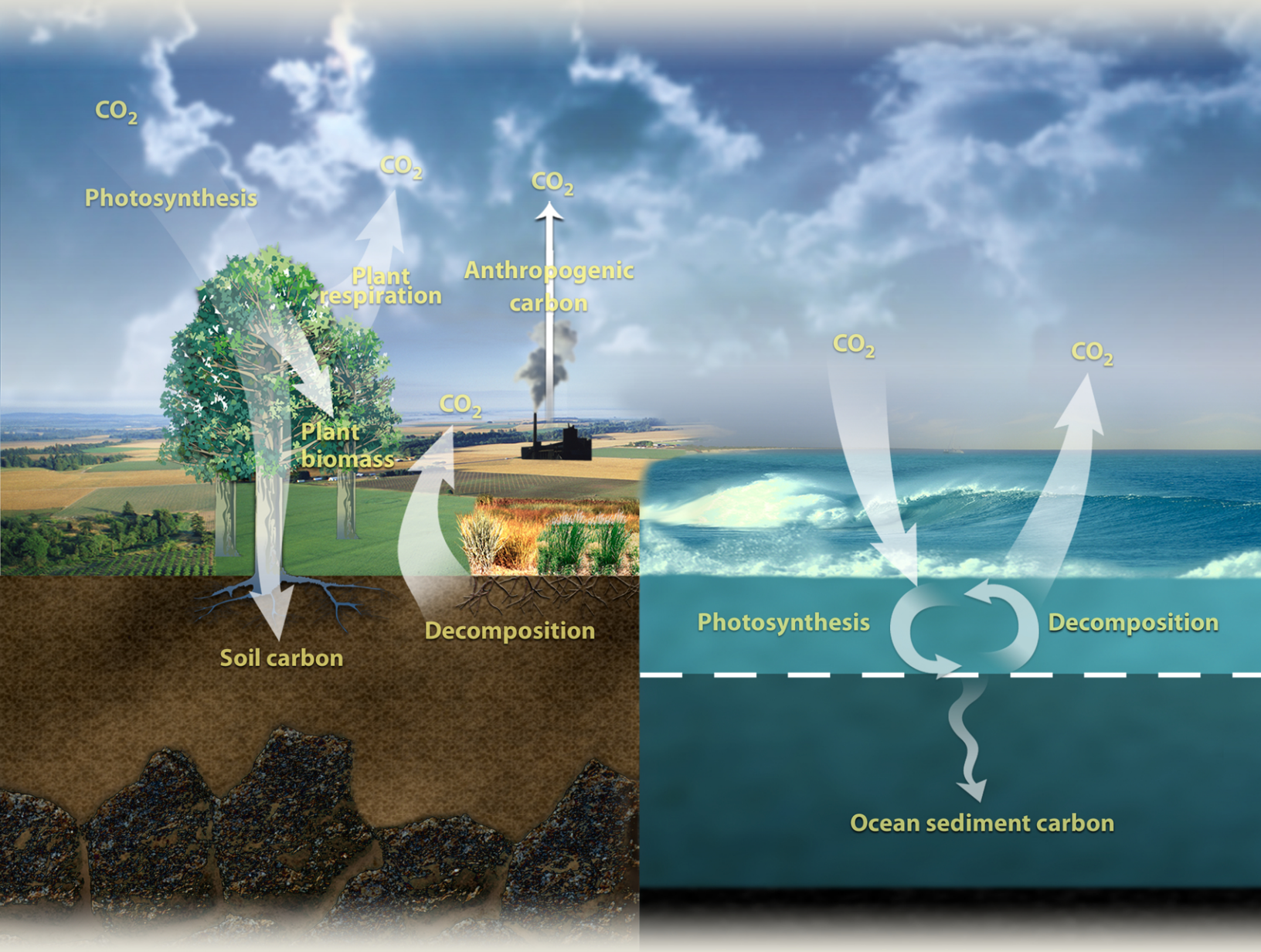
DOE/SC-108

Carbon Cycling and Biosequestration

Integrating Biology and Climate Through Systems Science

Report from the March 2008 Workshop



U.S. Department of Energy
Office of Science
Office of Biological and Environmental Research

U.S. DEPARTMENT OF ENERGY

Carbon Cycling and Biosequestration Workshop

Convened by

U.S. Department of Energy
Office of Science
Office of Biological and Environmental Research

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As part of the U.S. Department of Energy's (DOE) Office of Science, the Office of Biological and Environmental Research (OBER) supports fundamental research and technology development aimed at achieving predictive, systems-level understanding of complex biological and environmental systems to advance DOE missions in energy, climate, and environment. Specific OBER mission priorities include the development of biofuels as secure national energy resources, understanding relationships between climate change and Earth's ecosystems, and investigating the fate and transport of subsurface contaminants.

To develop research objectives in biological carbon cycling and biosequestration of carbon in ecosystems, OBER hosted the Carbon Cycling and Biosequestration Workshop in March 2008. Experts in terrestrial and ocean biogeochemical cycling, ecosystem science, research technology development, molecular biology, and modeling met to identify research needs and opportunities for understanding biological carbon cycling and biosequestration. Participants also assessed current science and technology and discussed fundamental research for pursuing OBER goals. This report outlines the workshop's findings and highlights key opportunities for research on biological aspects of the global carbon cycle.

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Executive Summary

One of the most daunting challenges facing science in the 21st Century is to predict how Earth's ecosystems will respond to global climate change. The global carbon cycle plays a central role in regulating atmospheric carbon dioxide (CO_2) levels and thus Earth's climate, but our basic understanding of the myriad of tightly interlinked biological processes that drive the global carbon cycle remains limited at best. Whether terrestrial and ocean ecosystems will capture, store, or release carbon is highly dependent on how changing climate conditions affect processes performed by the organisms that form Earth's biosphere. Advancing our knowledge of biological components of the global carbon cycle is thus crucial to predicting potential climate change impacts, assessing the viability of climate change adaptation and mitigation strategies, and informing relevant policy decisions.

Global carbon cycling is dominated by the paired biological processes of photosynthesis and respiration. Photosynthetic plants and microbes of Earth's landmasses and oceans use solar energy to transform atmospheric CO_2 into organic carbon. The majority of this organic carbon is rapidly consumed by plants or microbial decomposers for respiration and returned to the atmosphere as CO_2. Coupling between the two processes results in a near equilibrium between photosynthesis and respiration at the global scale, but some fraction of organic carbon also remains in stabilized forms such as biomass, soil, and deep ocean sediments. This process, known as carbon biosequestration, temporarily removes carbon from active cycling and has thus far absorbed a substantial fraction of anthropogenic carbon emissions.

Results from first-generation climate–carbon cycle models suggest that the capacity of the terrestrial and ocean biosphere to absorb anthropogenic CO_2 is likely to peak by mid-century. In some scenarios, large amounts of organic carbon currently locked in high-latitude permafrost, tropical forests, and other ecosystems may in fact be released back to the atmosphere. The rate and magnitude of photosynthesis and respiration, as well as the stability of carbon stored in ecosystems, are heavily influenced by climate variables such as temperature, CO_2 levels, availability of water and nutrients, and disturbances such as fire and pests. Given the immense quantities of carbon cycled by Earth's biosphere (210 gigatons annually), even relatively small shifts in the rates of carbon cycle processes and amounts of carbon biosequestered in ecosystems could have major impacts on atmospheric CO_2 levels.

Although it is critical to more accurately predict the impacts of shifting climate conditions on carbon cycling and biosequestration in ecosystems, most carbon cycle processes are either minimally represented or altogether absent from current climate models. Different models supplied with nearly identical human emissions scenarios have produced dramatically different projections for carbon uptake, storage, or release by land and ocean ecosystems. This problem is compounded by the limited set of experimental approaches aimed at validating the predictions of climate models. Resulting uncertainties diminish the models' predictive capabilities, decrease their level of resolution, and limit their general utility for anticipating and responding to climate change scenarios.

Understanding and predicting processes of the global carbon cycle will require bold new research approaches aimed at linking global-scale climate phenomena; biogeochemical processes of ecosystems; and functional activities encoded in the genomes of microbes, plants, and biological communities. This goal presents a formidable challenge, but emerging systems research approaches provide new opportunities to bridge the knowledge gap between molecular- and global-scale phenomena. Systems-level research emphasizes studies on the underlying principles of intact, complex systems and facilitates scaling of concepts and data across multiple levels of organization. Applying this approach to the global carbon cycle will require multifaceted but highly integrated research that incorporates experimentation on model organisms and systems, collection of observational data on communities and ecosystems, and mechanistic modeling of processes ranging from metabolic to global scales.

In March 2008, the U.S. Department of Energy's Office of Biological and Environmental Research (OBER) held the Carbon Cycling and Biosequestration Workshop. Operating within DOE's Office of Science, OBER is uniquely positioned to lead new national research initiatives aimed at understanding the interlinked systems that form the underpinnings of the global carbon cycle. OBER supports fundamental research and technology development aimed at achieving predictive, systems-level understanding of organisms, biological communities, ecosystems, and global climate. OBER research has been crucial in advancing modern genomics-based systems biology, understanding community and ecosystem-scale responses to climate change variables, and developing increasingly sophisticated models of global climate processes.

At the DOE Carbon Cycling and Biosequestration Workshop, researchers at the forefront of microbiology, plant biology, ecological research, and biogeochemical modeling identified research requirements necessary to (1) advance understanding of the biological processes that drive the global carbon cycle, (2) achieve greater integration of experimental biology and biogeochemical modeling approaches, (3) assess viability of potential carbon biosequestration strategies, and (4) develop novel experimental approaches to validate climate model predictions.

It is now widely recognized that we must confront expanding global energy needs while simultaneously reducing carbon emissions and minimizing negative climate impacts. Transformational breakthroughs are needed to increase the accuracy and resolution of climate change models that inform policy decisions, open new avenues to innovation in climate change adaptation and mitigation strategies, and assess the validity of potential solutions. Achieving an exponential increase in our understanding of the interwoven systems that control the ultimate fate of carbon in Earth's ecosystems is integral to meeting these challenges.

Overview

DOE Workshop on Biological Carbon Cycling and Biosequestration Research

The focus of climate research nationally and globally has shifted to establishing the capability to more accurately project climate change and its impacts, and to better define mitigation and adaptation options. The science to achieve these new and much more challenging goals revolves around the development of Earth System Models (ESM) and the science to support them. These models combine physical climate models, global biological processes, and human activities. Understanding the global carbon cycle across terrestrial and ocean environments and its responses to climate change is essential for the viability of these models. The global carbon cycle is a balance between natural processes and emissions from human activities. This knowledge will provide the scientific underpinnings for more robust climate change modeling and help to identify carbon biosequestration–based mitigation strategies and human adaptation options over the coming decades. The Department of Energy's (DOE) energy security mission is dependent on this modeling and research capability.

Increasing atmospheric CO_2 concentration is one of the most significant factors influencing future climate. There has been a rapid accumulation of heat-trapping CO_2 in the atmosphere [from 285 to 385 parts per million by volume (ppmv) since the Industrial Revolution], largely due to human activities—primarily fossil energy use. Strategies to minimize changes in climate will require that energy production and use be put in the context of Earth's natural biogeochemical cycling of carbon and other elements.

The DOE Office of Biological and Environmental Research (OBER) programs focus on increasing our understanding of carbon cycling in Earth's marine and terrestrial ecosystems, examining potential means of biological sequestration of carbon, and determining how climate change affects biological processes that influence carbon cycling and biosequestration (altered carbon cycling in managed ecosystems). Contributing to DOE's energy security mission, OBER supports research programs emphasizing the development of integrative, systems-level approaches to study the diverse natural capabilities and behaviors of plants, microorganisms, and the communities in which they reside. DOE OBER also facilitates development of breakthrough biotechnologies for urgent national priorities, including climate change research. Of particular interest is the interaction of genome-encoded processes in plant and microbial communities with environmental conditions. These studies will be critical in developing increasingly sophisticated models of global biogeochemical cycling and its response to climate change as well as informing potential carbon biosequestration strategies (see Fig. 1.1. Components of the Global Carbon Cycle, pp. 2–3).

To help develop program objectives in biological carbon cycling and biosequestration, OBER hosted the Carbon Cycling and Biosequestration Workshop in March 2008. Experts in terrestrial and ocean biogeochemical cycling, ecosystem science, research technology development, and modeling met to identify research needs and opportunities for understanding biological carbon cycling and biosequestration, assess current science and technology, and describe fundamental research that can

(text continued on p. 4)

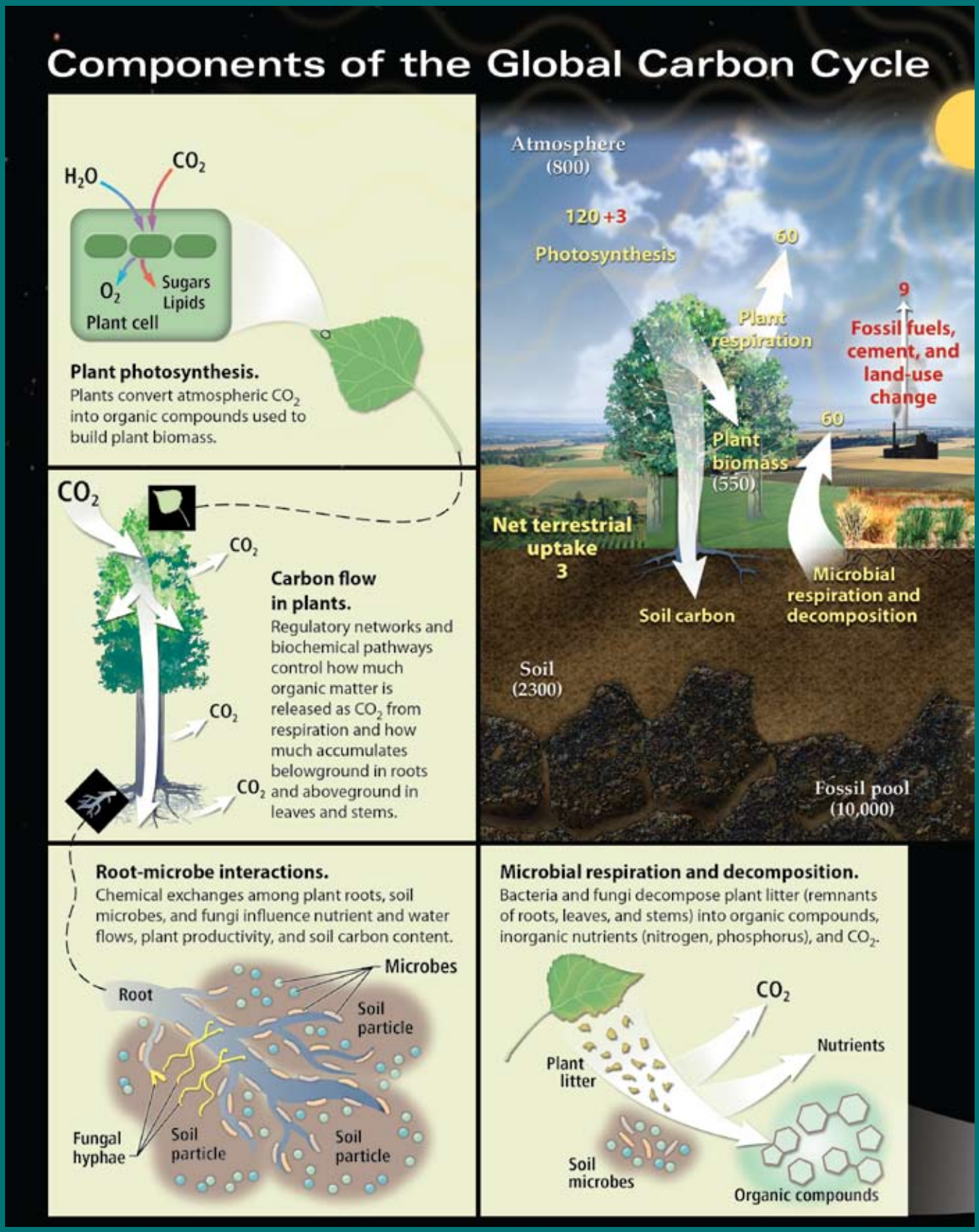
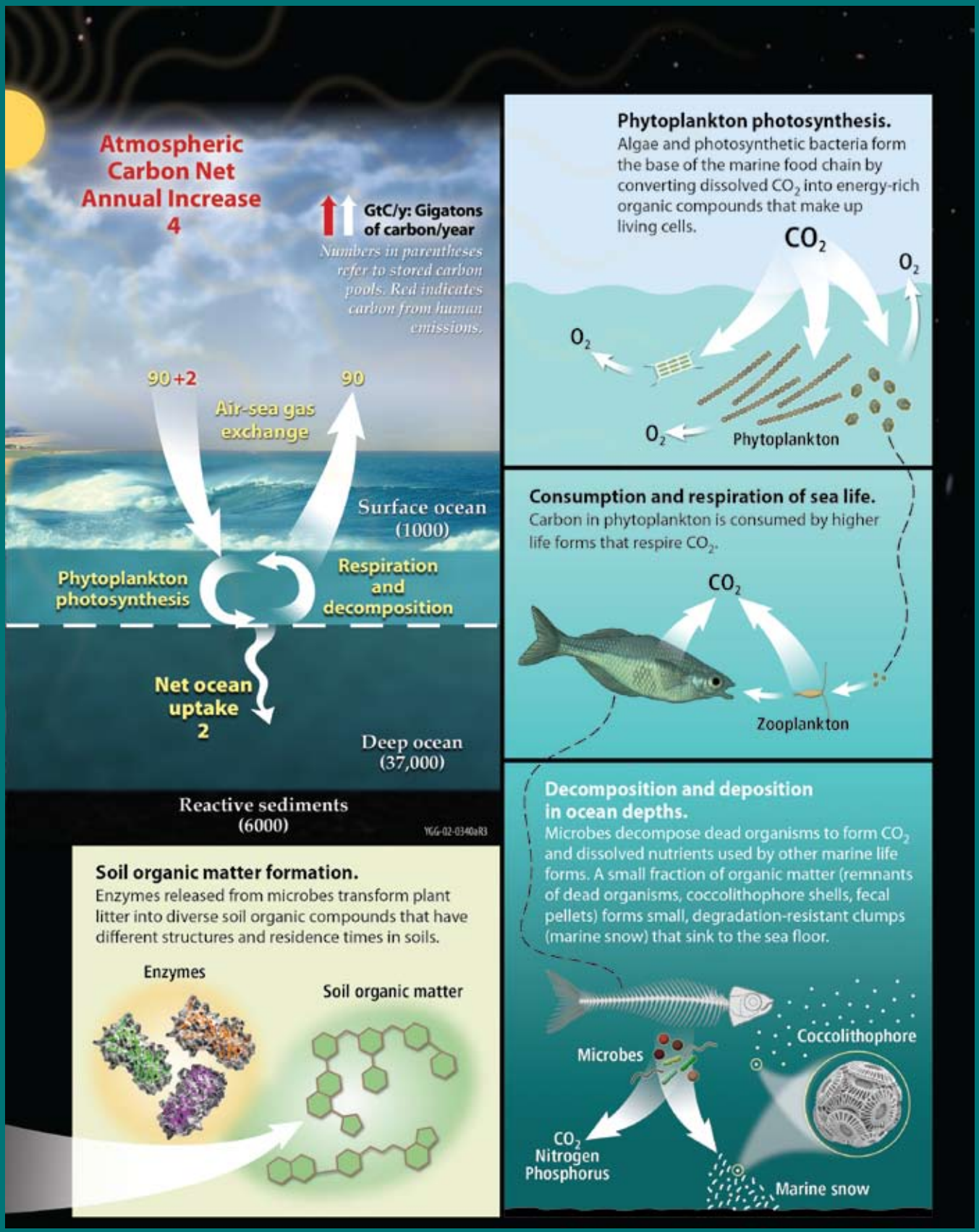


Fig 1.1. Components of the Global Carbon Cycle. A simplified representation of the contemporary global carbon cycle is shown in the center of this figure. Values in parentheses are estimates of the main carbon reservoirs in gigatons (GT) as reported in Houghton (2007). The natural flux between the terrestrial biosphere and the atmosphere is about 120 GT of carbon per year, and that between the oceans and atmosphere is about 90 GT per year (IPCC 2007). In the terrestrial biosphere, photosynthesis removes about 120 GT of carbon from the atmosphere; decomposition of biological material and respiration from plants and soil microbes return 120 GT of carbon. In the oceans, the marine biosphere does not take up CO_2 directly from the atmosphere. Each year the oceans absorb and release about 90 GT of carbon largely via diffusion across the air-ocean interface. The physical processes controlling the sinking of CO_2 into colder, deeper waters (where CO_2 is more soluble) and the mixing of ocean water at intermediate



depths are known collectively as the "solubility pump." Phytoplankton photosynthesis converts CO_2 into organic carbon that is largely returned to ocean water as CO_2 via microbial respiration and decomposition. The "biological pump" refers to the small fraction of organic carbon that forms into degradation-resistant clumps and sinks to the ocean floor. Together the solubility and biological pumps control the amount of carbon transported to ocean depths and the exchange of CO_2 between ocean and atmosphere.

Human activities (primarily fossil fuel use) emit about 9 GT of carbon each year. About 4 GT of this human-contributed carbon remain in the atmosphere; 3 GT are taken up by natural terrestrial processes, and another 2 GT are removed by the ocean (Canadell et al. 2007).

Peripheral boxes describe some of the biological processes (photosynthesis, partitioning, respiration, and organic-matter formation) discussed in this report that play key roles in regulating the flow of carbon in and out of terrestrial and ocean ecosystems.

be pursued to meet OBER goals. This report outlines the workshop's findings and highlights key opportunities for biological carbon cycling research.

Introduction

Energy production worldwide is significantly altering the atmospheric concentration of CO_2. When fossil fuels are consumed, carbon sequestered deep within the Earth for eons is added to the global carbon cycle. Fossil CO_2 emitted from smokestacks and tailpipes flows into one of three reservoirs with physical and biological components: the atmosphere, oceans, and terrestrial systems. The complex carbon flows and transformations among these major Earth system components make up the carbon cycle. These natural exchanges of carbon between the biosphere and Earth's physical components are many times greater than the 9 billion tons [gigatons (GT)] produced by humans each year (see Table 1.1. Annual Fluxes in Global Carbon, this page, and Fig. 1.1, pp. 2–3). The biological processes of photosynthesis and respiration largely control the annual flux of about 120 GT of carbon between the atmosphere and land. About 90 GT of carbon flow in and out of the ocean, primarily through air-sea exchange at the surface.

