative bacteria within the earthworm gut (Drake and Horn 2007; Bohlen 2006; and Daane, Molina, and Sadowsky 1997). Activities of these bacteria result in the in vivo emission of denitrification-derived dinitrogen (N_2) and the greenhouse gas nitrous oxide (N_2O) by the earthworm, affecting the fitness, culturability, and diversity of soil microbial biomes. Earthworms also facilitate lateral gene transfer in transiting microbes, serving as a biological factor assisting their cell-to-cell contact (Daane et al. 1996).

ecosystems (Strand et al. 2008). On the other hand, roots' critical contributions to the formation of soil organic matter are well established. Current estimates indicate fine-root turnover contributes a significant portion (more than 50%) to annual NPP in many terrestrial ecosystems.

Senescence is distinguished from other types of programmed cell death by the plant's recovery of carbon and nitrogen from dying tissue and the subsequent translocation of these nutrients to growing parts of the plant (e.g., developing seeds or perennial roots). A complete understanding of the genes, gene networks, and protein complexes facilitating this translocation is needed as is expanded knowledge of the molecular controls of fine-root mortality. Such insight on senescing roots will advance our understanding of terrestrial carbon cycling significantly.

Controlling Factors of Carbon Recalcitrance and Biosequestration

In some situations, microbial processing of plant-derived biomass can yield recalcitrant forms of carbon as a byproduct. Production and persistence of this stable carbon depend on both physical and chemical factors. For example, the longest-lived soil organic matter is aggregated with soil minerals. Formation of long-lived carbon is controlled by edaphic factors (i.e., soil characteristics, especially chemical or physical properties, that influence biota) as well as plant and microbial regulatory processes.

Soil Characteristics

Edaphic factors have important yet poorly understood effects on several plant processes regulating the persistence of carbon residues in soil organic matter. Processes affected by soil characteristics include production and composition of root exudates; root architectural patterns (and thus location of rhizodeposition); mineral-nutrient density; and the metabolism of phenolic compounds, silicon biocomposites, and other materials that may regulate litter-decomposition rates. Soil factors also significantly influence root turnover and rhizosphere communities, yet how they do so is unclear.

Environmental Conditions

The residence time of carbon is another key factor affecting the potential of its biosequestration in different soils. When the primary control on residence time is limited to decomposition induced by environmental extremes such as low temperature and oxygen, carbon inputs may be sequestered seemingly without constraint (e.g., in boreal peat deposits). However, such carbon is vulnerable to release from storage if environmental conditions moderate. Under a more biologically favorable environment, biochemical alteration and physicochemical protection (e.g., aggregates and sorption) are the primary mechanisms controlling residence of carbon in soil organic matter. Even with constant input, conditions or manipulations increasing residence time in soil can effectively sequester carbon. Identifying such conditions can help guide strategies to increase both carbon input rates and residence time, leading to enhanced carbon biosequestration.

Mineral Interactions and Aggregate Formation

Mineral interactions inhibiting chemical alteration of soil organic matter also significantly increase its residence time. In various stages of alteration, soil carbon can be protected from microbial degradation by an array of molecular associations with mineral surfaces. These largely chemical interactions depend on various factors such as SOM characteristics, reactivity and surface traits of soil minerals, base-cation status, pH, redox condition, and the presence of Fe and Al oxides. Numerous processes affect the physicochemical protection of soil carbon, including diffusion of soluble or colloidal carbon, advection of dispersed particles, mechanical actions of plant and fungal growth, mixing by soil mesofauna, localized hydration changes, freeze-thaw cycles, and mechanical disturbances such as tillage. Further SOM protection occurs when mineral-carbon aggregates physically impede microbial access to substrates or moisture conditions and soil structural controls on gas exchange inhibit decomposer activity.

Current widely used models simulating decomposition of soil organic matter are based on conceptual SOM pools described by first-order kinetics. Dating back to Olson (1963), this concept was first used in a multipool soil model by Jenkinson and Rayner (1977). Using such models has considerably advanced scientific knowledge of rate coefficient relationships with soil temperature and moisture conditions as well as interactions with nitrogen dynamics (including fixation by microbes in soil).

Also improved is our understanding of the relationships among particulate organic carbon (POC), mineral-associated organic carbon (MOC), dissolved organic carbon (DOC), and soil mineral particles. These carbon compounds and minerals form soil macroaggregates physically protecting POC from commutation and some types of decomposition. Moreover, such aggregates develop microsites in which organic matter is transformed less aerobically into humic compounds stabilized by intimate associations with mineral particles and formation of recalcitrant chemical compounds. This type of SOM protection and stabilization in most soils thus occurs in two stages: (1) macroaggregate formation that physically protects organic carbon and modifies the soil environment to enhance humification and (2) microaggregate formation that allows transformation of organic carbon into more stable humic compounds, thus shielding soil organic matter from decomposition. A process model broadly describing these dynamics has been suggested,

but improved understanding is needed (see section, Clays and Stable Humus and Fig. 3.8. Example of Soil Organic Carbon Model, this page).