Natural carbon sinks on land and in the oceans absorb about half the 9 GT of anthropogenic carbon emitted annually (Canadell et al. 2007). Although CO_2 emissions from human activities may seem insignificant relative to large natural carbon fluxes and stocks, they can shift the critical balance of the carbon cycle over time. The effective lifetime of CO_2 in the atmosphere exceeds centuries, so even relatively small imbalances can accumulate to significant atmospheric concentrations over hundreds of years. As anthropogenic CO_2 emissions continue to grow and atmospheric concentrations reach levels unprecedented in the last 650,000 years based on Antarctic ice-core data, the risks associated with perturbing natural carbon fluxes and the balance of Earth's climate system increase.

Table 1.1. Annual Fluxes in Global Carbon
(Gigatons of carbon per year)

Gross Natural Land-Atmosphere Carbon Fluxes[1]	
From atmosphere to plants	120
To atmosphere from plants	60
To atmosphere from soils	60
Gross Natural Ocean-Atmosphere Carbon Fluxes[1]	
From atmosphere to oceans	90
To atmosphere from oceans	90
Anthropogenic Carbon Emissions[2]	
To atmosphere from fossil fuel use	7.6
To atmosphere from land-use change	1.5
Total	9.1*
Fate of Anthropogenic Carbon Emissions[2]	
Added to atmosphere	4.1*
Absorbed by natural processes on land	2.8*
Absorbed by natural processes in oceans	2.2*

[1]Source: IPCC 2007.
[2]Source: Canadell et al. 2007.
*Rounded to whole numbers in Fig. 1.1, pp. 2–3.

These considerations define the pressing national need for a comprehensive understanding of the global carbon cycle across terrestrial and ocean environments. This knowledge will provide the scientific bases for more robust climate change modeling and help define options for carbon biosequestration over the coming decades. Greater understanding and predictive capabilities underpin six elements of national and international climate research strategies (see sidebar, Current Climate Research Strategies and their Dependence on Understanding the Global Carbon Cycle, p. 5).

Accurate Climate Projections, Effective Mitigation and Adaptation Strategies Depend on Understanding the Global Carbon Cycle

One of the major challenges for 21st Century climate research is decreasing the uncertainties associated with how oceans and terrestrial ecosystems will respond to a warmer, higher-CO_2 world. Results from first-generation coupled climate–carbon cycle models suggest that as the Earth continues to warm, the capacity of the ocean and terrestrial biosphere to absorb anthropogenic CO_2 could peak by

Current Climate Research Strategies and their Dependence on Understanding the Global Carbon Cycle

Yearly net atmospheric greenhouse gas emissions include carbon dioxide produced by human activity and that released naturally by terrestrial and ocean systems in the global carbon cycle. Contributing to this cycling of carbon among the atmosphere, land, and sea are carbon sources and sinks from physicochemical processes (e.g., the ocean's absorption and mineralization of carbon) and biological processes in terrestrial and ocean systems. Improved understanding of these components and their role in atmospheric retention of CO_2, especially that produced by energy use, is essential to DOE development of carbon mitigation and biosequestration strategies. Accurate climate projections and potential carbon biosequestration options critically depend on descriptive, predictive models of ocean and terrestrial ecosystems and their contributions to the global carbon cycle.

Current needs for climate projection and carbon biosequestration research strategies include:

1. **Incorporation of Increased Climate Knowledge into Models.** Better understanding and model representations of the historical climate record in the context of human activities, natural variability, and global processes that include the carbon cycle form the foundation for making meaningful climate projections and assessing human impacts on the global flow of carbon.

2. **Near-Term Projections of Climate Change and Impacts.** High-resolution spatial analyses of climate change over the next few decades will focus on understanding the effects of such change (e.g., potential shifts in precipitation and weather, including extremes) at the regional scale to support development of mitigation and adaptation strategies.

3. **Long-Term Projections of Climate Change and Ecosystem Feedbacks.** Earth System Models and others (e.g., dynamic vegetation simulations showing species succession in ecosystems) that incorporate advanced understanding of the global carbon cycle and other aspects of the biosphere affected by climate change will project potential climate shifts over centuries and longer. Projections for such time scales are critical for assessing the effects of increasing climate change, expanding human activities, and ensuing ecosystems-climate feedbacks. Such modeling endeavors require understanding not only the current and evolving physiology and functionality of ecosystems, but their transformations on various projected climate trajectories.

4. **Emission Budgets.** A better understanding of the global carbon cycle, under changing climate conditions, is necessary to accurately predict effects of future human activities and provide viable energy infrastructure options. All projection scenarios of atmospheric CO_2 concentrations require data on human emission budgets (e.g., energy generation and use, industrial activities, and land-use change) to support national and global energy options and strategies. Current climate modeling calls for emission and atmospheric-concentration trajectories that explicitly require quantifying carbon sinks and sources to derive atmospheric CO_2 concentrations.

5. **Definition of Viable Carbon Biosequestration Strategies.** Carbon biosequestration strategies require understanding the behaviors of sources and sinks under changing climate and environmental conditions to potentially manage them for optimum carbon capture and long-term storage and mitigation of anthropogenic CO_2.

6. **Projecting Impacts on Goods and Services Derived from Ecosystems.** Closely connected to understanding and quantifying carbon flow within ecosystems are the goods and services such systems provide to society. Understanding climate impacts on goods and services is necessary for assessing adaptation options. These impacts are measured in the variations in goods and services as climate varies. Goods include food, feed, fiber, fuel, pharmaceutical products, and wildlife. Services include maintenance of hydrologic cycles, cleansing (filtering) of water and air, regulation of climate and weather, storage and cycling of nutrients, provision of habitat, and aesthetics. Ecosystems' carbon-carrying capacity and stocks are becoming critical components of their services.

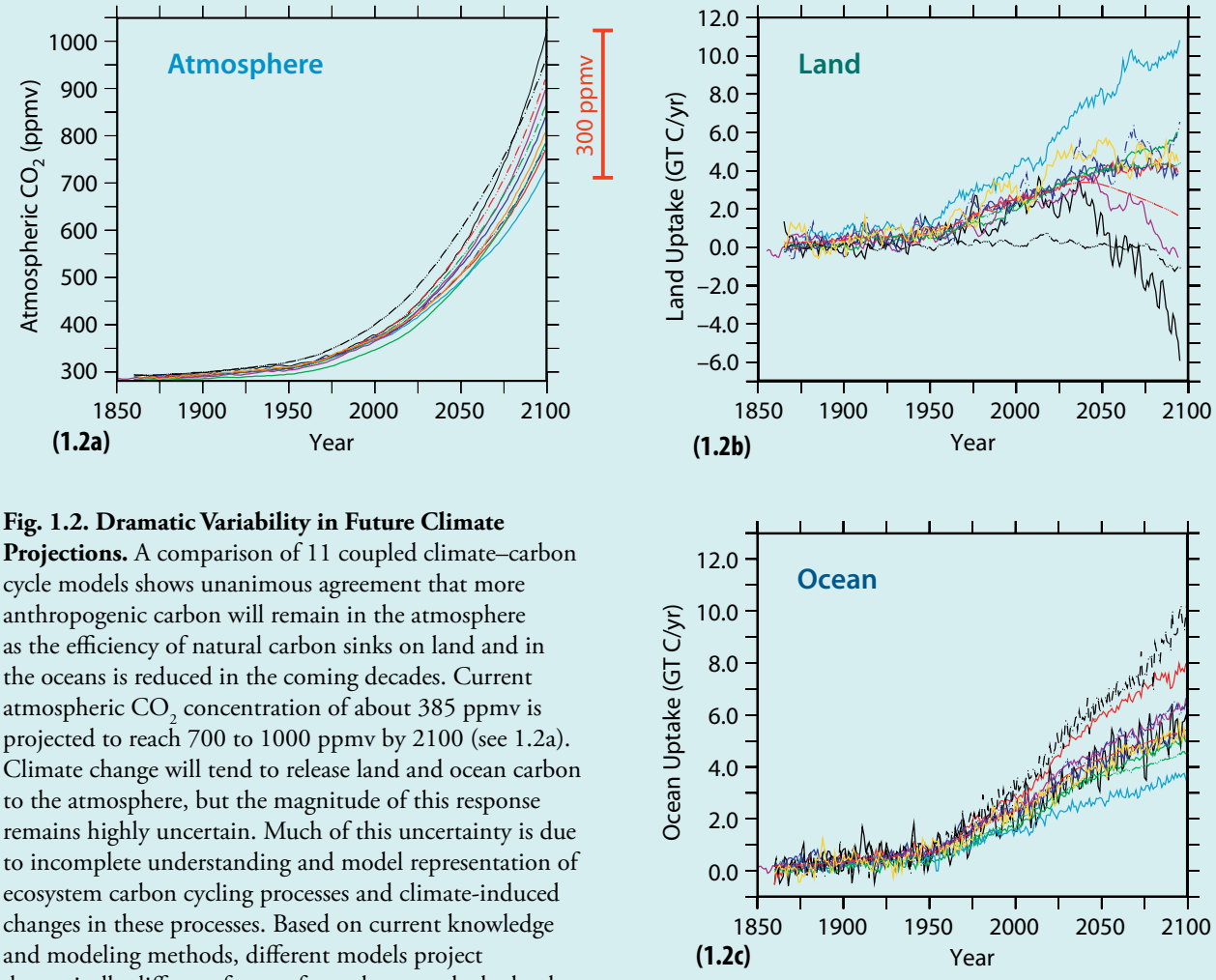


Fig. 1.2. Dramatic Variability in Future Climate Projections. A comparison of 11 coupled climate–carbon cycle models shows unanimous agreement that more anthropogenic carbon will remain in the atmosphere as the efficiency of natural carbon sinks on land and in the oceans is reduced in the coming decades. Current atmospheric CO_2 concentration of about 385 ppmv is projected to reach 700 to 1000 ppmv by 2100 (see 1.2a). Climate change will tend to release land and ocean carbon to the atmosphere, but the magnitude of this response remains highly uncertain. Much of this uncertainty is due to incomplete understanding and model representation of ecosystem carbon cycling processes and climate-induced changes in these processes. Based on current knowledge and modeling methods, different models project dramatically different futures for carbon uptake by land (see 1.2b) and ocean (see 1.2c). More observational and experimental data are needed to constrain these models and decrease the large uncertainties in future projections of climate-induced changes in the carbon cycle. [Source: Figure adapted from Friedlingstein, P., et al. 2006. "Climate–Carbon Cycle Feedback Analysis: Results from the C⁴MIP Model Intercomparison," *Journal of Climate* **19**, 3337–53. Reproduced by permission of the American Meteorological Society (AMS).]

mid-century and then stabilize or decrease (IPCC 2007). Terrestrial carbon sinks are projected to saturate, thus a better understanding of the temperature sensitivity of long-term soil carbon pools is needed. In oceans, rising temperatures and CO_2 levels are projected to decrease CO_2 solubility, increase acidification in surface waters, and reduce the vertical mixing of nutrients from the deep ocean, which would limit marine photosynthesis. Key uncertainties in the biological processes influencing these general projections remain.

In a recent comparison, when different climate-carbon models were supplied with nearly identical human emissions scenarios, these selected models—because they contained a variety of representations of global carbon cycle processes and treatments of interactions with climate—produced dramatically different projections for carbon uptake by land and ocean systems (Friedlingstein et al. 2006). For example, projections of CO_2 uptake by terrestrial ecosystems vary so widely that some models predict land to become a stronger sink, capturing up to 10 GT of carbon per year, whereas other models project land to become a carbon source, emitting up to 6 GT per year (see Fig. 1.2. Dramatic Variability in Future Climate Projections, this page). Two key

factors contributing to this wide variation in model output are (1) a limited understanding of potential biological responses and other feedbacks and (2) uncertainties in how to model these phenomena.

To improve the fidelity and accuracy of climate projections, the scientific community needs a better understanding of the fundamental mechanisms controlling carbon sources and sinks. In the past two decades, much progress has been made in understanding historical trends in atmospheric CO_2, and biogeochemical modeling of carbon in oceans and terrestrial systems continues to advance. However, current carbon cycle research still cannot *quantitatively* address several key questions, including the following.

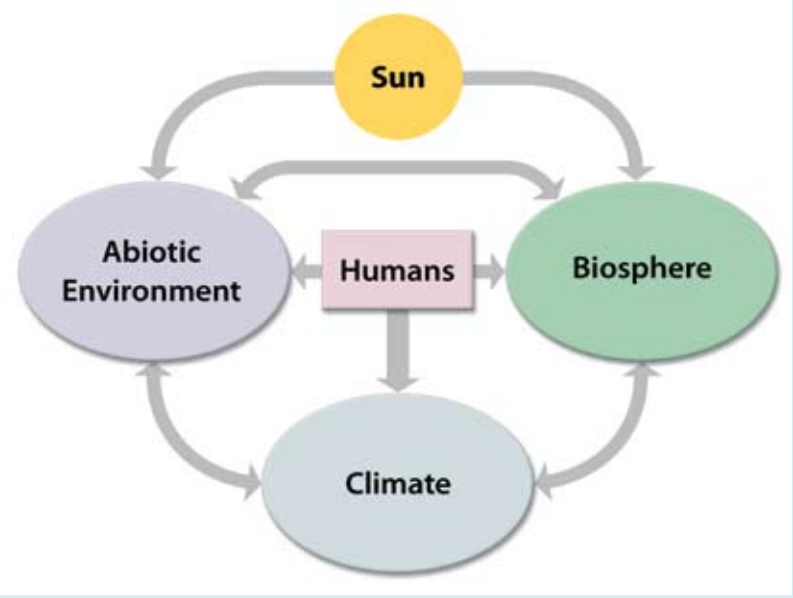


Fig. 1.3. Biosphere-Environment-Human-Climate Interactions.

- What are the fundamental processes controlling the behaviors of carbon sinks and sources in ocean and terrestrial systems?
- How will human activities and changing climate conditions affect these processes?
- Will current carbon sinks persist or become carbon sources in a warmer, higher-CO_2 world?
- How long will biologically sequestered carbon remain stored?

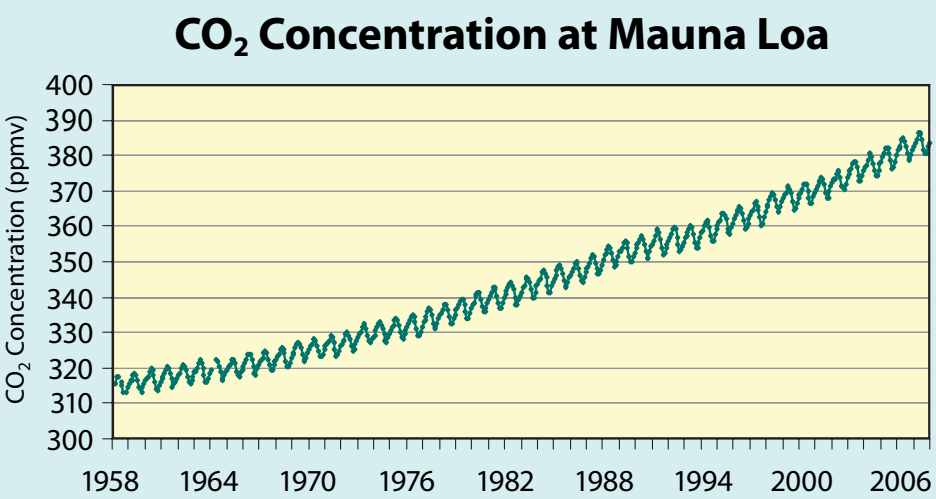
Climate is both a product and a catalyst of interactions between a region's physical environment and the biosphere, all of which are driven by the sun and affected by human activities (see Fig. 1.3. Biosphere-Environment-Human-Climate Interactions, this page). The challenge is relating all these factors. Quantifying photosynthesis, respiration, and other biological processes that are components of carbon cycling is difficult because the metabolic flux of material and energy through cells, organisms, and ecosystems is tightly linked to a particular region's abiotic environmental factors (e.g., temperature, precipitation amounts and timing, geographical features, nutrient availability, length of days and seasons, and sunlight exposure). The range of geographic and ecophysiological regions to consider in models is enormous, but to truly understand how climate will affect valued goods and services (e.g., food, fiber, fuel, water and air quality, wildlife habitats, recreation, and aesthetics), climate projections must have the required detail to guide management decisions at both global and regional scales.

Biology's Critical Role in the Carbon Cycle

Biological processes drive the carbon cycle and other elemental cycles in globally significant ways. Eons ago, microbial metabolism created the oxygen-rich atmosphere that sustains much of life today, and the net effects of biological processes are observed in measurements of atmospheric CO_2 levels (see Fig. 1.4. Biological Influence on Atmospheric Carbon Dioxide Concentration, p. 8).

The global carbon cycle is dominated by two tightly interlinked processes: photosynthesis and respiration. Photosynthesis by plants and marine microbes

Fig. 1.4. Biological Influence on Atmospheric Carbon Dioxide Concentration. The zigzag pattern in Mauna Loa atmospheric CO_2 measurements results from seasonal carbon flows between the atmosphere and biosphere. Greater landmass and deciduous vegetation in the Northern Hemisphere cause a drop in atmospheric CO_2 as photosynthesis fixes large amounts of CO_2 in spring and summer. In fall and winter, respiration and the decay of fallen leaves, combined with lower photosynthetic productivity, cause a net flow of CO_2 from the biosphere to the atmosphere. The upward slope of the trendline reflects the atmospheric increase of fossil CO_2 from human activities. [Source: Figure adapted from Keeling, R. F., S. C. Piper, A. F. Bollenbacher, and J. S. Walker. 2008. Atmospheric CO_2 records from sites in the SIO air sampling network. In *Trends: A Compendium of Data on Global Change*. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tenn., USA. http://cdiac.ornl.gov/trends/co2/graphics/mlo144e.pdf.]



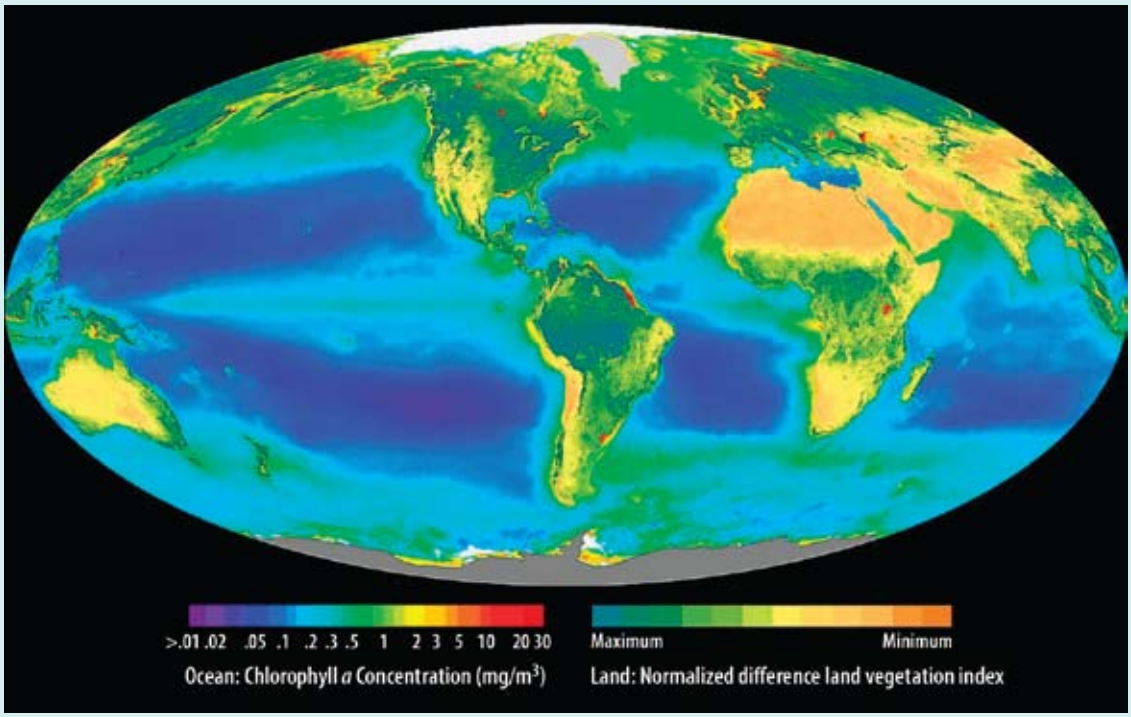


Fig. 1.5. Photosynthesis in the Global Biosphere. This NASA SeaWiFS image of the global biosphere shows the density of photosynthetic organisms on land and in the oceans. On land, the dark greens represent areas of abundant vegetation, with tans showing relatively sparse plant cover. In the oceans, red, yellow, and green regions depict dense blooms of phytoplankton (photosynthetic microbes), while blues and purples show regions of lower productivity. [Source: NASA SeaWiFS Project. http://oceancolor.gsfc.nasa.gov/SeaWiFS/.]

(see Fig. 1.5. Photosynthesis in the Global Biosphere, p. 8) removes CO_2 from the atmosphere and converts or "fixes" it into organic material. This step of the cycle is referred to as primary production. Photosynthesis by phytoplankton and cyanobacteria in oceans converts about as much atmospheric carbon to organic carbon as does plant photosynthesis on land (Fuhrman 2003). The rate at which all photosynthetic organisms for a particular region or across the globe convert CO_2 into organic compounds is known as gross primary productivity (GPP). Global GPP represents the largest flux of CO_2 out of the atmosphere.

Organic carbon produced by photosynthesis is then either incorporated into biomass or respired for energy generation and released as CO_2 and water. The rate of primary production that remains after accounting for losses through cellular respiration is called net primary productivity (NPP). Slight changes in the balance between photosynthesis and respiration can substantially impact atmospheric CO_2 concentration (see Fig. 1.6. Terrestrial Carbon Uptake and Storage, this page).

Greater insight into the biological processes controlling the balance of photosynthesis and respiration is needed because the net result of these processes influences the fate of carbon in ecosystems. Understanding partitioning and the fate of carbon fixed by photosynthesis is equally important to investigating the impacts of rising CO_2 levels on photosynthetic carbon assimilation. More research will be required to determine how plants regulate the allocation of fixed organic carbon used to increase biomass in photosynthetic plants or microbes versus the amount lost through cellular respiration and other processes.

Photosynthetic organisms are the original source of nearly all organic carbon in the biosphere. In terrestrial ecosystems, plants deposit detritus (plant and root litter) and root exudates into soils. In oceans, phytoplankton secrete cellular material into the water column, are lysed by viruses, and are grazed upon by zooplankton. A significant portion of the organic carbon liberated to the environment is rapidly respired by heterotrophic organisms and returned to the atmosphere as CO_2. More research is needed to determine how higher temperature, elevated CO_2, and other shifting environmental variables could alter the composition and metabolic activities of microbial communities that degrade organic carbon in soils and surface ocean waters.