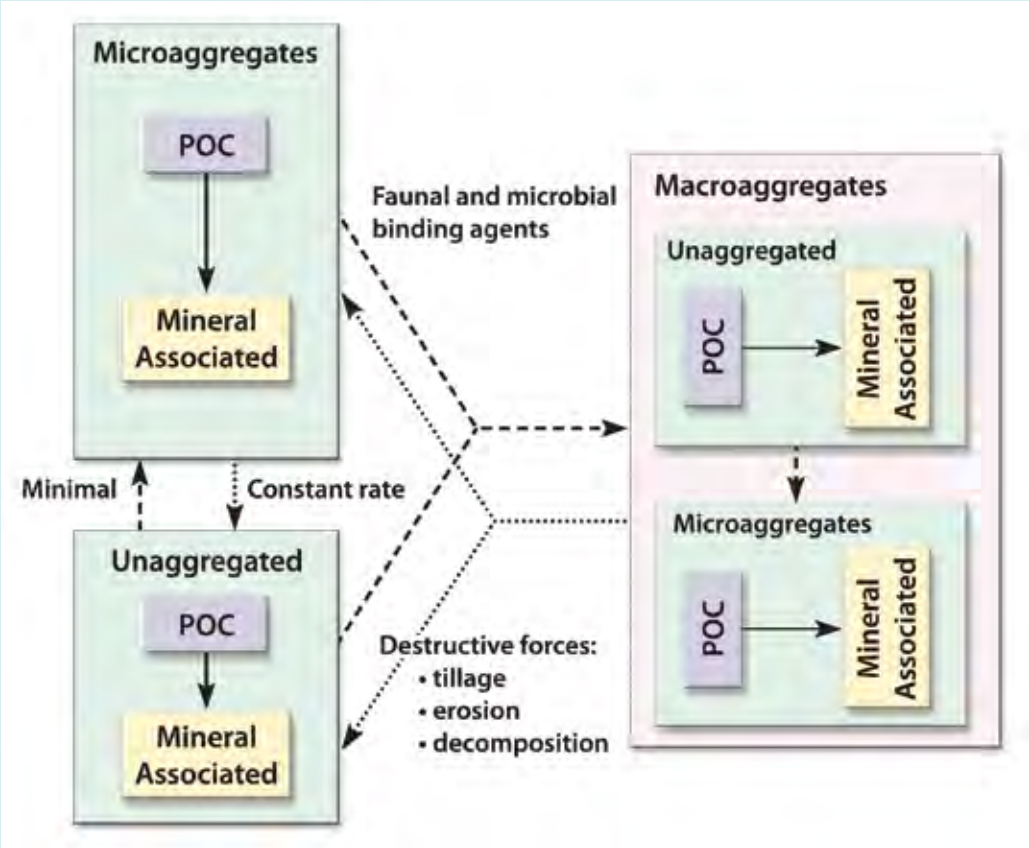
Clays and Stable Humus

Organic-matter decomposition releases into the soil humus and valuable nutrients accessible to and readily used by plants. When combined, humic acids, free nutrients, and clay produce stable humus, which in soil acts as a nutrient storehouse accessed by plants only when concentrations of decomposing organic matter are low. In addition to its critical role in plant nutrient availability, stable humus serves as a buffer for water and pH in soil. Equally significant to plant and soil health are the layers in clay, which provide important structural features, reactive surfaces, and space for microorganisms to function. Good soil structure facilitates nutrient uptake by growing plants.

Rates of Soil Carbon Stabilization and Destabilization

Various processes and changes, such as soil development, land-use shifts, and disturbance, influence carbon stabilization and destabilization rates. Table 3.1. Carbon Stabilization and Destabilization Rates Observed in Soils, p. 50, summarizes documented rates of change in soil carbon under several conditions. Understanding the processes controlling these rates is necessary for predicting how various conditions

Fig. 3.8. Example of Soil Organic Carbon (SOC) Model. Such models as the one depicted here are based on integrating soil aggregated dynamics and SOC kinetics, which provide the conceptual framework for modeling physicochemical processes in soil. Depicted are two classes of aggregates, macroaggregates (>250 µm) and microaggregates (53 to 250 µm), along with an unaggregated fraction of soil consisting of silt and clay particles and their interactions. Each aggregate contains two organic matter classes—particulate organic carbon (POC) and mineral-associated organic carbon (MOC). Each of these organic compounds can be extracted directly from soil with a combination of sieving, density flotations, and chemical digestion (see also Fig. 3.4. Microbial Communities and Soil Carbon Cycling and Storage, p. 37).



affect the stability of soil carbon and thus its potential biosequestration. On longer time scales, carbon-stabilization rates are associated with mineral transformations and SOM-mineral interactions. Explaining the decadal and more rapid changes observed in carbon stability is more difficult. For example, researchers debate whether observed changes in United Kingdom carbon stocks (Bellamy et al. 2005) and river DOC result from warming and associated decomposition or changes in ecosystem buffering capacity and rainfall pH. Also noteworthy to scientists are observed rates of change in nitrogen-amended tundra soil. Although mechanically undisturbed, this soil's vegetation has changed from tussock to shrub and shows large, rapid losses of centuries-old carbon. Such losses likely are associated with shifts in root depth and soil microbial communities rather than in soil temperature. (Some emerging ideas on processes determining carbon stability are discussed in Box 3.10, Emerging Theories of Soil Carbon Stability in Relation to Microbial Activity, p. 51.)

Vegetation shifts likely will occur on time scales similar to those of gradual warming (e.g., decades to centuries). Determining which of these changes is likely to have a more profound effect on soil carbon stocks will enable better prediction of the overall response of such stocks to climate change. Whether incubation studies (i.e., analyses of warming response in the absence of vegetation adaptation to altered climate conditions) offer the best option for making such predictions must be evaluated (see Box 3.11, Research on Soil Carbon Recalcitrance, p. 52).

Another challenge in predicting soil carbon stability is the lack of important but difficult to measure processes in plot-scale studies. For example, processes dominating soil carbon stocks within dynamic landscapes are not necessarily observed as such in plot-scale studies, which may assign greater importance to other factors. In particular, eddy covariance towers—measuring vertical turbulent fluxes including CO_2—by necessity are located on flat land, but most soil carbon stocks in a given landscape could be concentrated in poorly drained riparian zones (Davidson

Table 3.1. Carbon Stabilization and Destabilization Rates Observed in Soils

Process	Rate (MgC ha^{-1} yr^{-1})	Duration	Reference
Increase in organic matter during soil development (young soils 3000 to 10,000 years old)	+0.02	Thousands of years	Schlesinger 1990
CO_2 removal from atmosphere by organic matter accumulation and silicate weathering	+0.085 (young soils) +0.007 (old soils)	Thousands of years	Chadwick et al. 1994
Soil development (first 50 years)	+0.11 (surface litter) –0.03 to +0.3 (soil)	~50 years	Quideau et al. 2000
Carbon accumulation in surface litter after fire (boreal)	+0.03 to +0.3	~100 years	Trumbore and Harden 1997
Loss of carbon from upper 15 cm in United Kingdom soils	–0.7 to –1.2 (low-carbon soils) –5.5 (peat soils)	1973–2003	Bellamy et al. 2005; Schulze and Freibauer 2005
Drainage of peatland (Sacramento Delta)	~ –11.0	Decades	Deverel and Rojstaczer 1996
Conversion of tropical forest to pasture	–0.4 to +1.7	~20 years	Trumbore, Chadwick, and Amundson 1996
Nitrogen amendment (tundra soil)	–1.0	20 years	Mack et al. 2004
Aggregate stabilization and destabilization	+0.8	10 years	DeGryze et al. 2004

Box 3.10

Emerging Theories of Soil Carbon Stability in Relation to Microbial Activity

Soils store large amounts of carbon because microbes are unable to break down and mineralize all organic matter on short time scales. Carbon accumulation thus indicates constraints on soil carbon processing by microbial communities despite their genetic and metabolic diversity. Such constraints may be physical or biological, and the relative importance of each must be investigated in future experiments to predict how changes in microbial communities will affect carbon cycling.

The physical protection of organic matter against microbial breakdown has long been recognized as contributing to soil carbon storage (Sollins, Homann, and Caldwell 1996). Interactions between carbon compounds and soil minerals impede microbial and enzymatic access to organic compounds, regardless of their chemical form. Kleber, Sollins, and Sutton (2007) recently proposed that much of the stability resulting from these interactions is due to the formation of several distinct layers of organic material coating the surfaces of soil minerals.

Biological and chemical mechanisms also contribute significantly to soil carbon storage. Chemically complex and heterogeneous, soil organic matter has high concentrations of humic substances (MacCarthy and Rice 1991). The random chemical structure of such substances prevents microbes from easily targeting them with specific enzymes, thus allowing humic compounds to persist in soil (Allison 2006). However, this chemical structure has never been defined, and recent research suggests humics may not represent a distinct class of chemical compounds, but rather a complex mixture of known biopolymers, such as carbohydrates, proteins, and lignins (Kelleher and Simpson 2006; Lehmann et al. 2008). Nonetheless, the complexity of this mixture may constrain microbial decomposition because degrading any single constituent would require more energy than microbes could expend and still survive.