The efficiency of carbon storage in biologically driven reservoirs is closely linked to the cycling and availability of nutrients. For terrestrial ecosystems, very little is known

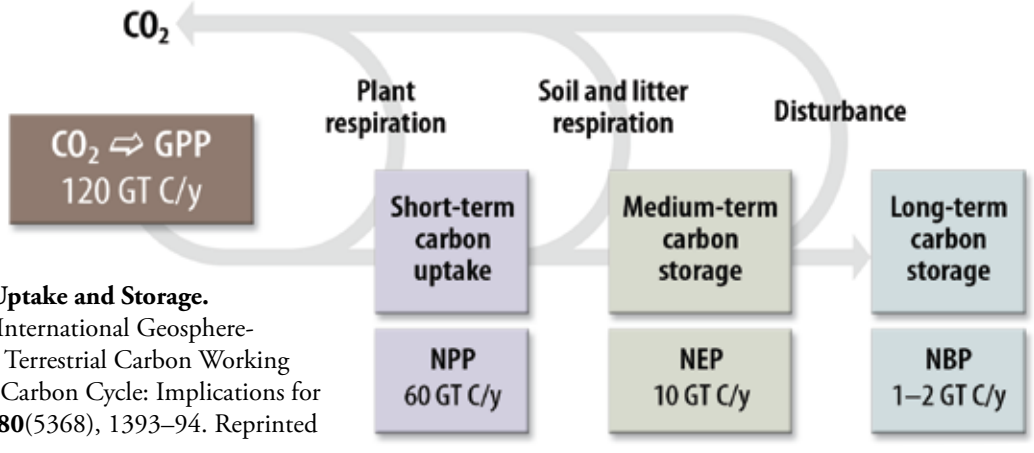


Fig. 1.6. Terrestrial Carbon Uptake and Storage. [Source: Figure adapted from International Geosphere-Biosphere Programme (IGBP) Terrestrial Carbon Working Group. 1998. "The Terrestrial Carbon Cycle: Implications for the Kyoto Protocol," *Science* **280**(5368), 1393–94. Reprinted with permission from AAAS.]

Box 1.1

Types of Ecosystem Disturbances

Disturbance: Any abrupt event that drastically changes ecosystem characteristics such as population diversity, behavior, or climate response. A large-scale disturbance that rapidly converts vast quantities of stable organic carbon (e.g., forests) into CO_2 can impact the carbon cycle significantly. An ecosystem's state must be put in the context of its disturbance history to be meaningful.

Climate-driven disturbance types

- Wildland fires
- Extreme events or severe weather (e.g., hurricanes and floods)
- Insects and disease
- Drought

Anthropogenic disturbance types

- Conversion of forest or grassland to agriculture by human-mediated fires (such activity is an important overlap with climate-driven fire disturbances) or other methods
- Burning of agricultural waste products
- Implementation of biofuels and carbon biosequestration strategies
- Wood harvesting (products, fuels)
- Urbanization

about the fundamental mechanisms by which limitations in nutrients—especially those other than nitrogen and phosphorus—affect processes related to photosynthetic productivity and, ultimately, carbon biosequestration. In oceans, the abundance and ratio of nutrients rising from the deep determine community composition and activity of surface phytoplankton. Understanding how nutrient availability and other factors limit distribution of marine microbial communities and their diverse suite of nutrient transformations remains a major challenge (Arrigo 2005). In addition to the nutrient transfers among primary producers, grazers, predators, and decomposers, the symbiotic relationships between microbes and higher organisms to obtain limiting nutrients also are important to understanding integrated nutrient cycling; yet most symbiotic associations remain poorly characterized.

Nutrient cycles traditionally have been studied in isolation, but a more comprehensive understanding is needed of how these interconnected cycles function together. The molecular machines that mediate the biochemical reactions within the global metabolic network represent a set of genes essential to life and the biogeochemical cycling of carbon and other elements (Falkowski, Fenchel, and Delong 2008). A critical point is that the interconnections among these cycles exist in the interactions between species in a complex series of trophic cascades.

From the great diversity of biological processes that shape and sustain the Earth, can we define a core set of genes or functions essential to the biogeochemical cycling of carbon? Some key biological processes warranting more detailed scientific understanding were identified at the DOE carbon cycle workshop and are summarized in the sidebar, Key Biological Carbon Cycling Research Areas, p. 11, and in Fig. 1.1. Components of the Global Carbon Cycle, pp. 2–3.

Ecosystem Response in a Changing Climate

Ecosystems undoubtedly will differ in their responses and vulnerability to climate change (IPCC 2007). While multiple factors may contribute to these differences, knowing the collective set of traits and functions present among different species within a community will be critical for determining rates and trajectories of ecosystem response, particularly net primary production and biosequestration of carbon. The ability of ecosystems to adapt to changing conditions will depend not only on a community's range of genome-encoded functions, but also on the sensitivity of organisms to alterations in nutrients and other limiting resources that regulate their fitness and abundance. The type and magnitude of resource alterations are the result of community response to environmental and anthropogenic shifts such as atmospheric nitrogen deposition, land-use change, habitat fragmentation, and variations in disturbance regimes.

Projections of mean carbon and nutrient stocks in vegetation, litter, and soil organic matter can vary greatly depending on the severity, frequency, and types of climate-related disturbances. Alterations in historical disturbance patterns resulting from a changing climate could have an overwhelming influence on the carbon cycle. More research is needed to quantify observed patterns in disturbance type, identify mechanisms driving the observed patterns, and develop a prognostic capability that can provide reasonable predictions for future disturbance patterns. Box 1.1, at left, Types of Ecosystem Disturbances, lists some of the most significant disturbances related to climate change and human activities.

Key Biological Carbon Cycling Research Areas

Terrestrial Ecosystem Processes

Plant Photosynthesis. Through photosynthesis, plants convert atmospheric CO_2 into organic compounds used to build plant biomass and drive metabolic and other processes. Research must reveal the impacts on enzymes and biochemical reactions underlying water loss and CO_2 exchange, nutrient uptake, and many other processes that control photosynthetic productivity as plants are subjected to changing levels of atmospheric CO_2 and climatic conditions.

Mechanistic Understanding of Respiration. Although the biochemistry of respiration and growth has been studied extensively, current understanding of respiration is limited by the lack of a mechanistic model. For certain levels of temperature increase, plants grown in high-CO_2 concentrations display increased biomass production and higher respiration rates, but the molecular and cellular mechanisms controlling this observed response need more detailed analysis. How plants acclimate to increasing temperature is another key area for investigation. In addition, distinguishing root respiration from microbial respiration in soils has proven especially difficult, yet doing so is important because each type of respiration responds differently to environmental signals, including those associated with climate change. New technologies for measuring carbon flux through metabolic pathways are becoming available and can help quantify respiratory carbon loss at cellular, microbial community, plant, and ecosystem levels.

Partitioning of Carbon in Plant Biomass. Carbon fixed by photosynthesis is translocated and partitioned among different plant compartments (e.g., leaves, stems, roots, and mycorrhizae), respired as CO_2, or released as exudates into soil. The pattern of partitioning has feedback effects on photosynthetic capacity via leaf area and nutrient-uptake capacity through root deployment. Residence times of carbon compounds in these compartments vary greatly. Simple carbohydrates are metabolized in minutes to hours. Plant structural compounds can persist for years to decades. Although most plant compounds released into soils are consumed and respired by fungi and bacteria, a small fraction may be stored in long-lived pools for thousands of years. The regulatory systems and molecular controls for partitioning carbon among plant structures, cellular respiration, or release into different soil pools must be better understood and represented in models.

Plant-Microbe Interactions in the Rhizosphere. In the narrow zone of soil surrounding the root (the rhizosphere), fungal, bacterial, and archaeal interactions with plant roots can impact plant growth and development significantly. In turn, rhizosphere microbes obtain carbon and energy for growth from root exudates. Fungi and bacteria can enhance plant productivity by providing nutrients such as phosphorus and nitrogen or by suppressing plant pathogens in the soil. Glue-like proteins and other molecules secreted by rhizosphere fungi and bacteria form stabilized soil structures that support plant growth by increasing soil moisture and organic carbon content. Explicit chemical communications between plants and rhizosphere microbes facilitate these interactions.

Characterization of the Plant Microbiome. Plant surfaces and internal passages are colonized by a diverse array of microorganisms (collectively called the "microbiome"), many of which confer beneficial properties to their hosts. Interactions between plants and their resident microbial communities can influence plant metabolism, improve resistance to stress, increase access to limiting nutrients, and deter pathogens. Understanding the nature and functions of the plant-associated microbiome and its potential importance to plant primary production is a key challenge.

Microbial Processing of Plant Materials. Soils represent the largest and most stable reservoir of carbon in terrestrial ecosystems and contain more than twice as much carbon as the atmosphere (Schlesinger 1997). Soil microbial communities mediate the multistep conversion of dead plant tissue and organic compounds exuded from plant roots into CO_2 or soil organic matter (SOM). The heterogeneous array of organic molecules composing SOM can reside in terrestrial ecosystems for decades to thousands of years. Microbial activity also contributes to the formation of mineral–organic matter complexes called microaggregates that physically protect organic carbon from degradation. Understanding the enzyme-catalyzed reactions and environmental conditions controlling the transformation of various SOM compounds into long-lived humic compounds or highly stable microaggregates could lead to opportunities for sequestering vast quantities of carbon in ways that improve soil quality and benefit the environment.

Oceanic Processes

Marine Microbial Photosynthesis. Phytoplankton (microscopic marine plants) and photosynthetic bacteria convert dissolved CO_2 into organic compounds in surface waters. By reducing the partial pressure of CO_2 in the upper ocean, photosynthetic marine microbes enhance the oceans' physical absorption of CO_2 from the atmosphere. Without phytoplankton photosynthesis, atmospheric CO_2 concentration would be 150 to 200 ppmv higher (Laws et al. 2000). Large oscillations in phytoplankton abundance, therefore, significantly affect the oceans' ability to take up atmospheric CO_2. Using metagenomics and other cultivation-independent techniques, scientists are just beginning to understand the composition of microbial communities dominating primary production in oceans. Differences in functional potentials of various photosynthetic microbes remain poorly understood, and predicting the effects of climate change on microbial communities and the marine carbon cycle is difficult.

Biological Pump. Although most organic matter produced in surface waters is consumed by heterotrophic microorganisms and other forms of marine life and then returned to the atmosphere as CO_2, carbon in the form of plankton, fecal pellets, calcium carbonate shells, and dead cells, for example, sinks to the deep ocean. Carbon in the deep ocean is effectively sequestered because it can remain there for thousands to millions of years due to the slow vertical mixing of ocean water. The process that results in transferring organic carbon into the deep ocean and sediments is known as the biological pump. The percentage of photosynthetically fixed carbon that is sequestered by the biological pump is difficult to measure and varies widely among different marine environments. Predicting the magnitude of future changes in oceanic carbon uptake (Falkowski et al. 2000) requires understanding factors controlling the efficiency of the biological pump.

Processing of Photosynthetically Fixed Carbon. The fate of organic carbon in marine systems is governed largely by microbial heterotrophs that are responsible for most carbon transformation, solubilization, and subsequent remineralization occurring in the water column. Despite microbes' crucial role in mediating these processes, only limited information is available regarding the identity of organisms and key genes and proteins involved in degradation of organic matter, as well as the relative degradation rates of various types of compounds.

Technical Strategy

Integrated Science for Predicting Carbon Cycle Responses

A Multidisciplinary Research Approach

There is a pressing national need for timely understanding of the carbon cycle across terrestrial and ocean environments to (1) provide the scientific foundations needed for more robust climate change modeling, (2) project climate change impacts on Earth's ecosystems, and (3) define options for carbon biosequestration. Scientific progress in these areas must be accelerated by joining diverse research communities in new ways and investing in and applying the latest advances in technology and computation. Identifying and describing crosscutting themes in carbon cycling and biosequestration research as well as key needs in science and technology were important objectives of the March 2008 DOE Carbon Cycling and Biosequestration Workshop. This chapter describes the emergent research themes and requirements for systems to be studied and the methods that can be employed.

Multiscale, Multidimensional Mechanistic Models

Advancing our understanding of the carbon cycle requires developing models that describe all levels of related systems—their states, components, and relationships—and predict these systems' functions and responses to climate change and disturbance. Such models also can be used as heuristic tools to develop insightful strategies for the ensuing multiscale research. System complexity, inadequate data or understanding, and computational limitations currently constrain researchers to simplifying and parameterizing system components in models, resulting in loss of crucial mechanistic details. Overcoming these limitations presents a considerable challenge to the scientific community.

Molecular to Global Scales

Genome-encoded molecular processes control the functions of cells, organisms, and communities and, in turn, influence ecosystem- and global-scale phenomena. However, connecting mechanisms across the molecular to organism levels and eventually to global climate represents a major research challenge. Historically, the research domains of climate, ecosystems, and molecular biology have differed widely in experimental and modeling approaches and methods, and results from single-discipline studies often do not translate well across scales. Integrating these results and discerning their connections across domains and scales will allow more precise projections of systems' behavior for the range of variables envisioned in climate simulations. The shift in biology from a reductionist to a systems paradigm provides an opportunity to bridge these gaps and will help facilitate an integrated approach such as is described in the sidebar, A Balanced and Comprehensive Application of Methods, pp. 14–15 (see also Fig. 2.1. Scales and Processes of the Global Carbon Cycle, p. 16).

A Balanced and Comprehensive Application of Methods

Observations, experiments, and computational modeling on natural and model ecosystems and their subsystems are essential components of a balanced approach to ecosystem research. Results from such an approach can be used to derive a mechanistic process understanding of ecosystems' state, function, and response to climate variables (see figure at right, Understanding the Response of Ecosystems and the Global Carbon Cycle to Climate Change: An Integrated Research Approach). This improvement in predictive understanding—based on molecular insights achieved using an integrated approach—in turn will enhance the accuracy of ecosystem simulation models. Results from such models can be extrapolated to less-studied ecosystems and conditions outside the range of available observations and experiments. When applied to climate models, these results also can broaden and improve predictions of ecosystem response to climate change.

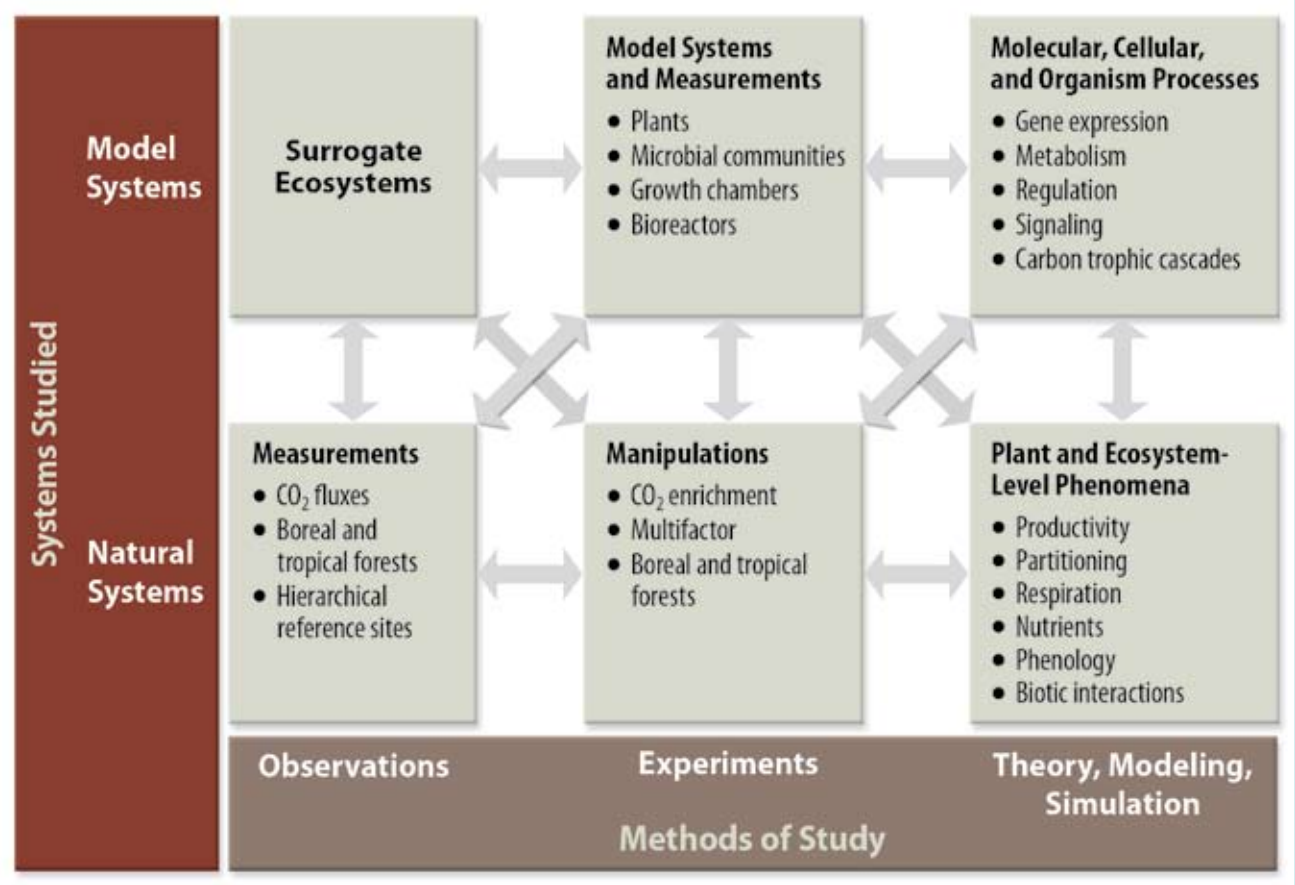
- **Developing "Reference Ecosystem Sites" for Integrated, Multidisciplinary Science.** Providing interdisciplinary scientists the opportunity to pool their experimental data and ideas is essential for meaningful progress in understanding complex ecosystems. One way to facilitate such collaboration is by establishing reference ecosystem sites that represent the diversity of natural ecosystems and ecoregions. These sites will enable an integrated view of plant, microbial, mesofauna, and collective physiology in the reference ecosystem, and resulting paradigms and mechanistic representations will improve extrapolations to the world's ecosystems and other conditions. Reference ecosystem sites provide an opportunity for investigating the extent to which manifold environmental and climate variables influence plant productivity, a crucial factor in understanding real-world carbon cycle processes across numerous environments.

- **Leveraging "Model" Systems Biology to Understand Natural Systems.** A "model" system has reduced complexity and is thus more amenable to integrated genetic, genomic, and functional analysis with emerging tools. Model systems, when used in conjunction with the study of natural systems, provide unparalleled opportunities to dissect the genetic and molecular mechanisms underlying ecologically important processes and enable experimentation on phenomena identified in natural systems. Although such an approach has yielded dramatic improvements to scientific understanding of organismal biology, this new knowledge must be scaled to higher levels of biological organization (e.g., communities and ecosystems) if scientists are to use it to anticipate the response of terrestrial and marine ecosystems to climate change.

- **Executing Long-Term Observational Strategies at Ecosystem Reference Sites.** A nested and fully instrumented hierarchical design in selected reference systems will enable long-term, space-time explorations of all types of processes in both terrestrial and ocean ecosystems. Such observational designs would include transects and disturbance clusters critical for understanding and modeling particular drivers of and responses to change. Furthermore, these reference sites can be used to examine natural ecosystem dynamics such as carbon fluxes and nutrient cycling and to track interannual variability and response to disturbance and climate anomalies (e.g., decadal drought). Observational capabilities thus provide a superb platform to test new approaches for coupling genomic-based, mechanistic systems biology to environmental and climate variables to explore linkages between biogeochemical processes and ecosystem function.

- **Using Manipulative Experiments to Measure How Primary Productivity and Related Properties in Different Biomes Respond to Climate Change.** In reference and model systems, the full range of data on factors contributing to shifts in primary productivity should be established—for example, CO_2, warming, changing hydrologic cycle, ozone, and nutrient availability. Accompanying such experiments should be a strong model-data integration component covering all aspects of biology and environmental variables during experimental design and throughout the experiment. There can be a valuable synergy in locating manipulative experiments adjacent to observatory sites.

Understanding the Response of Ecosystems and the Global Carbon Cycle to Climate Change: An Integrated Research Approach



- **Using Theory, Modeling, and Simulation (TMS).** The TMS process requires use of data-assimilation techniques to combine (1) varied types and levels of information on natural and model systems, (2) response functions from climate change experiments, and (3) measurements from observatory sites. As descriptive, predictive, and heuristic tools, TMS techniques can explore critical scenarios and variables to provide insight for research strategies, test the adequacy of scientific understanding and models, and develop hypotheses. Theory, Modeling, and Simulation also can create a virtual accelerator of global change to support modeled simulations exploring possible implications of altered carbon management or biosequestration strategies under future climate change. To reflect potential impacts accurately, climate modeling requires that coupled component models be transparently integrated across scales and processes.