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and Lefebvre 1993). The paper pointed out the importance of small pockets of histosols in determining soil carbon stocks in Maine. Further complicating efforts to evaluate soil carbon balance is an incomplete understanding of carbon dynamics at the regional scale. Achieving such an understanding requires assessing the magnitude of carbon fluxes associated with the fate of eroded or leached carbon and investigating recovery of SOM stocks in eroded lands, a matter of contention among researchers (e.g., Van Oost et al. 2007). Furthermore, long-term observations and models focusing on carbon balance at the stand level must be scaled up to estimate carbon fluxes at the regional scale.

Determining the importance of dynamic deep-soil carbon pools is also critical to advancing our knowledge of soil carbon biosequestration. Although comprising only a small percentage of the total carbon stock, such pools contain large volumes of soil and thus are potentially significant. Understanding their functions requires investigating root and rhizosphere processes, including fine-root dynamics studies

Box 3.11

Research on Soil Carbon Recalcitrance

Understanding all the factors involved in hierarchical aggregate dynamics requires experimental and observational methods to quantify rates of aggregate formation and dissolution and to discover details of processes facilitated by organic carbon interaction with soil minerals. Achieving this knowledge requires:

- Development of new laboratory and field experiments with associated in situ observations, sampling methods, and nondestructive analysis techniques to improve quantification of aggregate population dynamics.
- Establishment of measurement methods to identify microbial populations, enzyme activity, oxygen concentrations, and their distribution within aggregates and mineral particle surfaces.
- Development of approaches to capture long-term dynamics related to decomposition of coarse woody debris, biochar, and long-term soil organic matter.
- Use of emerging isotopic approaches to measure the role of soil organisms and microbial communities in soil carbon cycling.
- Spatial and temporal analyses of the relationship between microbial community structure and function and community response to disturbance. Metagenomic measurements of soil diversity and the expression of that diversity through function will be critical.

Key Research Questions

1. **How does aggregate turnover and stabilization affect carbon storage and turnover in various soils under different biological, edaphic, and environmental conditions?**
2. **What forms of carbon are stored in aggregates, and how stable are they?**
3. **How does vegetation type or species affect aggregation and resultant carbon biosequestration?**
4. **What are the saturation levels of various aggregate-associated carbon pools in assorted soils under different carbon biosequestration controls?**
5. **How can we optimize the role of aggregation in carbon biosequestration? What amendments to conditions might increase aggregation (e.g., calcium availability, organics, and increased root or fungal growth)?**
6. **How does aggregate size distribution and stability affect localized redox conditions, microbial habitats, and enzyme stability? How do these responses in turn affect humification?**
7. **Can we measure aggregate turnover? If so, does this predict carbon turnover?**
8. **What critical aggregate-associated properties must be measured routinely to best predict carbon turnover or stabilization?**
9. **Can aggregate properties, dynamics, and processes be incorporated into models to improve predictions of soil carbon dynamics?**

with new ideas on estimating and modeling (Guo et al. 2008), and assessing the importance of priming (Fontaine et al. 2007) versus physical or structural changes.

Potential Climate Impacts on SOM Stabilization Mechanisms and Carbon Pools

Several climate-related factors, including temperature, moisture, pH, and vegetation changes, potentially can affect SOM interactions, physical accessibility, and physical biochemistry, ultimately determining the stocks and stability of soil organic matter. Descriptions of these potential changes and their effects on soil carbon are given in Table 3.2. Potential Climatic Effects on Major SOM Stabilization Mechanisms, p. 53.

Table 3.2. Potential Climatic Effects on Major SOM Stabilization Mechanisms

Controls	Interactions (limited by sorptive protection)	Accessibility (limited by aggregation)	Physical Biochemistry (O_2 requirement, solubility, molecular size)
Moisture	1. Precipitation reactions result from desiccation. 2. Lack of solvent decreases stability of hydrophobic bonding (entropy-driven adsorption processes). 3. Moisture content may selectively control desorption processes: High moisture desorbs hydrophilics; low moisture releases hydrophobics.	1. Moisture affects mobility of bacteria, not so much that of fungal hyphae. 2. Excess moisture may destroy aggregates (slaking).	1. Lack of oxygen stabilizes SOM. 2. Nonpolar molecules aggregate in aqueous environments. 3. Van der Waals bonds are additive: Large hydrophobic organic fragments adhere better to surfaces than small fragments.
Temperature	1. Higher temperatures may enhance diffusion and increase mobility of solutes. 2. Loss of reactive, single-coordinated hydroxyls: Minerals may change crystallinity if exposed to elevated temperature (ferrihydrite → hematite at 40°C).	1. Increase in temperature may enhance mobility of organisms (e.g., bacteria). 2. Increases stimulate fungal hyphae growth (and vice versa).	1. Higher temperatures may promote abiotic condensation reactions (MnO_2). 2. Changes may lead to shifts in phase properties (i.e., "glass transition").
pH	1. Protonation and deprotonation change mineral surface reactivity. 2. Low pH removes bonding cations. 3. Extreme pH values may dissolve minerals: Toxic to Al and Mn below pH 5. 4. Changes in pH may affect ability to condition and standardize heterogeneous mineral surfaces.	1. pH selects for bacterial (high pH) versus fungal (low pH) dominance.	1. Ionization of organic functional groups affects solubility.
Vegetation Change	1. Exudation of low-molecular acids dissolves minerals.	Changes include: 1. Amounts and distribution of fine roots. 2. Plant-specific mycorrhizae with variable efficiency. 3. Exudates acting as "glue." 4. Root and shoot ratio (above- versus below-ground input). 5. Submicron-size aggregate formation.	Different carbon-allocation patterns may lead to: 1. Higher inputs of more labile materials (priming effect). 2. Hydrophobicity or hydrophilicity of plant inputs. 3. Changes in relative proportions of lignin and substituted fatty acids.

Technology Requirements for Research on Carbon Processing in Soils

Methodological Needs

- Library of known compounds for proteins (proteome) and metabolites (metabolome) to interpret mass-spectrometry data.
- Microsensors to make high-frequency, high-resolution spatial and in situ measurements of assimilable carbon, enzymes, and metabolites.
- In situ and real-time techniques to visualize the location, identity, and function of various taxa. Researchers must have ready access to such techniques and be informed of how these tools can help answer specific scientific questions.
- Model development at all scales.
- Real-time, in situ, and fine-scale synchrotron measurements to monitor compounds.
- Increased use of isotopes as tracers.

Infrastructure (Cyberinfrastructure and People) Needs

- Improved bioinformatic tools to provide better annotation, modeling-interface capabilities, and open access. Fungal and viral taxonomists must contribute to enhancement of such tools, and researchers with carbon cycling expertise should spearhead annotation.
- Cyberinfrastructure needed to keep information flowing easily.
- Increased accessibility to cutting-edge technologies (e.g., synchrotron, nano-SIMS, and atomic force microscopy) and better assimilation of data into structural models.
- New configurations of multidisciplinary research efforts, requiring collaboration and teams such as confederations in DOE's Genomics:GTL program.

New Molecular Capabilities (Genomic, Transcriptomic, and Proteomic Levels)

- Greater sequencing capacity for bacterial, archaeal, and fungal communities.
- Improved targeting techniques to isolate and separately sequence functional groups' nucleic acids to generate more interpretable data.
- Development of standard extraction protocols with quantifiable bias for DNA, RNA, and proteins.
- Efforts to expand data collection to include abundance as well as diversity information (both relative and absolute).
- More and better annotation, particularly of genes coding for degradative enzymes and those involved in environmental stress response to changes in moisture, pH, and redox, for example.
- Improved mRNA extraction and isolation techniques, especially for dry or low-biomass soils.
- Sequencing techniques able to handle small volumes of DNA and RNA without amplification or tools using amplification but reducing the associated biases.

Strategies for Optimizing Productivity and Carbon Biosequestration in Managed Ecosystems

Research to Support Carbon Biosequestration Strategies

Managed lands account for about 30% of current global terrestrial net primary productivity (NPP), and ongoing land-use changes will cause this percentage to increase steadily. Establishing a basis for optimization of carbon fixation and biosequestration requires a fundamental research approach resulting in molecular mechanistic strategies for carbon capture. Such an approach involves the following:

1. **Identifying basic processes** underlying gross primary productivity (GPP) and NPP of terrestrial plants, examining molecular controls on above- and belowground NPP components, and assessing areas in which knowledge gained through mechanistic studies could lead to enhanced carbon biosequestration in plant biomass and soils.
2. **Considering how efficient acquisition** and use of resources (e.g., nutrients and water) help maximize GPP and NPP rates in terrestrial plants; identifying the molecular basis of such efficiency; and assessing interactions between carbon and other resources potentially important in determining the rate, magnitude, or sustainability of biosequestration.
3. **Evaluating how GPP and NPP could be optimized** in plant populations and communities and considering the roles of genetic diversity and resource utilization in carbon biosequestration. One objective of such evaluations is maximizing NPP and litter input to soils over, for example, a growing season.
4. **Generating dynamic models** (e.g., in silico leaf and plant) that predict how changes in genetic regulatory networks might be used to enhance GPP or NPP by altering metabolic and developmental pathways in response to external perturbations or genetic manipulation.