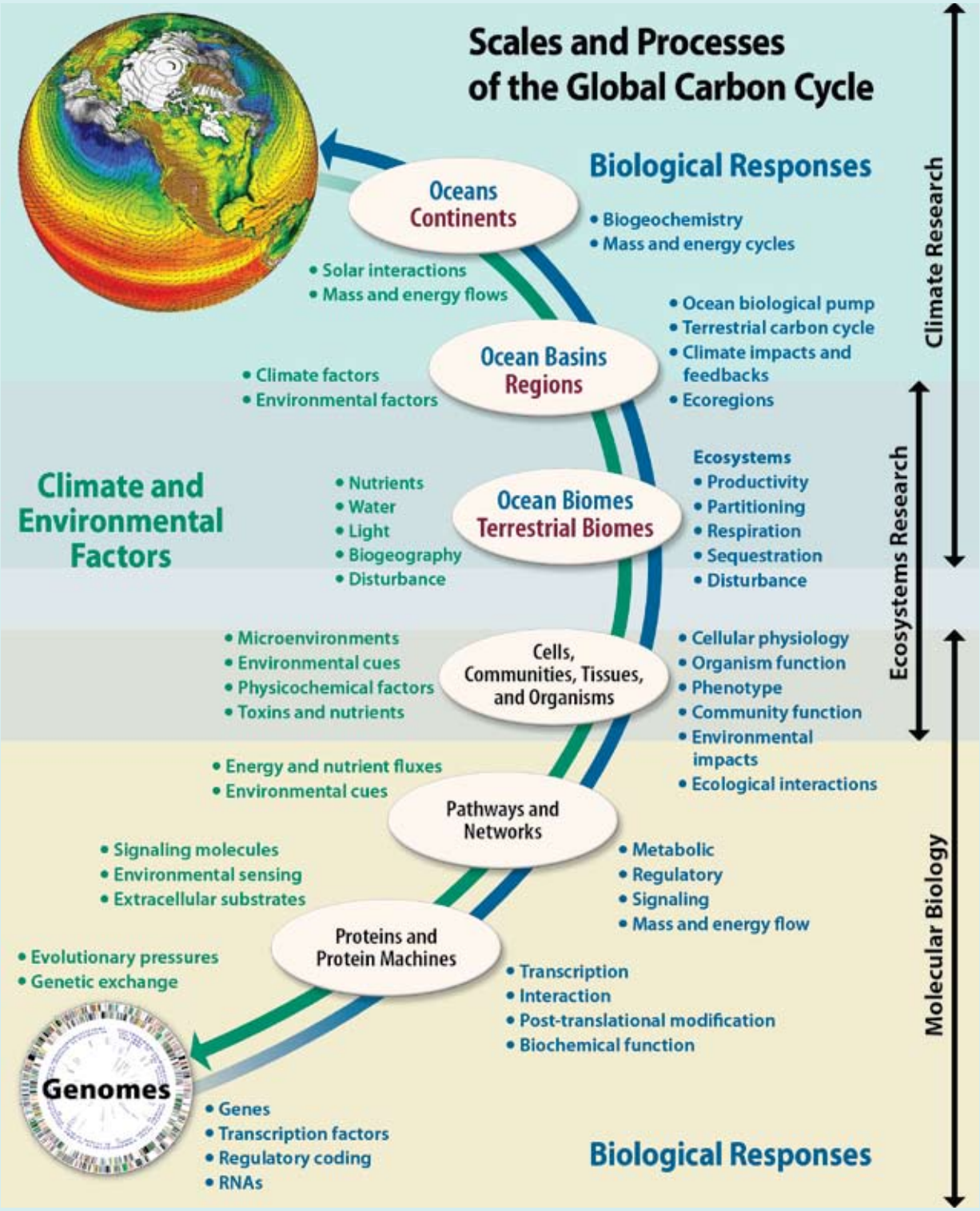


Fig. 2.1. Scales and Processes of the Global Carbon Cycle. The global carbon cycle is determined by the interactions of climate, the environment, and Earth's living systems at many levels, from global to molecular. Relating processes, phenomena, and properties across spatial and temporal scales is critical for deriving a predictive mechanistic understanding of the global carbon cycle to support more precise projections of climate change and its impacts. The domains of climate, ecosystem, and molecular biology research each has a limited reach in scales, constrained by the complexity of these systems and limitations in empirical and modeling capabilities. While linking comprehensively from genomes to global phenomena is intractable, many connections at intermediate scales are viable with integrated application of new systems biology approaches and powerful analytical and modeling techniques at the physiological and ecosystem levels. Biological responses (blue) are to the right of the systems ovals, and climate and environmental factors (green) are to the left of the systems ovals. [Note: Globe portion of figure courtesy of Gary Strand, National Center for Atmospheric Research, with funding from the National Science Foundation and the Department of Energy.]

Opportunities from Genomics and Systems Biology

Carbon cycling research and understanding can be greatly advanced by capitalizing on new and emerging insights from genomic and systems biology studies. Genome sequencing has ushered in a new generation of high-throughput or "omics" methods (e.g., transcriptomics, proteomics, and metabolomics) enabling systematic investigation of comprehensive networks of genes, proteins, and metabolites within cells. This systems biology approach—through modeling and simulation coupled with experiment and theory—aims to define organizing principles, emergent properties, and the resulting detailed organization that control the functions of organisms. Ecosystems carry out common, core functions and likely have a common set of principles and concepts encoded in their collective genomes. Even though specific functions vary from one system to another, the common fundamental principles allow the accumulated knowledge of regulatory, physiological, and metabolic functions developed for one biological system to accelerate knowledge discovery for other systems. Systems biology capabilities enable scaling to higher levels of biological organization, such as multispecies consortia, multicellular organisms, and even complex biological communities. Genomics and systems biology are hallmarks of DOE's Genomics:GTL program, whose ultimate scientific goal is achieving "a predictive, systems-level understanding of microbes, plants, and biological communities."

Linking genomic-based information to function requires both genome-scale data generation and systems biology tool development. Extending genomic understanding from model to nonmodel systems will be critical for identifying potentially useful organismal functions previously eluding study (for example, see Box 2.1, Metagenomics: Extending Genomics to Natural Systems, this page). Such efforts should be guided by larger-scale coupled models to acquire specific classes of data needed to populate component models. This data specificity, as opposed to indiscriminate accumulation of large volumes of information, will increase models' predictive capability and drive development of new theory.

Emphasis must be placed on developing and using genomic and systems biology approaches to model, for example, the regulatory networks controlling carbon processing (e.g., from assimilation by phototrophs to decomposition of organic matter by heterotrophs). Mechanistic (versus phenomenological) representations of such networks are critical for extrapolating ecosystems' properties and behaviors to a wide range of variables ideal for climate-simulation scenarios but historically outside the scope of observations. These representations are now tractable with the advent of genomic technologies that can supply a sufficient volume of information at many levels of organization and can couple that data with, for example, isotopic techniques that trace carbon flow through ecosystems.

The entire progression of data processing—from genome sequencing to determination of biogeochemical function—may be viewed as a unified (or potentially unifiable) information-sciences challenge. New instrumentation and methods for both biology and geochemistry, coupled with various visualization tools, are excellent catalysts for discovery and communication across disciplines and at multiple scales. The visualization aspect of data analysis is underappreciated but can be an

Box 2.1

Metagenomics: Extending Genomics to Natural Systems

Historically, biology has been confined to the study of individual organisms. New technologies such as metagenomics, metatranscriptomics, and metaproteomics offer a window into the metabolisms and lifestyles of vastly diverse microbes, including uncultivated organisms from environmental samples. Developing and pursuing metagenomic (or other "omic") research techniques not only will help capture the functional potential encoded in genomes, but also will enable new approaches for qualitative and quantitative measurements of active metabolic processes in the environment that then can be applied to mechanistic and predictive models.

important impetus for cutting-edge research. Furthermore, applying new tools and methods can improve the use of models to assimilate data, test understanding, and serve as heuristic and predictive tools.

Models: Predicting Carbon Cycle Behavior on Multiple Levels

A numerical model is a mathematical representation used in computer simulations to calculate the evolving state of dynamic, real-world systems. Models and simulations enable scientists to study complex phenomena difficult or impossible to examine under natural or laboratory conditions. Researchers also use models to represent and test current knowledge of a given system.

Models historically have been developed to address the needs of specialized, individual research communities. Improving the accuracy of climate projections, achieving a predictive understanding of biological carbon cycling, and assessing the feasibility of carbon biosequestration strategies require coordinated, multidisciplinary development of multiscale models and experiments that must inform and relate to one another. Three general scales of modeling—global, ecosystem, and organismal—are important to carbon cycling research. The array of models comprising each category helps expand and refine current knowledge as well as define areas requiring experimentation at multiple scales of biology.

Global Climate Models

Among all scientific computational challenges, global climate modeling is one of the most complex and computer intensive, requiring collective contributions from teams of modelers focused on different parts of the climate system. The most advanced global climate models currently available—Atmosphere Ocean General Circulation Models (AOGCM)—use mathematics and high-performance computing to couple component models for atmosphere, ocean, land, and ice. Extraordinarily sophisticated, AOGCMs incorporate phenomena ranging from volcanic eruptions' effect on temperature patterns to the impact of shifting sea ice on reflectance of atmospheric sunlight. The behavior of atmosphere, ocean, land, and ice is represented by a system of mathematical algorithms based on parameterized component systems' behaviors and the fundamental laws of physics and chemistry. However, as climate impacts become more pronounced and human presence and activities expand, model complexity must evolve to the next level: Earth System Models (ESM).

Earth System Models

As extensions of general circulation models, ESMs include biogeochemical processes, vegetation changes, and human influences to more completely simulate the multitude of factors influencing climate in all its complexity (see Fig. 2.2a. Terrestrial Ecosystem Parameters Important to Earth System Models, p. 19). Accurately predicting future CO_2 feedbacks and concentrations is a key objective driving development of ESMs. Central to meeting this goal is a detailed understanding of the global carbon cycle, including how its sources and sinks behave and respond to climatic and atmospheric change.

Connecting the Scales of Climate

Applying experimental results and observations across process, spatial, and temporal scales is the primary challenge of global carbon cycle research. Environmental scientists can measure ecosystem functions and phenomena (see Fig. 2.1. Scales and Processes of the Global Carbon Cycle, p. 16) but have difficulty relating results to higher and lower scales and extrapolating behavior outside the range of observations.

Fortunately however, scientific research is addressing these challenges. A new generation of ecosystem-level analyses and emerging genomic information hold promise for improving our mechanistic understanding of and, ultimately, ability to scale important carbon cycle and climate change processes. Key crosscutting areas of interest are new multifactor ecosystem manipulations that analyze climate change effects on carbon cycling at the ecosystem level and the potential of genomic data to inform representations of critical biological processes and parameters (e.g., mechanisms, rate constants, and submodels of metabolism and regulation). Such genomics-enabled advances are necessary for removing the "black box" of understanding surrounding the biology element of ecosystems. For example, research must fill significant knowledge gaps in the complex processes controlling

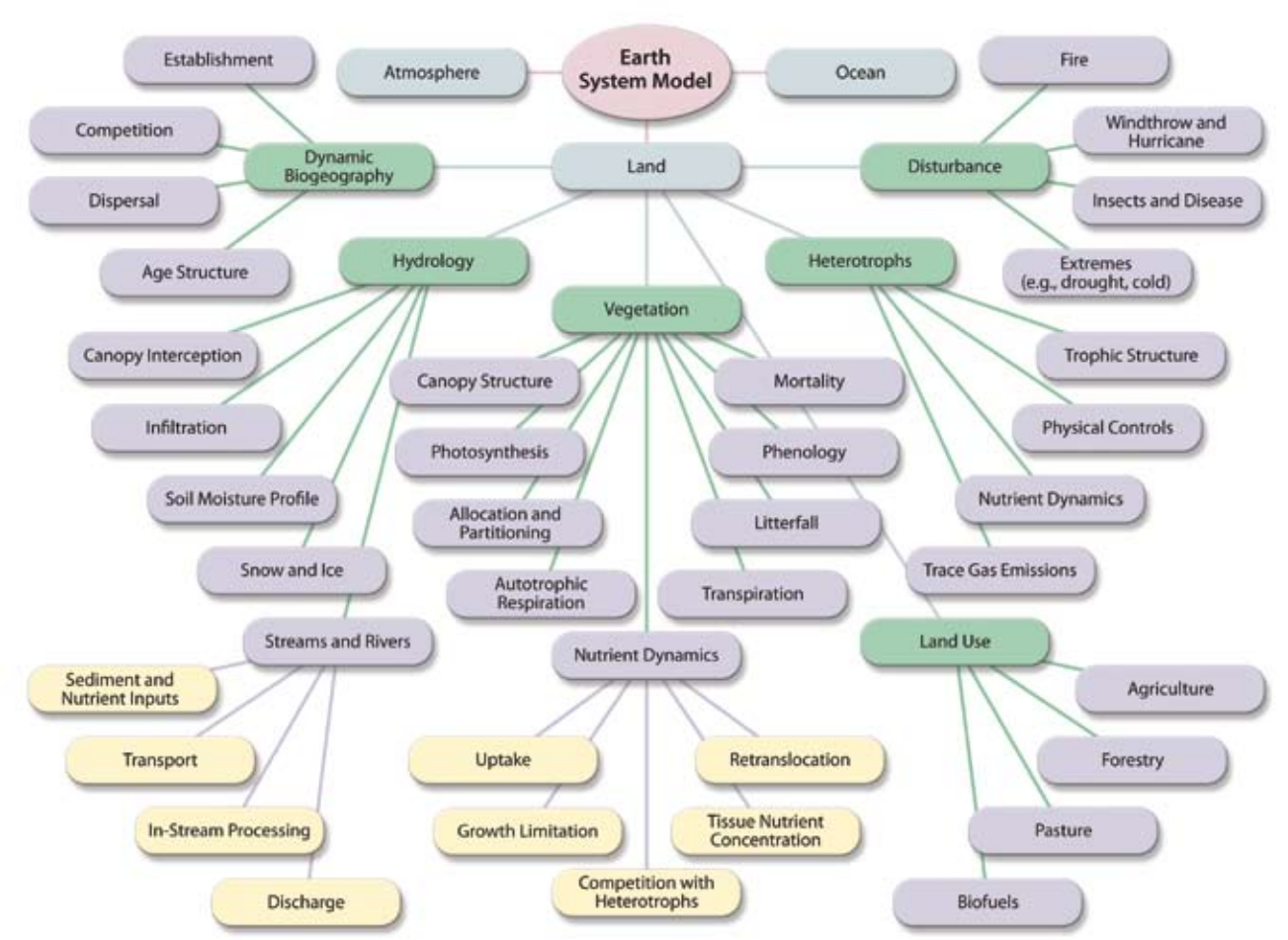


Fig. 2.2a. Terrestrial Ecosystem Parameters Important to Earth System Models.

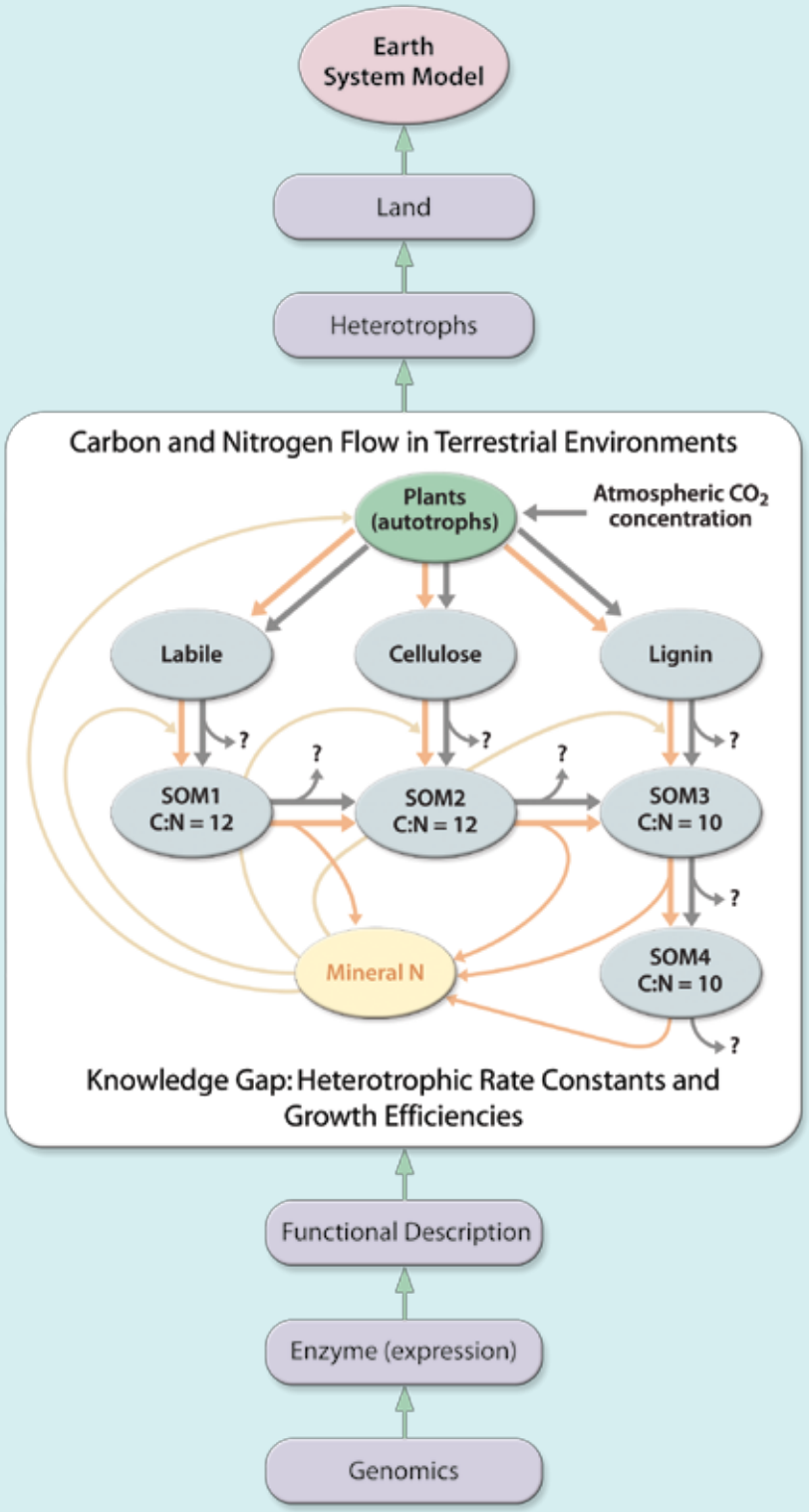


Fig 2.2b. Knowledge Integration and Synthesis.

soil carbon dynamics. Particularly needed are the rate constants and detailed mechanistic knowledge of the processing of plant litter to long-lived soil organic matter by soil mesofauna and heterotrophic microorganisms (see Fig. 2.2b. Knowledge Integration and Synthesis, this page). Current model representations of these processes are highly parameterized using rate constants from radiolabeled (^{14}C) from biomass-decomposition experiments in microcosms that lack mechanistic detail and links to actual environmental conditions.

The dynamic interplay between the functional potential encoded in a biological community's collective metagenome and the physicochemical conditions of the surrounding environment governs molecular processes controlling cellular, organismal, ecosystem, and ultimately global phenomena (i.e., phenotypic traits are the product of genome-environment interactions; phenotype = G × E). However, connecting mechanistic understanding at the molecular level to the physiological changes observed in organisms, ecosystems, and global climate represents a major challenge for these difficult-to-reconcile approaches (see Fig. 2.1. Scales and Processes of the Global Carbon Cycle, p. 16).

As computational capabilities become more powerful, models are able to incorporate greater detail about climate processes, yet including all real-world details is impossible. Thus, approximations—often based on insights from laboratory and field experimentation—or parameters derived from process calculations of more complex components are used to represent processes too small in scale or too complex to be resolved in large-scale models. Many assumptions in current global models about carbon fate in a changing climate may not be valid considering the limited understanding of the biogeochemical cycling of carbon. (For example, see the broad range of modeling parameters in Fig. 2.2a. Terrestrial Ecosystem Parameters Important to Earth System Models, p. 19.) However, larger-scale models can be enriched by more detailed, smaller-scale research to provide hypothesis-driven

experiments, measurements, and observations needed to validate and refine assumptions and parameters. For instance, research on the genetic regulation and molecular mechanisms controlling root proliferation could inform root-turnover rates used in terrestrial ecosystem models. Further model refinement will require greater insight into biogeochemical processes, particularly those yielding the largest potential feedbacks—either positive or negative—of atmospheric greenhouse gases. Thus, research strategies targeting these processes and quantifying their feedbacks are high priorities.

Ecosystem Models

Ecosystem models—categorized as either biogeographical or biogeochemical—represent interactions between biotic and abiotic components of a particular environment. Biogeographical models represent how populations in a particular region change over long time scales. Biogeochemical models represent biologically mediated transformations and flows of carbon and other materials within an environment.

Biogeographical Models. One type of biogeographical model is the Dynamic Global Vegetation Model (DGVM), which is used to study how general categories of plant functional types are established and respond to competition, disturbances, and other factors. DGVMs coupled to global climate models play a key role in projecting changes in land surface and terrestrial carbon storage. Improving DGVMs and carbon cycle models requires refining representations of response functions that link alterations in community structure to global change factors at different time scales. For example, rising atmospheric CO_2 concentration, climate warming, altered precipitation, and nitrogen deposition likely will alter the amount of organic matter transferred from plant to litter to soil (see Fig. 2.2b, p. 20). This could lead to concomitant shifts in the balance and structure of plant and microbial communities. Advancing our understanding of these responses and incorporating resultant insight into DGVMs will improve predictions of climate effects on terrestrial carbon flow.

Biogeochemical Models. These models are developed independently for ocean and terrestrial systems, and those produced by global climate–modeling communities are coupled to larger general circulation models (sometimes called global circulation models) for atmosphere and ocean.

Terrestrial biogeochemical models are based on current knowledge of carbon-transfer processes that partition photosynthetically fixed carbon into several pools. However, partitioning among plant parts and soil pools versus plant respiration is poorly understood and thus requires further research for improved model representations. Another limiting factor in biogeochemical modeling is inadequate understanding of nitrogen-climate interactions. Nitrogen availability is a key regulator of CO_2 assimilation, and although more models are incorporating nitrogen processes, insight into how nitrogen availability shifts in response to atmospheric and climate change is very limited and warrants further study. Another need is identifying—as a function of soil depth—more dynamic linkages between root deployment and soil responses, including nutrient and water uptake, decomposition, and biosequestration of carbon and nitrogen. Further progress can be made by expanding information on nitrogen fixation in natural ecosystems under steady state and in response to elevated CO_2, climate change,

Key Research Questions

1. **What is the carbon-handling capacity of global ecosystems, and how will it be affected by climate change and human activities?**
2. **Where and in what form is the carbon in global ecosystems?**
3. **What are the mechanisms at molecular, ecosystem, and global scales by which carbon is cycled into and out of ecosystems?**
4. **How long will carbon reside in various pools, and why?**
5. **What are the potential factors controlling ecosystem carbon flow (e.g., nutrients, soil physics and chemistry, soil microbial processes, temperature, and moisture)? To what extent do such factors influence carbon cycling?**
6. **How are global ocean and terrestrial carbon cycling linked to each other and climate via atmospheric processes?**
7. **What are the atmospheric factors in ecosystem productivity and carbon biosequestration, and how do such factors affect the integrated carbon-nutrient-water cycles?**
8. **How will carbon pools and biosequestration mechanisms respond to the full range of climate change variables, including CO_2, temperature, modified water regimes, nutrients, and radiation?**
9. **What opportunities are available to optimize carbon biosequestration and extend pool lifetimes?**

and disturbances. Equally important is advancing our understanding of how denitrification, leachage, volatilization, and other nitrogen-loss processes respond to elevated CO_2 and climate change. Critical to effective biogeochemical models is incorporation of several key regulatory mechanisms underlying ecosystem response to warming (e.g., adaptation of photosynthesis and respiration and nutrient dynamics). However, most models are incapable of including such information, a limitation that must be overcome for better predictive capabilities.