Potential Strategies for Leaf-Level Manipulation of Carbon Fixation in Managed Ecosystems

Emergent mechanistic and systems-based GPP models are providing potential opportunities to substantially increase carbon fixation in managed ecosystems, impacting DOE strategies for fulfilling carbon biosequestration and biofuel missions. The following are examples of such strategies:

1. **Modifying Diffusion Resistance to CO_2 Transport in Leaves.** The resistance to CO_2 diffusion between a C_3 leaf compartment and the active site of Ribulose-1,5-bisphosphate carboxylase/oxygenase [(RuBisCo), a critical enzyme in carbon fixation] is referred to as mesophyll resistance. Because it significantly limits carbon acquisition (24% reduction) and water and nutrient use efficiencies, robust research is needed to understand the physical and biological basis of this phenomenon.
2. **Suppressing or Bypassing Photorespiration.** Originating in an ancient, oxygen-free atmosphere, RuBisCo evolved without the ability to discriminate between its primary substrate, CO_2, and oxygen. RuBisCo's reaction with O_2—in photorespiration—results in a 35% reduction in carbon capture (Ainsworth and Rogers 2007). Strategies to enhance fixation include redesign of RuBisCo or development of more-efficient carbon and energy pathways to manage the oxidation products of photorespiration (Kebeish et al. 2007).
3. **Engineering Maladapted RuBisCo in Plants.** RuBisCo in current C_3 plants is optimized for historic CO_2 concentrations of 200 ppmv (Zhu, Portis, and Long 2004). Introducing into C_3 plants the RuBisCo from other species having greater catalytic activity (thus better suited for higher CO_2 concentrations) would increase carbon gain dramatically despite C_3 plants' inferior ability to discriminate CO_2 and O_2.
4. **Optimizing Nitrogen Distribution within the Photosynthetic Apparatus.** Nearly half the nitrogen invested in soluble protein within leaves is in RuBisCo. An analysis of the optimal distribution of nitrogen resources among enzymes involved in carbon metabolism projected that manipulating the partitioning of such resources (e.g., in the regenerative phase of the Calvin cycle) could enhance carbon acquisition greatly without increasing the total nitrogen requirement (Zhu, de Sturler, and Long 2007).

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Strategies for Optimizing Carbon Productivity, Partitioning, and Biosequestration at the Plant Level

1. Minimize Carbon-Sink Limitations and Negative Feedback on Photosynthesis.

Carbon source-sink interactions significantly impact photosynthesis and plant growth. New experiments, including studies examining elevated atmospheric CO_2, show that limited sink capacity decreases photosynthetic rates in leaf tissue. For example, C_3 plant productivity often is limited by sink capacity. Productivity will decrease further as elevated atmospheric CO_2 concentration rises. One of the most pronounced and universally observed responses of C_3 plants to elevated CO_2 concentration is accumulation of foliar carbohydrates, even when root volume is unrestricted (Long et al. 2004). Large increases in soluble carbohydrates in leaves usually indicate carbon sinks are replete, a condition having two important implications. First, plants could use additional carbon to improve productivity or biosequestration potential. Second, since carbohydrates diminish photosynthetic capacity, plants may be unable to fully exploit the benefit of rising CO_2 concentration. Moreover, simple shading experiments in which a portion of photosynthetically active leaves are wrapped in foil to eliminate their contribution to carbon assimilation have demonstrated sink-stimulated increases in the photosynthetic rates of unshaded leaves. Taken together, these results show photosynthetic activity is tightly regulated by sink demand. Thus, reducing sink limitations on photosynthetic rates will increase plant productivity.

Opportunities to reduce these limitations arise from recent experiments suggesting sink regulation of photosynthesis is mediated by alterations in phloem loading (Chiou and Bush 1998; Vaughn, Harrington, and Bush 2002). For example, decreased sink demand leads to sucrose accumulation in the companion cells of leaf phloem. A sucrose-sensing system detects this increase and represses expression of the proton-sucrose symporter that loads the phloem (Vaughn, Harrington, and Bush 2002). Transcriptional repression, combined with high rates of symporter turnover (Ransom-Hodgkins, Harrington, and Bush 2003), lowers phloem loading. This in turn causes sugar accumulation in the mesophyll, leading to hexose-mediated decreases in photosynthetic gene expression and lower rates of photosynthesis (Goldschmidt and Huber 1992; Krapp et al. 1993; Krapp and Stitt 1995; Sheen 1994). Increasing sink capacity and uncoupling photosynthesis from sink regulation by controlling phloem loading thus offer significant strategies for enhancing plant productivity and ultimately carbon biosequestration.

2. Optimize Carbon-Nitrogen Metabolism to Increase Plant Productivity.

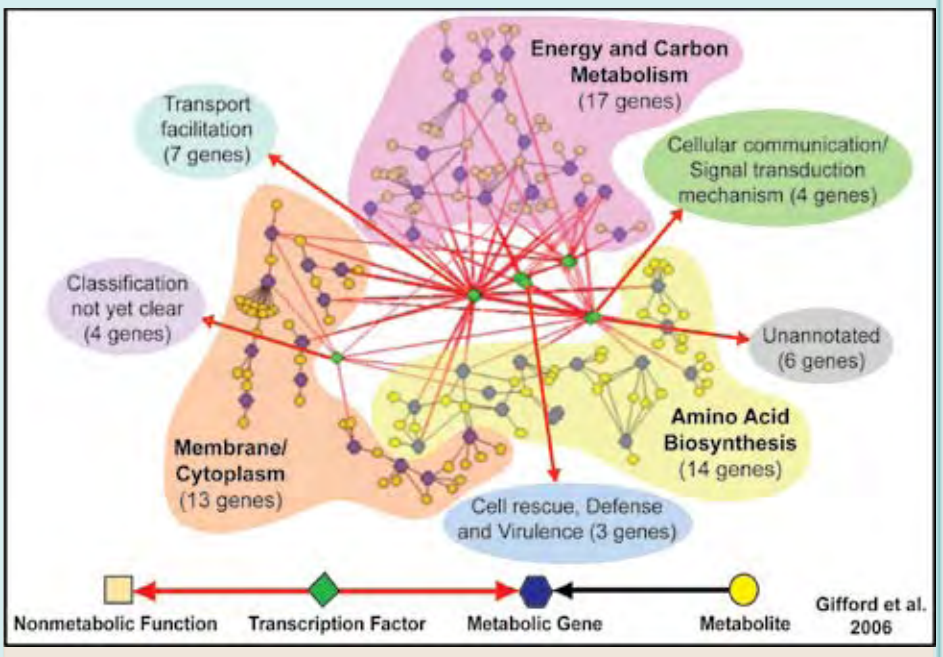
The relationship between CO_2 and nitrogen assimilation is critical to plant productivity. Assimilation of inorganic nitrogen into its organic form requires photosynthetically derived carbon skeletons to serve as backbones for transforming nitrogen into amino acids. These amino acids are used for DNA and protein synthesis and the formation of metabolic systems, whose subsequent activity determines plant capacity for growth and productivity. Linked to such productivity is resource partitioning, which also is influenced by plant central metabolism. Metabolic responses of carbon and nitrogen to genetic and environmental cues largely determine the partitioning of these resources among major biosynthetic pathways. However, the link between metabolism, productivity, and partitioning is poorly understood. Improved understanding of central metabolism is thus needed to guide strategies for enhancing productivity or altering partitioning.

Achieving such insight requires integrating physiological, biochemical, and genomic data using systems biology approaches. Such approaches expand our mechanistic understanding by linking different fields of scientific investigation (and biological organization). For example, ample evidence suggests carbon metabolites (sucrose and glucose), acting as "signals" of carbon status, interact with signals for nitrogen status (e.g., nitrate) to control genes directing metabolic and developmental processes such as nitrogen assimilation and amino acid synthesis as well as germination, shoot and root growth, and flowering. While recent genomic studies confirm the existence of complex carbon- and nitrogen-responsive gene networks in plants, the mechanisms for carbon and nitrogen sensing and signaling remain largely unknown. Systems biology approaches are just beginning to identify gene regulatory networks controlling the coordination of plant carbon and nitrogen metabolism with other cellular processes (Gutiérrez et al. 2007, 2008; see figure, Multinetwork Analysis of a Carbon- and Nitrogen-Responsive Metabolic Regulatory Network, at right). Expanding these genomic

and systems biology approaches to identify regulatory networks coordinating carbon and nitrogen metabolism with development should reveal key regulatory hubs whose alterations in transgenic plants may be used to enhance carbon and nitrogen use efficiency, energy use, and plant productivity.

3. Modify Carbon Partitioning in Plant Organs and Soil Organic Matter.

Modification of plant morphology and phenology can have substantial impacts on productivity. Morphological changes can enhance gas exchange in shoots and increase nutrient and water acquisition in roots. The controls of root architecture are poorly understood yet represent great potential for increased productivity and carbon biosequestration in soil. Similarly, the timing of leafing out, canopy closure, and leaf senescence have not been major targets in efforts to increase biomass yields, yet all three offer significant opportunities for improving plant productivity and biomass generation.