Oceanic biogeochemical models currently have relatively simple structures. These models may incorporate active cycling of nitrogen, phosphorus, and oxygen and are based on numerous variables such as dissolved oxygen; nutrients, including nitrate and phosphate; detritus particles; and a few general categories of organisms (e.g., phytoplankton and zooplankton). Given a particular scenario for anthropogenic CO_2 emissions, ocean ecosystem models can be used to project changes in surface-water partial pressure of CO_2, planktonic biomass, the concentration of biologically available nitrogen in the water column, or subsurface oxygen concentrations. In many cases, however, these models are based on parameter fitting to empirical data rather than mechanistic modeling and thus are less reliable in predicting dynamic responses to climate change.

Biological Models

Although powerful systems biology approaches have achieved some success in predicting gene regulatory networks that control bacterial response to genetic and environmental perturbations (Bonneau et al. 2007), modeling of cellular systems is still in its infancy. In fact, no comprehensive model of an organism or even a bacterial cell yet exists. Cells and the molecular processes driving life are so complex, building a complete model of even a single cell requires a combination of multiple modeling approaches. Examples of strategies for modeling different cellular processes and networks are (1) metabolic models using differential equations to describe enzymatic reactions and associated reaction rates, (2) gene regulatory network models characterizing gene expression and interactions between transcription factors and the genes they regulate, and (3) signal-transduction models describing information flow in cells via a cascade of chemical transformations in response to a few critical biomolecules. A key challenge for systems biology research is integrating data and information from these diverse cellular processes to create a predictive model for the behavior of whole cells and ultimately larger-scale biological systems.

Summary of Research Requirements for Biological Carbon Cycling and Biosequestration

Several consistent themes have emerged that together frame and set the requirements for the next generation of carbon cycling research. This research must aim to (1) understand and predict the behavior of the global carbon cycle and its interactions with climate, (2) improve climate change projections, and (3) create the foundations of carbon biosequestration strategies. Every element of this approach applies to each of the specific research examples outlined in this report, which integrates molecular biology, ecology, and climate modeling to address the molecular- to global-scale processes directing the carbon cycle. Specific research objectives follow.

- **Apply Diverse Scientific Approaches to Natural and Model Systems, Observations and Experimentation, and Modeling.** Using molecular and genomic approaches to investigate model systems (e.g., experimentally tractable organisms, artificially constructed communities, microcosms, mesocosms, and managed ecosystems) will generate methods and hypotheses that can drive experimentation on natural systems. Such hypotheses also can be used to determine whether model-system results can be translated to other systems. The mechanistic understanding resulting from these experiments will be used to create predictive models—at the process, ecosystem, and climate levels—that will stimulate a new generation of model-driven research. Conversely, direct observation of natural systems can generate hypotheses that can be tested more easily and rigorously in model systems (e.g., individual organisms, low-diversity constructed communities, microcosms, and mesocosms). Taken together, these activities lead to iterative refinements of experimental approaches, models, and theories.
- **Obtain a More Detailed Understanding of Major Carbon Pools.** Elucidating global carbon-carrying capacity and understanding the dynamics and response of the global carbon cycle require research that focuses on the world's major carbon pools and areas of primary productivity. These areas include boreal regions with massive stores of carbon in peat (see Fig. 2.3a. Global Carbon Storage in Vegetation and Fig. 2.3b. Global Carbon Storage in Soils, p. 24), tropical rainforests, and oceans, all of which dominate global primary productivity. Identifying and characterizing major carbon pools for particular regions within the United States can help define national strategies for mitigation and adaptation and could provide insight into global pools.
- **Characterize and Ultimately Predict Biological Response to Climate Change with Genomics and Systems Biology.** Biological structure and function at all scales are determined by the collective genome (or metagenome) of a system and its interactions with the environment. High-throughput, genomic-based tools (e.g., transcriptomics, proteomics, metabolomics, and interactomics) will be used to characterize the functions of biological systems and develop a predictive, mechanistic understanding of various systems across required spatial and temporal scales.
- **Design Experiments Addressing Multiple Climate Factors.** Research has demonstrated that the effects of multiple climate factors are not always additive combinations of the same factors taken individually but are nonlinear, complex results of shifting variables acting in concert. Research on ecosystem response

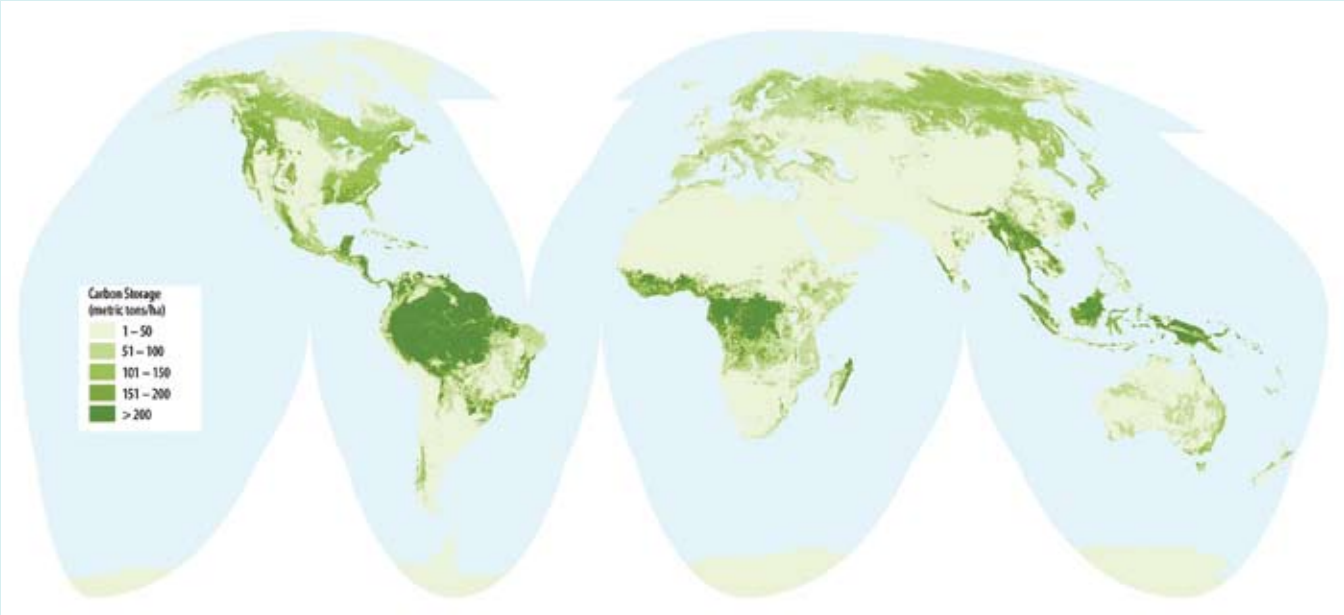


Fig. 2.3a. Global Carbon Storage in Vegetation. Estimates of carbon storage in the world's above- and belowground live vegetation are shown at a 10-km resolution. This live vegetation includes woody tissue, leaves, fruits, flowers, and root systems. The greatest carbon stores in live vegetation are observed in tropical and boreal forests. Temperate forests and tropical savannas also store significant quantities of carbon in their vegetation. [Source: World Resources Institute. 2000. EarthTrends: Environmental Information. Available at http://earthtrends.wri.org/text/climate-atmosphere/map-225.html. Washington, D.C.: World Resources Institute.]

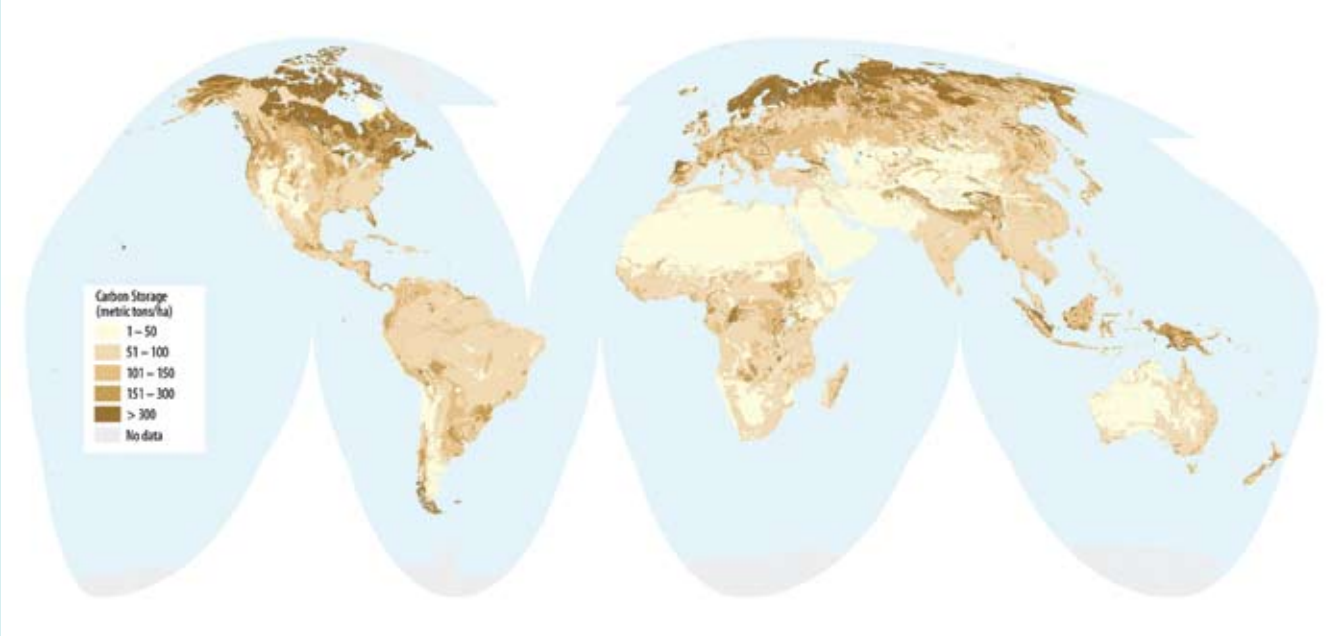


Fig. 2.3b. Global Carbon Storage in Soils. The greatest soil carbon stores are found in high latitudes (e.g., boreal forests and tundra), with other important stores located in tropical forests, tropical savannas, and temperate grasslands. [Source: World Resources Institute. 2000. EarthTrends: Environmental Information. Available at http://earthtrends.wri.org/text/climate-atmosphere/map-226.html. Washington, D.C.: World Resources Institute.]

must therefore employ a balanced scheme of observations and experiments incorporating the full range of climate factors (e.g., CO_2, temperature, precipitation, nutrients, ozone, and cloudiness) critical for predicting scenarios of future climate change.

- **Link Carbon, Nutrient, and Water Cycles.** Carbon cycling cannot be studied in isolation. Photosynthetic productivity and respiration are limited by water and nutrient availability, thus research must integrate carbon, nutrient, and water cycles. For example, the *Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (IPCC 2007) characterized the CO_2-fertilization effect as a negative climate–carbon cycle feedback and regarded this conclusion as robust, even though the studies on which this result was based used coupled climate–carbon cycle models that excluded nutrient cycles. Several studies have suggested that incorporating nutrient cycles can change the magnitude and even sign of this feedback.
- **Address Multiple Scales of Time and Space for Processes Underlying Climate Change.** A major challenge in climate research is integrating information about processes occurring at various spatial scales—from molecular (nanometer scale) to ecosystem (meter to kilometer scale) to global levels. Time scales for these processes extend from picoseconds to centuries. Along with empirical and theoretical methods, modeling and simulation techniques must transparently bridge these vast spans of space and time.
- **Apply Advanced Instrumentation and Methods.** Critical for carbon cycling research are new generations of tools for investigating biological mechanisms and measuring the flux of carbon and other materials at molecular, cellular, organismal, and ecosystem scales. Needed tools include suites of sensors and techniques for field-scale, in situ, and remote-sensing observations as well as those enabling laboratory- and facility-based measurements.
- **Understand the Interactive Genomic, Environmental, and Climatic Influences on Plant Productivity.** The mechanistic bases underlying ecosystem productivity are the consequences of interactions between global genomic potential (biological capabilities) and environmental and climate factors. (Phenotypic traits are the product of genome-by-environment interactions.) Research must elucidate the effects of all such factors on the underlying biological metabolic, regulatory, and physiological processes of plant productivity. For example, greater insight is needed into carbon partitioning, community composition, and how different community members are affected by climate change. A mechanistic understanding of these and other relevant processes is necessary for better representation of them in models. Environmental variables defining agroecosystems and biomes are important to plant productivity, such as soil mineralogy, physiology and chemistry, topography, hydrology, latitude, length of season, and length of day. Significant climate factors include temperature, precipitation, radiation, cloudiness, humidity, CO_2 and other gases, and nutrient inputs. Because interactions between these variables and a community's collective genome can affect productivity profoundly, rigorous investigation is required.
- **Determine the Role of Disturbance in Ecosystem Dynamics.** Ecosystems' carbon-carrying capacity and dynamics are influenced greatly by their disturbance history. Thus, global ecosystem inventories must be calibrated in terms of such histories, and the effects of climate change and human activity on

disturbance frequency and severity must be more precisely understood and predicted (see Box 1.1, Types of Ecosystem Disturbances, p. 10).

Interdisciplinary Projects and Training

Addressing current and future challenges in climate change science requires cross-disciplinary interactions and training as well as integration of modeling and experimentation. Particularly essential is incorporating classical ecosystem science and biology into climate and biogeochemical modeling in a synergistic manner. Solving complex problems in carbon cycling requires interdisciplinary teams focused on a common set of questions and working toward a shared goal (a grand challenge concept). Effective collaboration comprises the appropriate mix of skills and capabilities to facilitate linkage of different disciplines, theory, experiments, and models at various scales. A shared vision of producing more-predictive models should be developed, with specific needs depending on the particular ecosystem aspect under investigation. For example, climate modelers, biogeochemists, soil scientists, microbial ecologists, and molecular biologists and bioinformaticians together could address the problem of connecting climate-modeling needs with soil biology to provide critical kinetic mechanisms and parameters for processing of soil organic matter (see Fig. 2.2b. Knowledge Integration and Synthesis, p. 20).

Systems biology and modeling in general require cooperative, cross-disciplinary efforts performed in close collaboration with experts in, for example, computer science, engineering, statistics, physics, and information visualization. Moreover, collaborations among groups studying various organisms and across multiple scales (from molecules to ecosystems) should enable development of approaches agnostic to the source of the data. Specific workshops and cross-disciplinary projects, programs, and training should be developed to foster interactions among groups using systems approaches. Such opportunities would promote modeling and data integration across platforms and scales, including genomic, organismal, environmental, ecosystem, and climate.

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

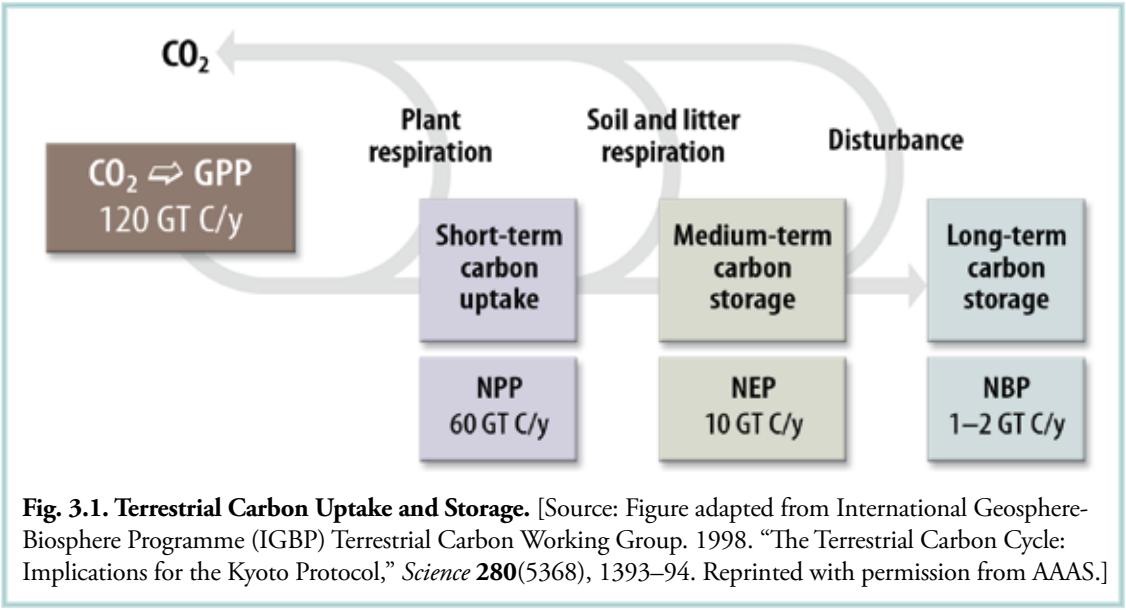


Fig. 3.1. Terrestrial Carbon Uptake and Storage. [Source: Figure adapted from International Geosphere-Biosphere Programme (IGBP) Terrestrial Carbon Working Group. 1998. "The Terrestrial Carbon Cycle: Implications for the Kyoto Protocol," *Science* **280**(5368), 1393–94. Reprinted with permission from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

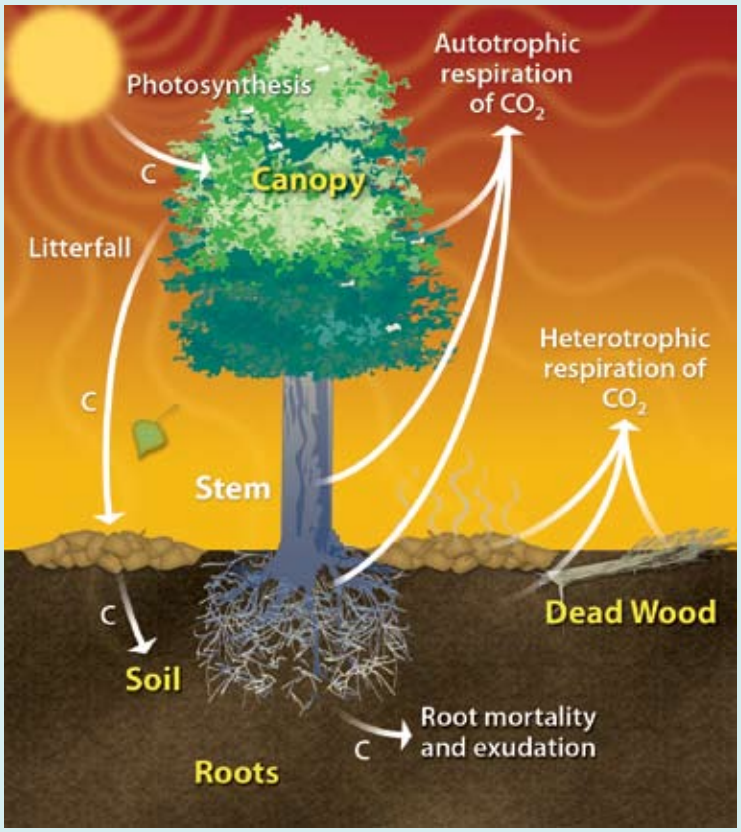


Fig. 3.2. Terrestrial Photosynthetic Carbon Cycle. [Source: Image adapted from and used courtesy of N. Scott and M. Ernst, Woods Hole Research Center, http://whrc.org.]

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.

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?**

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

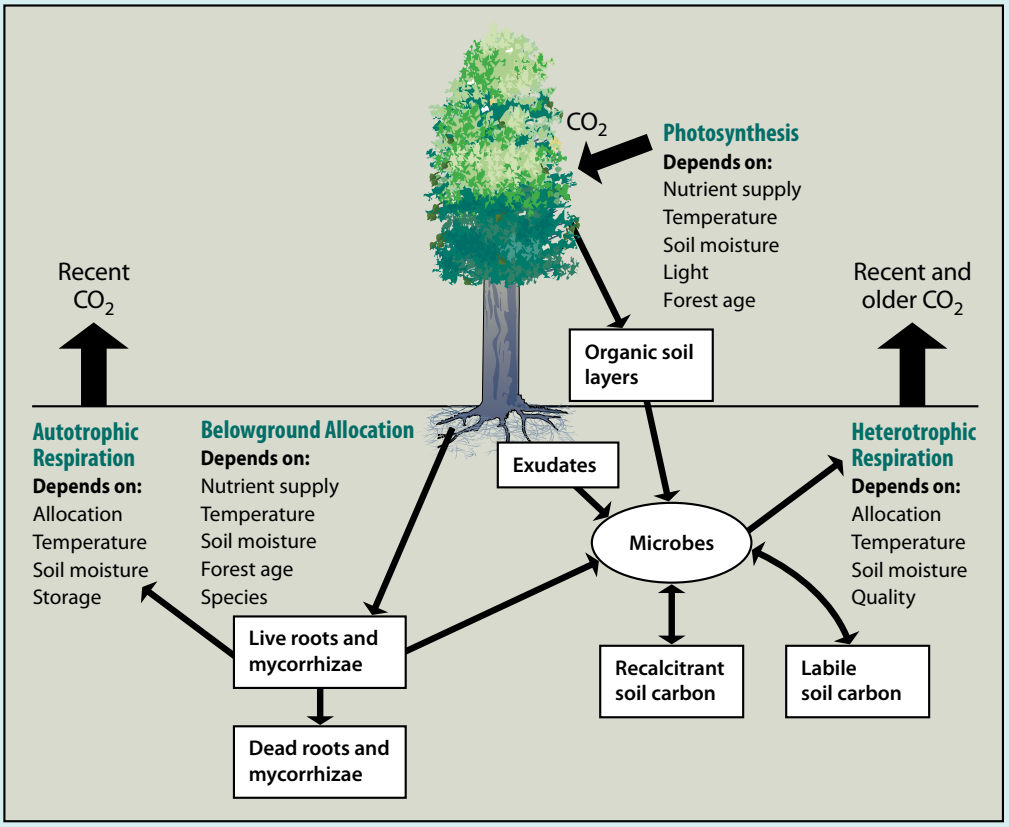


Fig. 3.3. Conceptual Model of Components and Responses of CO_2 Efflux from Soil. Both the autotrophic and heterotrophic components of soil respiration are strongly controlled by substrate availability—phloem transport of carbohydrate supply for root and mycorrhizal respiration and dead organic material 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

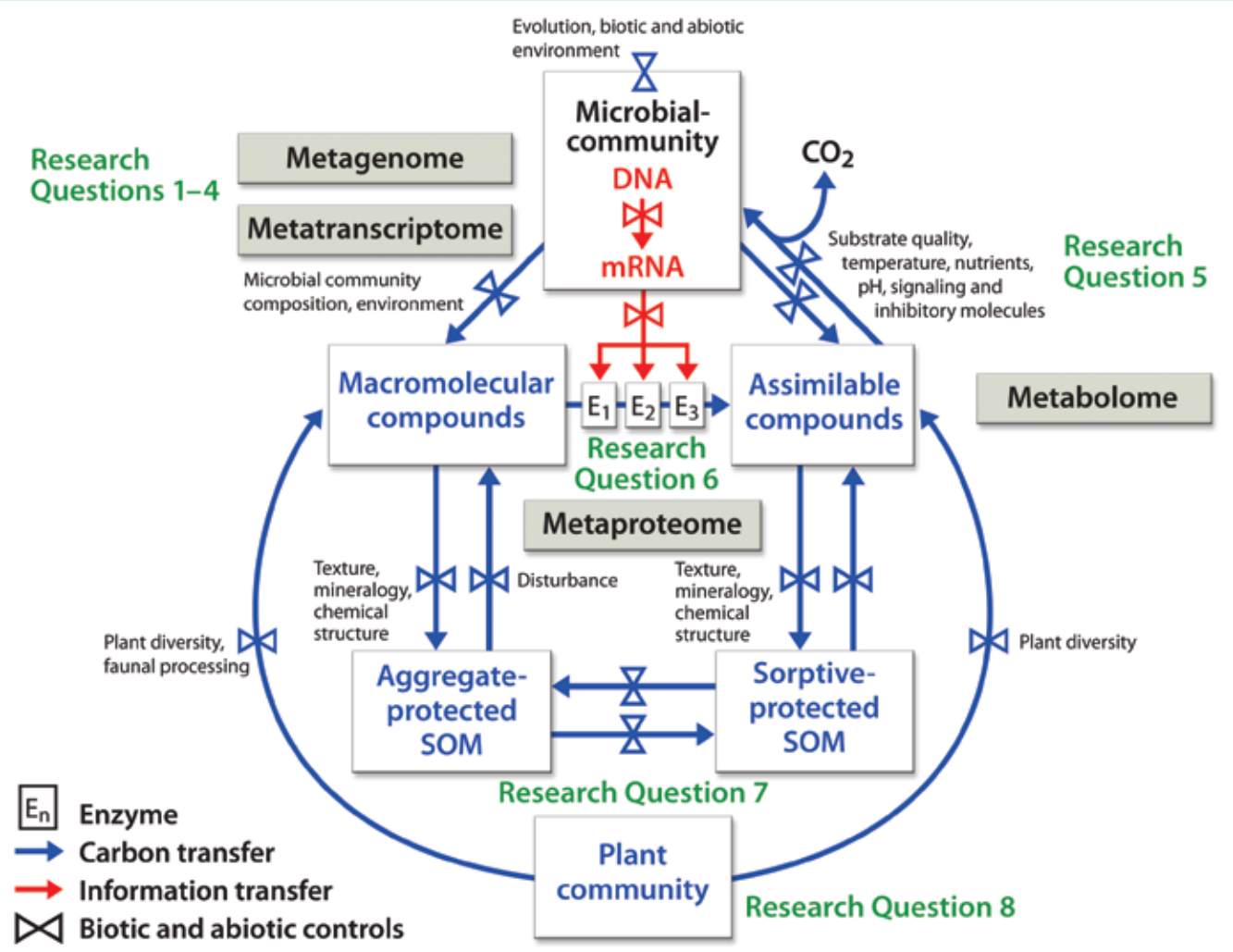


Fig. 3.4. Microbial Communities and Soil Carbon Cycling and Storage. This conceptual diagram provides a framework for integrating genomic, transcriptomic, proteomic, and metabalomic information with biogeochemical process data to better understand the relationship between microbial communities and soil 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.

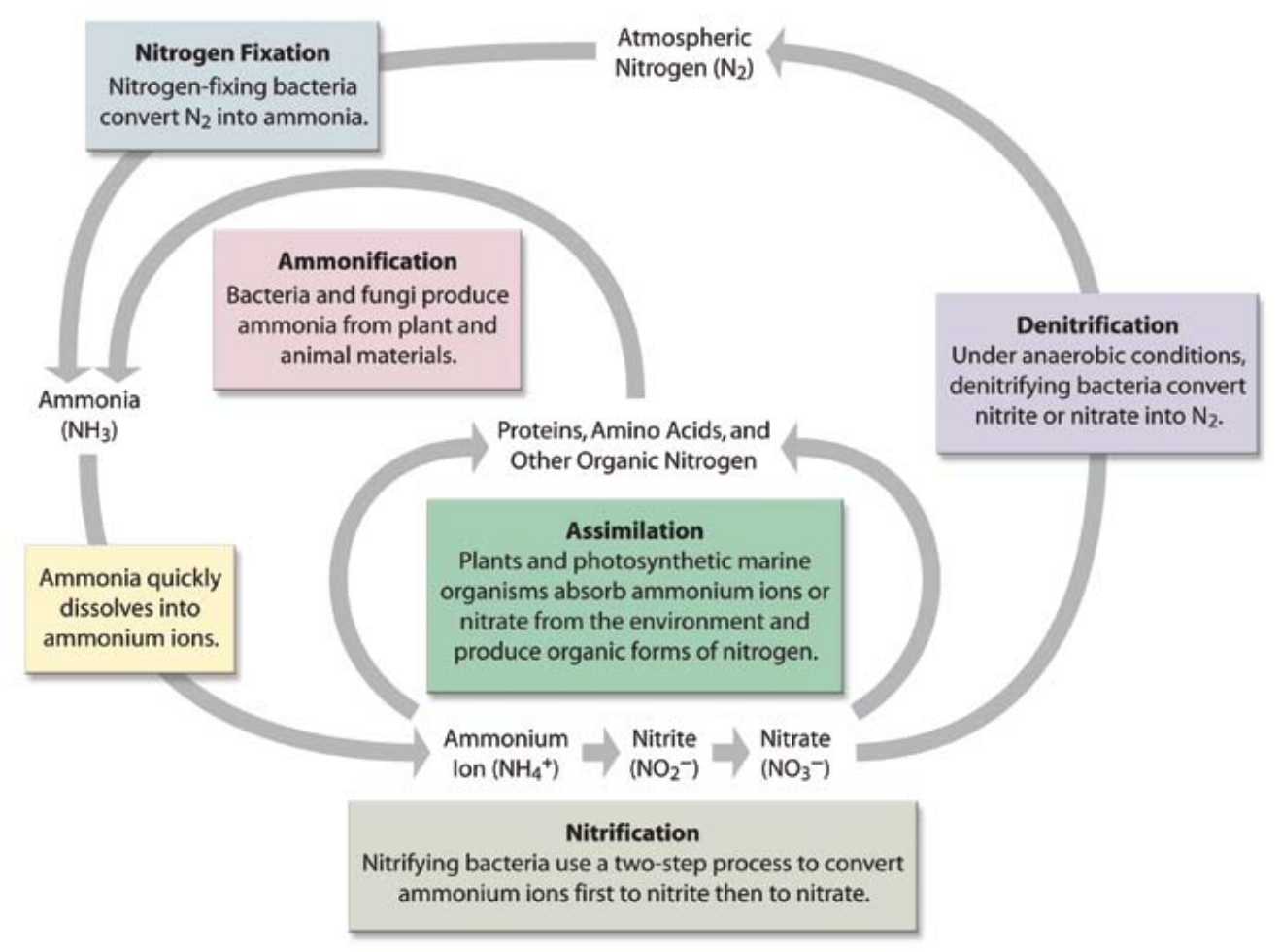


Fig. 3.5. Nitrogen Cycle.

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

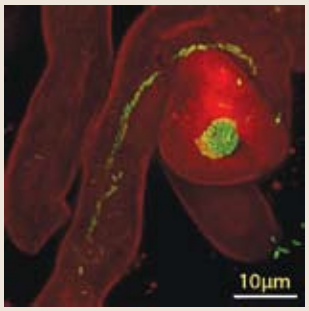
Plant-Microbe Symbioses for Nitrogen Fixation

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.

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.



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.]

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.

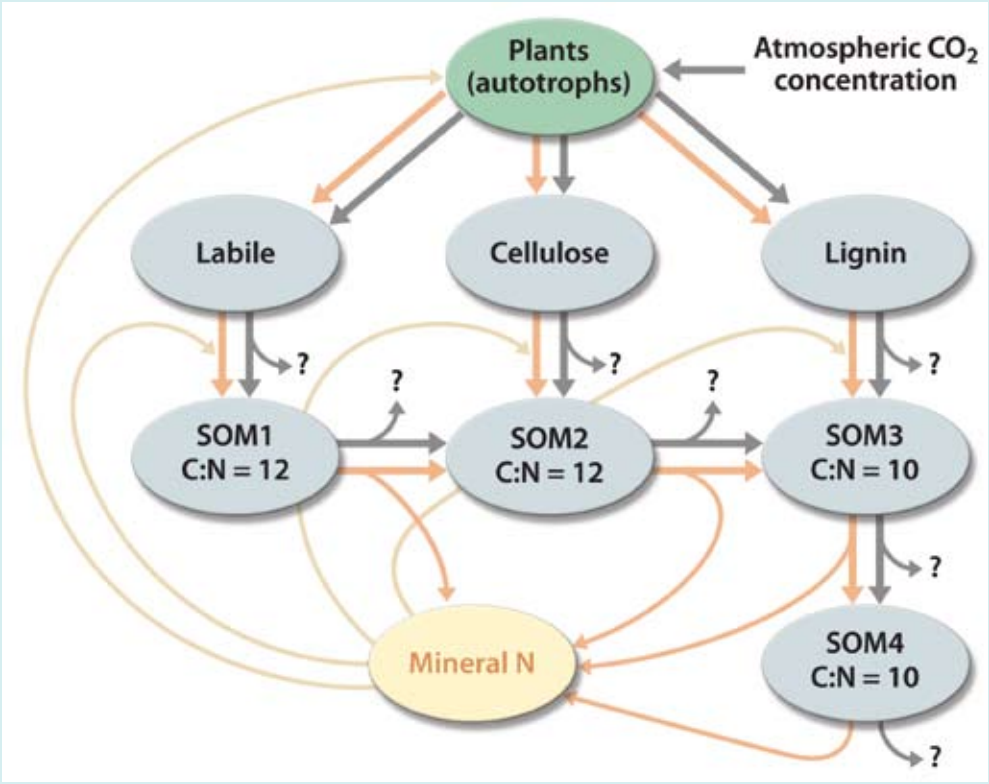


Fig. 3.6. Soil Carbon and Nitrogen Dynamics: Heterotrophic Cascade in the Decomposition of Plants. Heterotrophic decomposition of different plant material produces various pools of soil organic matter (SOM) and nitrogen. Better knowledge of the biochemical mechanisms and kinetic parameters associated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

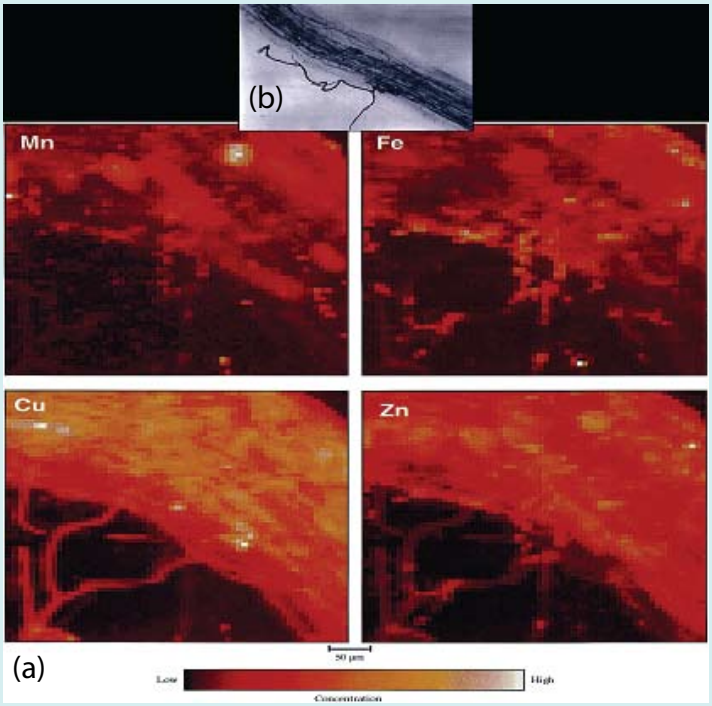


Fig. 3.7a–b. Distribution of Micronutrients in Plant Roots and Associated Fungal Hyphae. (a) Selected element-specific XRF images of a hydrated *P. lanceolata* root infected by the mycorrhizal fungus *G. mosseae*. The concentration ranges for the individual images are 0.03 to 0.87 mg cm^{-2} for Mn, 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).

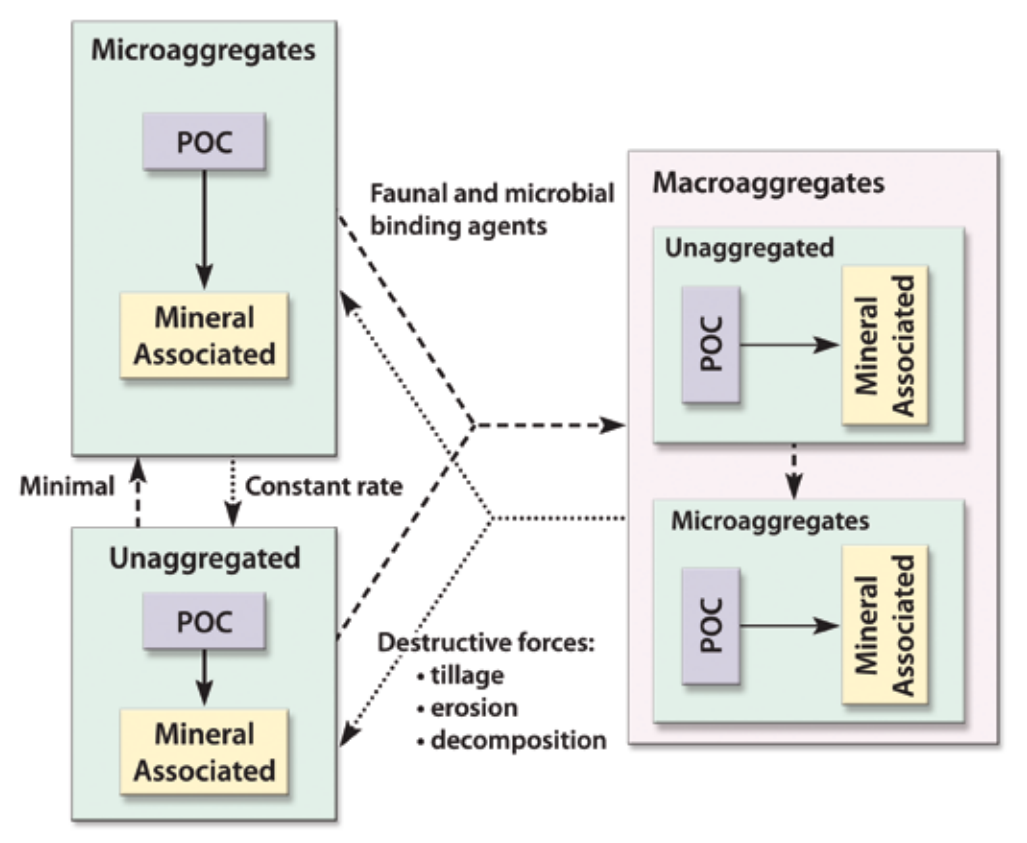
Clays and Stable Humus

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 belowground 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.

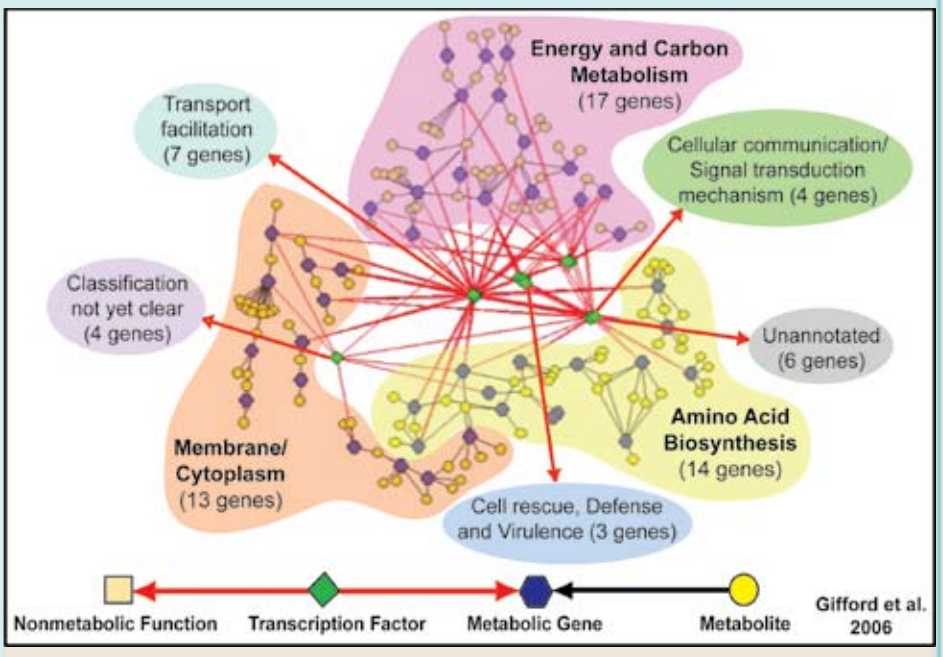
The relationship between CO_2 and nitrogen assimilation is critical to plant productivity. Assimilation of inorganic nitrogen into its organic form requires photosynthetically derived carbon skeletons to serve as backbones for transforming nitrogen into amino acids. These amino acids are used for DNA 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)

Continued from p. 57, Strategies for Optimizing Productivity and Carbon Biosequestration in Managed Ecosystems

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.

Ecosystem and Plant Response to Environmental Variables and Climate Change

Biotic and Abiotic Interactions in Ecosystems

Plants live in complex environments where many biotic and abiotic factors limit or promote productivity and carbon biosequestration. Temperature and water extremes, nutrient and other resource availabilities, microbe-induced diseases, and insect attacks can limit plant productivity. At the same time, this productivity intimately depends on plant interactions with certain beneficial microorganisms (as described in Chapter 3, Carbon Flows in Ecosystems, p. 27) and access to appropriate temperature, water, and light regimes. Furthermore, these biotic and abiotic factors can influence each other, giving rise to complex environmental conditions to which plants must adapt. This complexity is illustrated by millions of acres of bark-beetle outbreaks triggered by warming climates that in turn increase winter survival of bark-beetle populations. In the coming decades, such ecological complexities will pose increasing challenges in both agricultural and natural ecosystems (see Chapter 5, Ecosystem Dynamics, p. 71, for a discussion on the impacts of climate change on the frequency and severity of such disturbances).

Uncertainty about how abiotic and biotic factors interact at a mechanistic level limits a comprehensive understanding of plant and ecosystem productivity. This knowledge gap reduces our ability to interpret observations, make meaningful projections concerning disturbance and its impacts, and develop the strategies needed for intervening in an ecosystem's response to abiotic and biotic interactions. Thus, determining which abiotic and biotic factors most affect plant productivity, the mechanisms by which these factors act, and whether particular factors influence the quality of biomass accumulation (e.g., transient versus stable biomass) is essential for predicting ecosystem response. Moreover, such knowledge could reveal strategies to either enhance or diminish the extent to which particular interactions affect improved carbon biosequestration.

Consequently, achieving increased plant productivity and carbon biosequestration requires studying and managing abiotic and biotic processes and interactions at multiple levels of organization—from molecular biology to whole-organism phenotypes to ecological communities to global factors that influence Earth's carbon cycle and climate change.

Water Factors in Ecosystem Productivity

Potential alterations in water availability arising from climate change will have significant implications for plant productivity. For example, climatic changes are expected to affect the overall rainfall quantity in many parts of the world, undoubtedly influencing plant growth. More subtle shifts in rainfall patterns throughout the year also might profoundly impact plant and plant-community growth patterns. Additionally, climate warming will alter soil water balance

Key Research Questions

1. **What are the abiotic and biotic factors and interactions that determine nutrient availability?**
2. **How are the carbon, nutrient, and water cycles linked, and how do such linkages determine ecosystem productivity, carbon biosequestration, and response to climate change?**

irrespective of changes in precipitation. The extent of such alterations will depend on soil type. For example, soils unable to absorb and retain moisture may be affected more severely than others able to do so. Moreover, changes in soil water content will have downstream effects on microbial communities and chemical and nutrient mobility in soils. Likewise, climatic changes altering rainfall pH (i.e., acidification of rainwater) could have broad impacts on the chemical composition, bioavailability of inorganic nutrients, and microbial communities in soil. A comprehensive understanding of these complex, climate-induced changes is needed for accurate, predictive modeling of the effects of altered water availability on plant productivity.

Plant Traits and Strategies for Combatting Drought

Plants possess a variety of strategies—some more successful than others—for dealing with water limitation. Such strategies fall into one of three general categories: (1) drought escape, reflecting plants' ability to alter their life cycle to escape periods of water shortages; (2) drought avoidance, in which plants adjust internal processes to maintain their internal water supply; and (3) drought tolerance, which enables plants to continue to grow, though perhaps in an altered manner, despite reduced water (Bray 1997). Limited understanding of the mechanisms directing these strategies impedes our ability to optimize plant traits and productivity amid water deprivation. However, two traits—water use efficiency (WUE) and root systems—have been focuses in research to enhance plant productivity during drought.

Defining Genes and Processes that Determine Water Use Efficiency

Water is central to the distribution and productivity of plants in ecosystems and agriculture. Changing rainfall and temperature patterns give impetus to determining the molecular and developmental mechanisms that influence water use efficiency and plant productivity during drought. Experimental approaches in model plant species and crops have begun to identify causal influences on drought tolerance arising from various plant WUE strategies, including adaptations of traits for stomata, transpiration, root architecture, and other diverse physiological mechanisms. The role of symbiotic fungi in water-deprivation adaptation also must be considered. Combined research approaches using systems biology, omics technologies, spectral analyses of water-stressed plants, and whole-plant phenotypic analyses of natural genetic variation offer great potential for understanding and manipulating drought tolerance in plants. Such an integrated understanding and subsequent optimization strategies would have important implications for plant productivity and carbon biosequestration.