Multinetwork Analysis of a Carbon- and Nitrogen-Responsive Metabolic Regulatory Network. An *Arabidopsis* multinetwork (Gutiérrez et al. 2007) was used to generate a regulatory network consisting of metabolic genes (blue hexagons) regulated by carbon, light, and nitrogen treatments. This predicted metabolic regulatory network, containing genes involved in energy metabolism and amino acid biosynthesis, is connected by several transcription factors (green diamonds) that may act as network hubs to coordinate regulation of carbon and nitrogen metabolic genes. The processes associated with nonmetabolic genes, also potentially regulated by these transcription factors, are listed in colored ovals; numbers of genes within each category are shown in parentheses. Importantly, these genes include some of unknown function, which can now be associated with nitrogen regulatory networks. [Source: Gifford, M. L., R. A. Gutiérrez, and G. M. Coruzzi. 2006. "Modeling the Virtual Plant: A Systems Approach to Nitrogen–Regulatory Gene Networks," Essay 12.2, Chapter 12: Assimilation of mineral nutrients. http://4e.plantphys.net/article.php?ch=12&id=352. In *A Companion to Plant Physiology, Fourth Edition* by Lincoln Taiz and Eduardo Zeiger. Sinauer Associates, Inc., Publishers, Sunderland, Mass. (See also http://www.virtualplant.org and Gutiérrez, R. A., et al. 2007.)]

As critical components in the acquisition of diffusion-limited nutrients, root hairs—subcellular extensions of root epidermal cells—also profoundly contribute to productivity. These organs are active sites for rhizosphere modification via plant exudates and therefore rhizodeposition of fixed carbon. Genotypic variation in root-hair length and density, which differ substantially among and within species, is highly correlated with phosphorus uptake, thus influencing plant growth and competitive ability in low-phosphorus soils. Quantitative trait loci—stretches of DNA linked to genes of particular interest, in this case those controlling root-hair length and density—have been identified in crop plants and explain about half of phenotypic variation. Modern genetic tools (e.g., association mapping and map-based cloning) allow researchers to identify major genes controlling these root-hair properties that clearly influence carbon biosequestration.

Understanding plant perennialism and exploiting the mechanisms directing it also have the potential to greatly increase carbon production and storage. Perennial crops, particularly their root systems, offer many advantages over annuals. These superior root systems allow rapid, robust plant growth in spring while reducing soil erosion and the need for energy-demanding agricultural inputs such as fertilizer. Two major crops, maize and wheat, have perennial

(continued next page)

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relatives that can serve as starting points for introgression of perennial traits into annuals. However, little is known about the molecular basis of perennialism, making vigorous research essential to fully using perennial mechanisms to improve plant productivity.

4. Use Genetic Approaches to Discover Genes Controlling Biomass.

Identifying previously unknown genetic loci directing plant productivity holds great promise for using such discoveries to increase biomass yield and thus carbon biosequestration. Researchers are using genetic, genomic, and systems biology approaches to screen plant genomes for genes and gene segments linked to increased plant biomass. Such screens could reveal new insight into water and nutrient use efficiencies and photosynthesis (e.g., light reactions, RuBisCo activity, and carbon metabolism). Novel pathways and master regulatory genes also may emerge from such investigations. Examples of screening techniques and associated approaches follow.

- **Genetics:** EMS mutants, enhancer traps, and T-DNA.
- **Natural Variation:** Screen accessions and RI lines (for identifying multigenic traits).
- **Genomics and Systems Biology:** Integrated networks and regulatory hubs (for integrating carbon regulation with other processes).

Results of harvest index (HI) science also raise important questions about genetic control of biomass production. Over the past 100+ years, HI-driven plant breeding (with greater biomass of reproductive tissue as the defining variable) has contributed significantly to dramatic increases in crop yields. In many cases, an enhanced harvest index has been achieved by selecting for dwarf plants (i.e., stem and leaf carbon shifted to reproductive tissue) and usually has resulted in increased biomass per unit area. Such observations suggest the genetic potential for enhanced biomass (productivity defined as total carbon per unit area rather then seed yield) has not been explored fully. Thus, systematic screenings for biomass genes hold considerable promise for identifying novel genetic loci associated with productivity.

5. Optimize Biomass Productivity versus Respiration.

Partitioning of carbon between respiration and biomass production varies at the ecosystem, population, organismal, and tissue levels. Current understanding of the biochemistry of growth and respiration is limited severely by the absence of a mechanistic model of the latter process. New omic technologies, along with radioisotope, stable-isotope, nuclear magnetic resonance, and metabolic-flux techniques, should allow more-extensive analyses of substrate- and end-product limitations on respiration. Transformational progress could be made if molecular and biochemical physiologists use these techniques for understanding respiration mechanistically. Researchers could then incorporate this mechanistic understanding into models using a complete differential equation approach similar to photosynthesis. A final and key step would involve conducting a sensitivity analysis that provides the simplest framework possible for evaluating respiration in a whole-plant and ecosystem context. Such an evaluation would advance ecophysiological researchers beyond trying to understand respiration as either a fraction of gross plant productivity or an empirical function of nitrogen tissue and carbohydrate status.