Transpiration and Nutrient Acquisition

Many global climate change variables—including precipitation, temperature, length of growing season, humidity, and radiation intensity—likely will affect water availability and use by plants. The significant and direct impact on primary productivity from climate-induced shifts in plant water status is commonly recognized. Less widely known, however, is that altered soil water availability and transpiration will have important secondary effects on plant acquisition of soluble nutrients, especially nitrate-N, calcium, magnesium, sulfur, and silicon.

Regulation of Root-System Architecture for Water Acquisition

Numerous studies have shown that elevated CO_2 changes root architecture, or the spatial configuration of root systems. Architecture traits significantly correlate with a plant's ability to survive under water stress. For instance, lateral-root density, a key trait in determining productivity, is linked to plant performance when water is limited. Denser lateral roots facilitate more water uptake, thus allowing plants to perform better during drought. Root systems and the mechanisms by which they increase water capture vary widely among drought-tolerant plants. In some plants, roots extending well below the surface obtain water deep into the soil profile. In others, shallow root systems allow rapid capture of rainwater before it is lost to evaporation. Some plants are highly plastic, having root systems that change in response to water availability or shift during development to adjust to seasonal fluctuations in soil water distribution. Moreover, hydrotropism, though poorly understood, is a process allowing roots to sense and grow toward water. These various types of root systems are clearly important to plant productivity and survival during water stress. Thus a thorough understanding of the mechanisms regulating their development and the potential consequences of climate change on root architecture is critical. Also needed is greater insight into how root-system architecture and interactions with rhizobia change in response to shifts in water distribution.

Root Architecture and Nutrient Acquisition

The role of root architecture in mediating plant response to climate change will depend on ecosystem edaphic constraints—the limitations arising from specific soil conditions. Most terrestrial ecosystems have multiple such constraints, including low availability of nitrogen, phosphorus, and often calcium, as well as excessive levels of aluminum, manganese, or salinity. Although root-system response to elevated CO_2 and nitrogen has received some research attention, few studies have investigated how shifts in root architecture affect other nutritional constraints. For example, architectural changes arising from elevated CO_2 may have very different impacts when comparing plant acquisition of phosphorus and calcium, nutrients often limiting in forest soils. Phosphorus is diffusion-mobile, and calcium moves by mass water flow. Thus, architectural changes resulting in finer branching or root proliferation in topsoil may increase phosphorus acquisition, and those resulting in root extensions into deep areas with greater water availability might enhance calcium uptake. Greater analysis is needed of root-system interactions with specific nutrients and edaphic limitations prevalent in most native soils. Without such understanding, making general statements will be difficult when predicting how elevated CO_2 and other climate change variables will alter root architecture and how these alterations will affect nutrient acquisition in future atmospheres.

Rhizodeposition of Root Exudates

About half a plant's belowground carbon allocation is deposited in its rhizosphere or root zone. Most of this carbon material consists of dead root tissue, but a portion contains compounds exuded by living cells. Compounds in these exudates—including mucilage, organic acids, phosphatases, phytosiderophores, and protons—protect growing roots from aluminum stress and, in concert with soil microbial symbionts, mobilize relatively insoluble nutrients such as phosphorus and iron. Interactions of global change variables with root exudates thus

should have important consequences for plant growth in acidic and alkaline soils. However, the few studies examining climate change impacts on root exudates have produced conflicting results. For example, in some studies, elevated CO_2 had no effect on root exudates, while in others it decreased exudates and altered their composition. Further uncertainty surrounds observed increases in rhizosphere respiration amid elevated CO_2, but whether these increases are due to additional exudates per unit root surface area or simply greater root growth is unclear. Adding to the complexity of exudate functioning and composition are interactions with light, temperature, and other variables affecting photosynthesis. Though challenging, the complex interplay of root exudates with root photosynthate supply, root growth and architecture, and the rhizosphere deserves further study because of the importance of these interactions for plant adaptation to acidic and alkaline soils comprising much of Earth's land surface.

New methods must be developed in plant physiology, soil microbiology, biochemistry, and systems biology for improved understanding of these interactions at genomic through organismal scales. Models to support simulations of systems must be written to capture this new level of integrated understanding and thus accurately represent, at organismal to global scales, plant-soil interactions and their link to global carbon cycling.

Temperature and Light Impacts on Plant Productivity

Shifts in temperature arising from climate change have serious implications for plant productivity and thus carbon biosequestration. Climate warming affects almost all physical, chemical, and biological processes. Several key regulatory mechanisms underlying ecosystem response to such warming include acclimation of photosynthesis and respiration, phenology, nutrient dynamics, and ecohydrological regulation (Luo 2007). Despite the importance of these basic processes, most models still are incapable of representing how they are affected by climate change.

Even small changes in temperature can have profound impacts on chemical reactions determining plant productivity. Understanding how temperature affects these processes is thus critical, particularly when making global-warming projections. For example, shifts in soil temperatures might accompany changes in microbial communities, rates of SOM degradation, and soil chemistry. These in turn may alter nutrient supplies to plants. Furthermore, microbes, plant roots, and degrading litter facilitate the release of a complex array of chemical substances (e.g., proteins, amino acids, and phenolic compounds) whose interactions with each other may be affected by shifting temperatures. Aboveground temperature changes also might influence gas-exchange kinetics in leaves. Moreover, since the plant itself has no buffer against temperature changes, chemical reactions within plant cells may be fundamentally altered.

Equally important to plant productivity is light, and thus understanding how climate change can influence it is critical. A key area requiring further study is climate change effects on cloudiness and aerosols, factors that influence radiation incident on ecosystems. Changes in this primary energy input for plants, therefore, would impact ecosystem growth significantly. Furthermore, the quality, intensity, and spectral distribution of light affect carbon fixation and flux in ways not completely understood. Light quality, for example, triggers signaling cascades in plants that

regulate important aspects of development, including organ morphology, overall shoot and root proliferation, and flowering time. A deeper understanding of such mechanisms and how climate change might affect them is needed to predict future plant productivity and carbon biosequestration.

Change in Growing Season and Resultant Phenology

Changes in the length of growing seasons have been detected over broad areas and are some of the more obvious manifestations of climate change effects on ecosystems. Increased growing-season length will have not only phenological impacts, directly affecting both photosynthesis and respiration fluxes, but also a range of indirect effects (e.g., changes in herbivore-plant interactions, litter quality, and stocks of nonstructural carbohydrate reserves in plants). For many ecosystems, the net effect of growing-season changes on carbon balance is not yet known on decadal time scales. Such changes could influence the effectiveness of forest management for carbon biosequestration in unexpected ways, such as the interactions mentioned above. Shifts in the length of growing seasons present both a modeling challenge and potential test for models of carbon allocation and residence time in ecosystems, especially for examining interactions of changing seasonality with elevated CO_2 and nitrogen deposition.

Experimental Responses of Different Biomes to Atmospheric and Climatic Change

Experimental Results and Extensions to Tropical and Boreal Systems

Manipulative field experiments have been used to quantify the response of net primary productivity (NPP) to elevated CO_2 and simulated climate change in different ecosystems. Synthesizing the results of four Free-Air CO_2 Enrichment (FACE) experiments in forest ecosystems, Norby et al. (2005) concluded that the response of forest NPP to elevated CO_2 concentration is highly conserved across a broad range of productivity, with stimulation at the median of 23% ± 2%. At low leaf-area indices, much of the enhanced productivity was attributed to increased light absorption, but as leaf-area indices expanded, the response to elevated CO_2 concentration was wholly caused by greater light use efficiency. The surprising consistency of response across diverse sites provides a benchmark to evaluate predictions of ecosystem and global models.

For example, in exploring the ramifications of CO_2 fertilization in simulations of future climate change using an intermediate-complexity coupled climate-carbon model, Matthews (2007) simulated the four forest FACE experiments. The model response of NPP to elevated CO_2 concentration was remarkably close to experimental results, lending increased credibility to the model's formulation. Similarly, Hickler et al. (2008) found that the LPJ-GUESS dynamic vegetation model reproduced the magnitude of observed NPP enhancement at the forest FACE sites. However, predicted NPP enhancement in tropical forests is more than twice as high as in boreal forests, suggesting that currently available FACE results are not applicable to tropical ecosystems. This prediction highlights important differences among biomes in their response to elevated CO_2 concentration and sets forth the hypothesis that, relatively, tropical-forest NPP will be more responsive

and boreal-ecosystem NPP less responsive to future CO_2 concentration increases. Testing this hypothesis with manipulative experiments in tropical forests [where gross primary productivity (GPP) is highest] and boreal ecosystems (where more carbon is stored) is a critical research need that likely could clarify important uncertainties about the carbon cycle.

More difficult to address in manipulative field experiments are ecosystem responses to climatic warming. Using meta-analysis, Rustad et al. (2001) reported that aboveground plant productivity increased in response to warming in high-latitude systems but declined as latitude decreased. Unfortunately, no data were available for assessing ecosystems at latitudes lower than 34°. Despite a lack of data on how warming affects GPP and NPP in tropical ecosystems, the most pressing research need is understanding productivity responses in boreal systems. These ecosystems store a large amount of carbon, and climate change, particularly warming, could accelerate decomposition, leading to massive loss of carbon and a positive carbon feedback to the climate system. On the other hand, NPP response to CO_2 fertilization and extended growing seasons caused by warming could produce a negative feedback on atmospheric CO_2. The net effect of warming in boreal systems, including permafrost melting, encroachment of woody shrubs, and altered albedo, is impossible to predict with current data and understanding. Manipulative warming experiments in boreal ecosystems, which thus far have been too small in scale, must be expanded greatly to provide better guidance.

Nutrient Availability and Soil Moisture as Determinants of CO_2 Response

The apparently robust conclusion from FACE studies that forest NPP is enhanced by elevated CO_2 masks several significant sources of variation that could be especially important in determining how a specific site will respond to rising CO_2 concentration. At the Duke University FACE site, a wide range of NPP responses to CO_2 enrichment across replicate plots correlated with differences in soil nitrogen availability. Under low nitrogen availability, CO_2 enrichment increased NPP by 19%, whereas under intermediate and high nitrogen availability, NPP rose 27% (Finzi et al. 2002). When soils are poor in nutrients or experience prolonged water limitation (represented by only within-site variation in the Duke dataset), forests may have limited capacity to support any response to CO_2 enrichment (Oren et al. 2001). Furthermore, concurrent increases in tropospheric ozone could negate productivity increases from elevated CO_2 concentration (Karnosky et al. 2001; King et al. 2005). Nitrogen availability is not only a factor in spatial variability (e.g., how specific sites respond to such conditions), it also may influence whether NPP responses observed at the Duke site can be sustained for decades (Hungate et al. 2003; Luo et al. 2004).

The exclusion of nutrient interactions limits confidence in model conclusions simulating the complex feedbacks between carbon cycling and climate change. In fact, although one summary conclusion from the *Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (IPCC 2007) regarded a particular negative climate–carbon cycle feedback as a robust result, the studies on which this conclusion was based used coupled climate–carbon cycle models that excluded nutrient cycles. Several studies have suggested that incorporating nutrient cycles into these coupled models can change not only the magnitude of the feedback, but whether it is positive or negative as well. Current observations and experimentation are not comprehensive enough to constrain this source of modeling uncertainty. IPCC

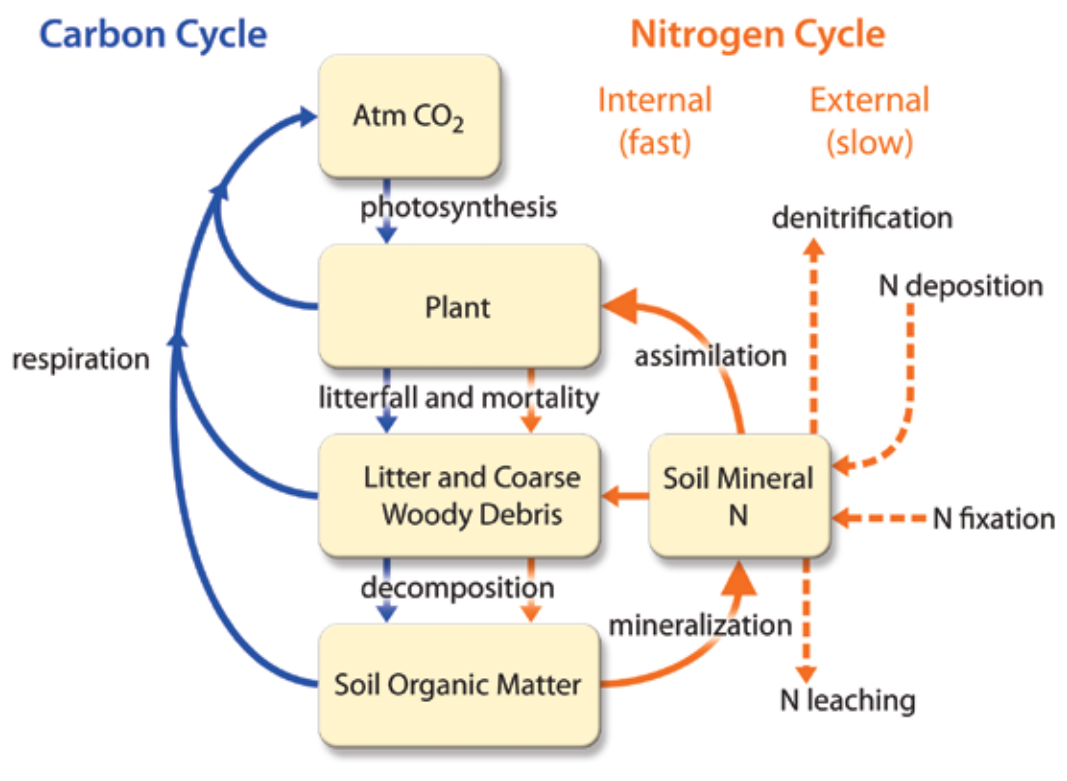


Fig. 4.1. Coupling of the Carbon and Nitrogen Cycles.

(2007) models included essentially independent responses of photosynthesis and ecosystem respiration to warming. However, introducing nutrient cycling (nitrogen as a first step) into models changes system dynamics by coupling photosynthesis to heterotrophic respiration through mineralization of nitrogen from soil organic matter (see Fig. 4.1. Coupling of the Carbon and Nitrogen Cycles, this page). Central to coupling these cycles in models is also coupling plant and microbial communities in ecosystems through microbial decomposition of detritus and biological nitrogen fixation.

Plant-Soil Interactions (Soil Physicochemistry)

Plants display remarkable plasticity in many processes contributing to GPP, NPP, and the role of terrestrial ecosystems in carbon cycling and biosequestration. This plasticity is driven by various molecular mechanisms and phenotypic traits. Such traits (see discussion in the section, Plant-Trait Variation, NPP, and Carbon Biosequestration, p. 29) are determined by a multitude of genome-by-environment interactions (phenotypic trait = G × E), underscoring environmental and edaphic factors' tremendous potential to modify plant characteristics. Having varying physical and chemical components at local to global scales, soils, in particular, can influence plant traits and thus productivity significantly (see Fig. 4.2. Global Soil Regions, p. 66, and Table 4.1. Soil Types and Their Properties, p. 67).

Furthermore, these chemical and physical factors control processes related to recalcitrance and the fate of carbon in soils around the globe. For example, rhizodeposition, root mortality, and chemical composition of roots are all likely affected by plant-soil interactions. Understanding how soil physicochemistry affects plants is thus critical for assessing carbon biosequestration and the significance of these interactions in regulating GPP and NPP.

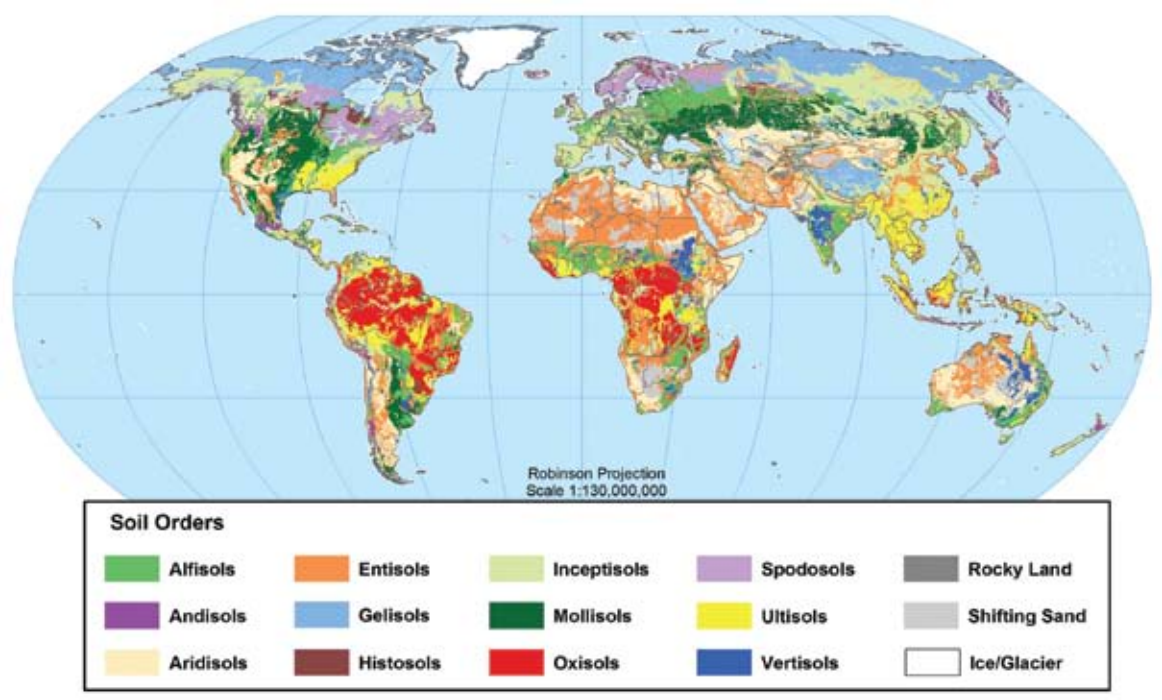


Fig. 4.2. Global Soil Regions. [Source: U.S. Department of Agriculture, Natural Resources Conservation Service. http://soils.usda.gov/use/worldsoils/]

Plant Responses to Multiple Nutrient Limitations in Soils

Little is known about the fundamental mechanisms by which limitations in nutrients—especially those other than nitrogen and phosphorus—affect processes related to plant primary production. Advancing our understanding of these mechanisms requires research and accompanying mechanistic models investigating plant response to multiple stress factors, including availabilities of 16 essential nutrients and exposure to 6 common ion toxicities (see Box 4.1, Metal Roles in Photochemistry: Global Limitations to Photosynthetic Carbon Assimilation, p. 68).

Mineral Stress Limitations on Primary Productivity

Mineral stress is prevalent in native soils. In fact, many natural and agricultural ecosystems are characterized by ion toxicities and suboptimal availability of mineral nutrients. Much terrestrial vegetation, for example, is supported by highly weathered tropical soils with low availability of phosphorus, calcium, and magnesium as well as aluminum and manganese toxicities. On the other hand, dense plant communities in more fertile soils face intense competition for nutrients. Predominant global soils having various toxicities and nutrient constraints represent complexes of mineral stresses. As major limitations to global primary productivity, such stresses warrant vigorous research to quantify the extent and severity of their effects on terrestrial ecosystems (see Table 4.1, p. 67).

Scientific understanding of plant response to stress from individual minerals is limited. However, only just beginning to be revealed is how plants and their associated microbial symbionts respond to concurrent multiple stresses—and in the context of climate change. Today's conceptual models of plant response to multiple resource limitations are inadequate for accurately representing the combination of mineral

Table 4.1. Soil Types and Their Properties*

Soil Name	Characteristics	Environmental Properties
Alfisols	Slightly acidic fertile surface layer over mineral- and clay-rich subsoil	Semiarid to humid climates; forests and mixed vegetation
Andisols	Rich mineral content with little orderly crystalline structure; volcanic origins	Cool, moderate- to high-precipitation environments near volcanoes
Aridisols	Dry with low organic material content; possible high salt content or mineral formation	Deserts and arid regions; hot and cold; low-population rangelands
Entisols	Recently formed, lack of soil horizon development; possible high rates of erosion or deposition	Diverse environments: dunes, steep slopes, river valleys, exposed bedrock, floodplains
Gelisols	Permafrost near surface; accumulated organic matter; reduced microbial activity	Freezing temperatures at high latitudes or elevations
Histosols	Anoxic and mostly saturated; accumulated organic matter	Wetlands at all latitudes
Inceptisols	Moderate soil horizon development; diverse characteristics	Various semiarid to humid climates; crops, timberlands, mountains, rangelands
Mollisols	Dark-colored surface horizon; high base and organic matter content	Grasslands, prairies, steppes; moderate to marked seasonal moisture loss
Oxisols	Highly weathered; rich in low-activity minerals such as metal oxides	Subtropical and tropical forests, crops; slash and burn often applied
Spodosols	Acidic with sandy texture; high organic matter, iron and aluminum oxides in subsoil	Cool humid or temperate; mostly coniferous forests
Ultisols	Acidic and highly weathered; reddish to orange, clay-rich subsoil with minerals	Humid climates; forests
Vertisols	Expanding clay when moist and shrinking when dry to form cracks	Subhumid and semiarid; long dry seasons; rangelands, crops

*Information from U.S. Department of Agriculture, Natural Resources Conservation Service. http://soils.usda.gov/technical/soil_orders/ and The Missouri Cooperative Soil Survey. http://www.soilsurvey.org/tutorial/page4.asp.

stresses typical of most terrestrial ecosystems. To create robust models, greater insight is needed into how mineral stresses structure communities, underpin competition and fitness, and are integrated through adaptive and maladaptive responses at organismal and cellular scales to determine carbon assimilation and use.

Mineral Stress Interactions with Climate Change

Mineral stresses likely have important, complex, yet poorly understood interactions with global climate change variables. Each of these stresses has complex yet distinct interactions with global change variables, complicating predictions of how plants in these environments will respond to possible future climates. Though sources of great uncertainty, important interactions between mineral stress and climate variables include the effects of transpiration on root acquisition of soluble nutrients, particularly calcium and silicon; impacts of altered root architecture on the acquisition of immobile nutrients, especially phosphorus; consequences of altered root-exudate production on aluminum toxicity and transition-metal acquisition; and the interaction of photochemical processes with transition-metal availability.

Box 4.1

Metal Roles in Photochemistry: Global Limitations to Photosynthetic Carbon Assimilation

Metals are required for biological redox reactions and thus are integral to light harvesting in chloroplast membranes. For example, metals contribute to this process through the magnesium ion in the center of chlorophyll through hydrolysis in Photosystem II (PSII). Metals also are needed as cofactors for antioxidant enzyme systems that detoxify reactive oxygen species (ROS) generated in chloroplasts by the combination of excited electrons and molecular oxygen. Particularly important in ROS detoxification are iron in catalase and ascorbate peroxidase and the various transition metals in superoxide dismutase (SOD) isoforms. Imbalances in metal supply to chloroplasts generate dysfunctions in electron transport during photosynthesis that lead to increased ROS formation and persistence, damaging photosynthetic tissues in what is known as photo-oxidative stress. This damage is exacerbated by environmental conditions such as temperature extremes, intense visible or ultraviolet (uv) radiation, and ozone. Metal imbalance is common in many terrestrial ecosystems. For example, in acidic soils supporting most terrestrial vegetation (e.g., those in tropical and subtropical forests as well as many humid temperate systems), low calcium and magnesium availabilities as well as aluminum, manganese, and iron toxicities are widespread. In alkaline soils typical of drier systems, iron, manganese, copper, and zinc availabilities often are suboptimal. These various limitations and toxicities may disturb leaf photochemistry, thereby limiting photosynthetic carbon assimilation.

Substantial genetic variation controlling tolerance of metal imbalances exists within and among species. However, with the exception of aluminum tolerance in crops and New England tree response to calcium, the genetic controls for coping with these imbalances are little researched and poorly understood. Genetic differences among plants are manifest in variations in metal acquisition, metabolism, and compartmentation as well as in tolerance to photo-oxidative stress via altered antioxidant metabolism. Such variations provide interesting opportunities for new research into the genetic influences on plant response to stress. For example, a research area deserving further investigation is the role of manganese toxicity as a key constraint to light utilization, photosynthetic carbon assimilation, and species composition in the eastern forests of North America where acid deposition, logging, and soil erosion are increasing metal imbalances. Molecular aspects of this research might relate to antioxidant systems, ion channels, or rhizosphere exudates that account for genetic variation in tolerance. Understanding this variation likely will become increasingly important in light of future climate change that could include more temperature extremes and altered ozone and radiation intensity.

Key Research Question

1. **How do transition-metal toxicities and deficiencies interact with plant photochemistry to limit carbon assimilation, especially in the presence of other photo-oxidative stresses such as ozone, uv and visible light, and temperature extremes?**

   Example-specific hypotheses to be tested using the example of manganese toxicity include:

   a. **Genetic taxa with greater antioxidant capacity are more tolerant of manganese toxicity. (This may be useful as a molecular marker of manganese tolerance across species or for selection and transgenesis of manganese-tolerant plants.)**

   b. **High temperature, ozone, and uv radiation are synergistic with manganese toxicity in susceptible taxa. (Synergy of light intensity and manganese toxicity already has been demonstrated.)**

   c. **Genetic taxa with greater uptake capacity for magnesium, zinc, copper, and iron are more tolerant of manganese toxicity.**

Photochemical Processes

Toxic levels of reactive oxygen species (ROS) can form in chloroplasts under certain conditions. Important to both the generation and detoxification of these species are metals involved in the light reactions of photosynthesis (e.g., manganese, magnesium, iron, and copper) and in antioxidant enzyme systems [e.g., zinc, copper, and manganese in superoxide dismutase (SOD) and iron in catalase]. Several global change variables, including ozone, high light, ultraviolet (uv) radiation, temperature extremes, and drought, can increase ROS formation. Thus plants suffering suboptimal availability of magnesium and transition metals because of high soil pH, base imbalances, and aluminum and manganese toxicity may be more sensitive to global change than healthy plants (see Box 4.1, p. 68).

Genomic Approaches to Understanding Plant-Soil Interactions and Edaphic Stress

Plants have evolved multiple mechanisms to maintain nutritional homeostasis in diverse edaphic environments. Some of these responses can be genetically simple, with only a single or a few gene products contributing to a phenotype. For example, nutrient transporters or enzymes such as phosphatases are phenotypic traits determined by the action of a single gene product. However, most traits facilitating tolerance to edaphic stress are genetically complex, including products of biosynthetic pathways (e.g., root exudates), morphological changes (e.g., shifts in root architecture), and symbiotic associations (e.g., mycorrhizae and nitrogen fixation).

Advancing genomic-level understanding of plant responses to edaphic stress would be valuable in two general ways. First, such insight would provide basic knowledge of plant-environment interactions, leading to discovery of tolerance mechanisms for edaphic stresses. For example, antioxidant gene arrays could be designed to test whether interactions between metal toxicity and uv light induce oxidative stress. Where robust and consistent plant-environment relationships are identified, regulation of selected genes could then be used to monitor environmental change over time relative to an established baseline. This monitoring approach could employ sentinel organisms amenable to genetic analysis or, as molecular methods advance, could focus on genetic signals conserved across species. A second benefit of progress in genomics-based understanding of plant tolerance will be the increased availability and use of more genetic targets of known function to enhance crop response to edaphic stress.

The sequencing and functional analysis of plant genomes are major scientific efforts aimed at understanding plant genetic complexity. Expression profiling using microarrays is a powerful tool for examining how genes respond to experimental variables, although distinguishing primary and secondary responses from such data is exceedingly difficult. Microarrays also are being used to examine genomic responses to mineral deficiencies and toxicities deduced from the up-regulated expression of genes with known function. Significant progress in understanding genetic response requires using functional genomic tools in future studies to focus on linking known edaphic stress factors—either alone or in combination with climate change variables—to resulting phenotypic traits. Resulting genomic information can be used to identify molecular markers linked to genes of interest for crop and natural-ecosystem adaptation to mineral stress.

Key Research Questions

1. **How do abiotic adaphic factors influence the nature, development, productivity, and response of ecosystems to climate variables?**
2. **How do plant-microbe associations facilitate adaptation to local climate and adaphic conditions to balance the carbon, nutrient, and water cycles?**

Analyzing plant responses to multiple and interacting edaphic variables at the organismal and physiological levels has proven to be extremely complicated. Attempts to understand these complex systems at the levels of gene metabolic and gene regulatory networks underlying these higher-order plant responses add yet another dimension to the challenge. Moreover, phenotypic responses are the sum of multiple and interacting gene products passing through several levels of regulation (e.g., transcription, translation, and post-translation modifications); even within the same plant, genetic responses vary widely from one tissue type to another.

At the genetic level, quantitative traits are of paramount importance, and substantial genotypic variation is apparent. Thus diversity among haplotypes (a segment of DNA containing closely linked gene variations inherited as a unit) could be more important than the population mean for a species' ability to tolerate stress. While this argues for using genomic rather than physiological approaches, in which typically only a few genotypes are observed, it also poses a challenge considering the immense functional complexity of numerous haplotypes of a multitude of interacting genes. At the cellular level, researchers are discovering a complex system of interacting signaling responses associated with environmental stress. At the tissue, organ, and organismal levels, greater insight into photosynthesis and water relations has been gained, but much remains unknown concerning, in particular, roots and the rhizosphere, where many key processes appear to occur.

Finally, scientific understanding of mineral metabolism, apart from nitrogen, is substantially less than that of photosynthesis and leaf responses to light, temperature, and CO_2. Genomic and molecular biology investigations must be coordinated with classical ecosystem research to determine to what extent stress interactions and responses may be generalized across species and ecosystems. Such research also will reveal whether the functional importance of genetic changes applies only to a unique organismal and ecological context.

Ecosystem Dynamics

Ecosystem responses to manifold biotic and abiotic influences, both natural and anthropogenic, are the overriding factors in productivity and carbon biosequestration. A system's dynamics—including age-related natural variations in carbon use efficiency (CUE), responses to chronic stress arising from global change and interannual variability, and modified functions triggered by climate and man-made disturbances—significantly affect the fate of ecosystem carbon. Further study is needed to determine the combined impact to Earth systems from these influences and other system factors discussed previously, including plant traits, soil characteristics, and microbial populations.

Stand Development in Forests: Baseline Maturation and Aging of Ecosystems

The terrestrial biosphere is a mosaic of plant communities with widely divergent characteristics. As communities develop on a plot of land, gross primary production (GPP) and the relationships between it and net primary production (NPP) change. Since this natural variation must be understood amid a changing climate, anticipating the trajectory of these relationships is especially important for predicting the future productivity of long-lived, woody communities (i.e., forests). Comprising a central role in the global carbon cycle, forest ecosystems sustain about 80% of terrestrial NPP and 50% of global NPP and thus are a major part of the terrestrial carbon sink that removes some 30% of anthropogenic carbon emissions each year. Consequently, understanding the complex dynamics directing carbon flow through forests is critical.

An important measurement of this flow is carbon use efficiency. Defined as the ratio of NPP to GPP, CUE is a measure of the capacity of forests to transfer carbon from the atmosphere to terrestrial biomass. CUE for forests is widely assumed in many landscape-scale carbon cycling models to be a constant value of 0.5—that is, about half of GPP is made into biomass. To achieve a constant CUE, tree respiration must be a constant fraction of canopy photosynthesis. However, a literature survey of research indicated that CUE values calculated from independent estimates of GPP and NPP were not constant but varied, ranging from 0.23 to 0.83 for different forest types (DeLucia et al. 2007), a finding consistent with theoretical considerations (Amthor 2000). This uncertainty in observed or experimental values is significant because a 20% error in current estimates of carbon use efficiency used in landscape models (0.4 to 0.6) could misrepresent an amount of carbon equal to total annual anthropogenic emissions of CO_2 when scaled to the terrestrial biosphere (DeLucia et al. 2007).

Some of the variation in forest CUE probably is related to the stage of stand development. For example, aboveground forest NPP certainly declines with age, potentially diminishing the capacity of old-growth forests to sequester atmospheric CO_2. Although poorly understood, the mechanisms governing the age-related decline in forest NPP are embodied in two competing hypotheses.

The "respiration hypothesis" [see Fig. 5.1. "Respiration Hypothesis" (a) and "GPP Hypothesis" (b), p. 72] suggests that GPP remains constant but NPP declines following canopy closure early in stand development because of increasing

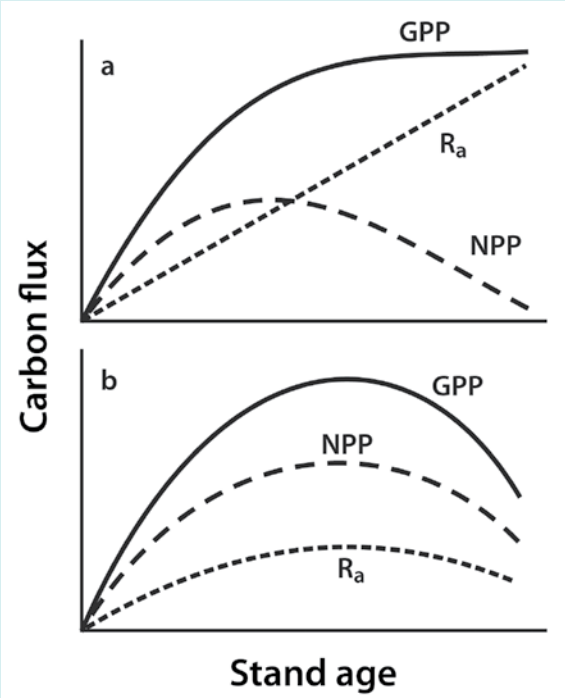


Fig. 5.1. "Respiration Hypothesis" (a) and "GPP Hypothesis" (b). Graphs a and b describe how changes in autotrophic respiration (R_a) and gross primary production (GPP) contribute to age-related decline in forest net primary production (NPP). For additional information, see Barnes et al. (1998) and Ryan et al. (2004) for (a) and Ryan, Binkley, and Fownes (1997) and Gower, McMurtrie, and Murty (1996) for (b).

autotrophic respiration (R_a) associated with the accumulation of woody biomass. This hypothesis has been modified to include increased partitioning of carbon below ground as a factor contributing to the decline in NPP as forests age. The decrease in CUE with stand age suggests that increasing R_a does have a role in age-related NPP decline.

In contrast, the "GPP hypothesis" [see Fig. 5.1 (b), this page] posits that R_a is a fixed fraction of GPP whose age-related decline causes NPP to decrease. Several factors, including increasingly unfavorable water relations and nutrient limitations in large trees, may contribute to GPP decline in old forests. The near-constant GPP fraction partitioned to R_a supports this hypothesis and should lead to a constant CUE with stand age.

Further research is necessary to gain a clear understanding of the factors affecting NPP as forests age. In addition to accurately discerning the relationships among GPP, NPP, and R_a during various stages of stand development, greater insight is needed into the controlling regulatory and metabolic processes in primary producers and their symbiont microbial communities. The ability to model how forest carbon cycling will respond to global change depends critically on a thorough understanding of all these factors.

The Role of Plant-Trait Variation in Ecosystem Response to Chronic Stress Arising from Climate Change

Ecosystems undoubtedly will differ in their responses and vulnerability to global climate change (IPCC 2007). A mechanistic understanding of the complex interplay of various factors dictating these responses is critical for forecasting climate effects on plant productivity and carbon biosequestration (see Fig. 5.2. Factors in Species Composition of Ecosystems, p. 73). Such an understanding could reveal the incredible variability in how ecosystems react to chronic alterations in resources and how particular ecosystem attributes influence a system's ability to adjust to these and other shifts brought on by climate change. Knowing how ecosystems differ in their response and susceptibility to changes in a single resource—and eventually multiple types of resources—will improve capabilities for simulating trajectories of climate change impacts. Also important is how different resource types and amounts will vary the shape, direction, or rate of such response trajectories. Further influencing these projections are multiple ecosystem attributes, such as the variation in phenotypic traits within populations and among plant and other species, sizes and turnover rates of nutrient pools, the nature and responsiveness of soil biota, and trophic complexity. Thus, to advance understanding of the nature and pace of ecosystem reactions to climate change and improve predictive capabilities, new research must examine the relative importance of the different mechanisms underlying response trajectories [see Fig. 5.3. Hierarchical Response Model (HRM), p. 73]. Specifically needed is more insight into ecosystem response to key aspects of dynamic climate

change, such as elevated CO_2, warming, ozone level, and altered precipitation regimes, as well as interactions with other global shifts.

Numerous factors are expected to contribute to ecosystems' different responses and vulnerability to climate change. However, variation in traits within populations of and among different plant species is likely critical for determining rates and trajectories of ecosystem response, particularly NPP and carbon biosequestration. For example, global climate change represents chronic and directional shifts in resource availability, either directly via elevated CO_2 and altered precipitation regimes or indirectly as, for instance, through the impacts of warming and elevated CO_2 on water balance. Ecosystem reactions to these chronic resource

Fig. 5.2. Factors in Species Composition of Ecosystems.

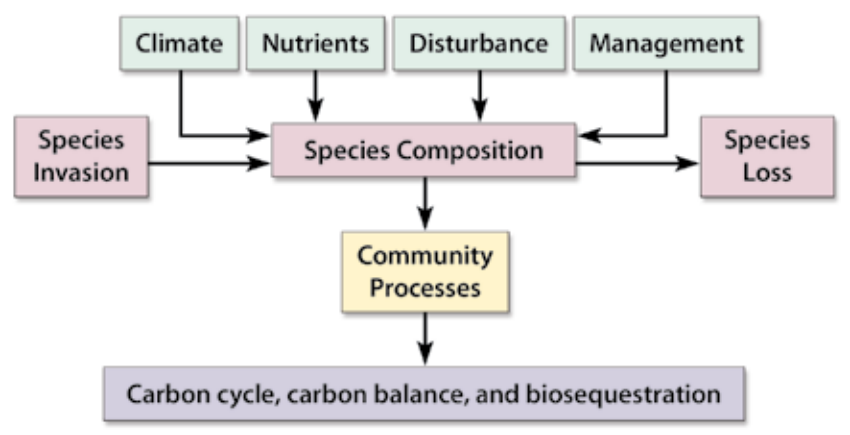
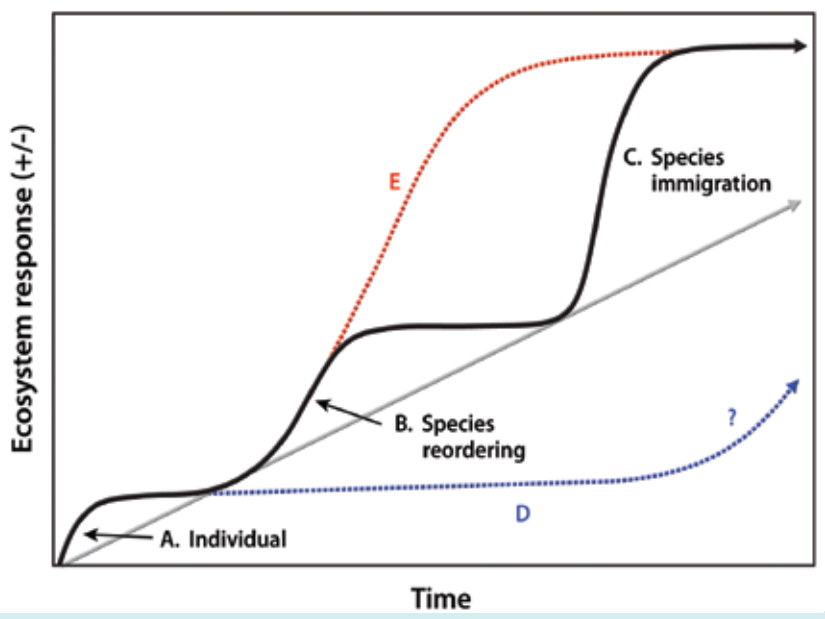


Fig. 5.3. Hierarchical Response Model (HRM). This graph depicts the hierarchy of mechanisms underlying ecological change (black line) as ecosystems are exposed over time to chronic resource alterations associated with global changes (e.g., elevated CO_2, nitrogen deposition, and climate shifts). Fairly modest initial ecosystem responses may reflect relatively rapid individual-level responses (A), with the magnitude and extent of these initial responses limited by traits of the resident species. Larger shifts in ecosystem response are expected with reordering of species (B) in the community (e.g., shifts in relative abundance). The timing and duration of this phase may vary depending on variation in traits and the rate of population turnover or may be attenuated depending on internal interactions. Finally, immigration of new species better suited for altered resource levels may result in further change in ecosystem response (C). Timing may depend on the regional species pool and dispersal limitation. Other responses to chronically altered resources are possible, including gradual linear change (thin grey line) if the magnitude and rate of change were similar for all three mechanisms (A, B, and C). The HRM has potential exceptions. For example, ecosystems dominated by very long lived species with slow turnover rates, such as forests, may appear to be resistant to change (D) as resources accumulate over time. Conversely, ecosystems that become susceptible to invasion by exotic species or pests and pathogens due to resource alterations may bypass changes driven by individual-level responses or community reordering and could experience large shifts in structure and function in a relatively short period of time (E). [Source: Figure modified from Smith, M. D., A. K. Knapp, and S. L. Collins. "A Framework for Assessing Ecosystem Dynamics in Response to Chronic Resource Alterations Induced by Global Change," *Ecology*, in review.]



Key Research Questions

1. **Which phenotypic trait or suites of traits are most important in determining ecosystem response to change? What are the relevant genomic markers for phenotype?**
   a. **What is the relative importance of phenotypic-trait variation within populations (i.e., genetic level) versus among species (i.e., species level) in determining ecosystem response to change?**
   b. **What is the relative importance of ecosystems' physiological versus transformational responses in determining productivity, carbon biosequestration, and carbon-pool stability?**

alterations are expected to be driven, in part, by plant and associated symbionts' responses determined by phenotypic-trait variation and occurring at different hierarchical scales.

The primary and most rapid response to chronic resource alteration is expected to occur at the individual level (see Fig. 5.3, A, p. 73). This response is driven by traits related, for example, to physiology, metabolism, growth, and stress tolerance that in turn are expected to affect NPP, carbon biosequestration, and other processes over the short term. The impacts of altered resources could be either positive or negative depending on the suite of traits represented in a community and the effects of these traits on NPP and carbon biosequestration. As resources continue to shift and in some cases accumulate over time, some species or populations are expected to increase in abundance as a consequence of possessing traits favorable to the new environmental conditions. Meanwhile, those less suited for such conditions are expected to decline. This species- or population-reordering phase of response (see Fig. 5.3, B, p. 73) also likely will affect NPP and carbon biosequestration, with the impact expected to be nonlinear and large as a consequence of rapid population growth and alterations in species or genotype interactions (May 1986; Frost et al. 1995; Blenckner 2005; Ives and Carpenter 2007). Finally, with continued resource alteration, new species or genotypes are expected to immigrate into the community. These species will possess novel suites of traits potentially favorable to the new conditions and contribute in different ways to NPP, biosequestration, and other ecosystem processes. As a result, the immigration phase (see Fig. 5.3, C, p. 73) is expected to elicit the greatest ecosystem response, increasing productivity nonlinearly due to rapid population growth from immigration of new species and subsequent alterations in species interactions (Hobbs et al. 2006; Ives and Carpenter 2007; Knapp et al. 2008).

An important challenge for researchers is determining the nature and relative importance of ecosystem physiological and transformational responses that control productivity, carbon biosequestration, and carbon-pool stability. If, in climate change scenarios, conditions such as resource alterations continue to evolve, then the processes of growth and alteration can be expected to continue, with stands transforming continually.

Variation in rates of change and durations of lag periods between the transitions depicted in Fig. 5.3, p. 73, will in part determine different ecosystems' vulnerability to change. This variation will depend not only on trait diversity at the population level and among ecosystems' plant species, but also on the ability of species to adapt to changing conditions. Other ecosystem attributes and phenomena also will influence the pace of change and the time between transitions. These factors include the magnitude, rates, and types of resource alterations; interactions with other environmental and anthropogenic changes such as atmospheric nitrogen deposition, altered land use, and habitat fragmentation; and shifts in disturbance regimes.

Interannual Variability: Episodic Stress

Carbon-flux data from long-term site studies are invaluable for detecting trends in terrestrial ecosystem responses to episodic phenomena such as interannual variability (e.g., El Niño and La Niña). Consequently, such data are increasingly valuable as sites operate longer and grow in number. Long-term data can be used

to detect scale-emergent processes operating at multiple temporal scales (Urbanski et al. 2007; Dunn et al. 2006) and to characterize complex and nonlinear behaviors as switches, pulses, lags, and hysteresis. For example, these data can provide insight into the dependency of light use efficiency on diffuse radiation; the role of growing-season length, stand age, and drought on net ecosystem exchange (NEE) of CO_2; and the impact of rain pulses on ecosystem respiration and interannual variation in NEE (NEE equals NEP plus CO_2 sources and sinks not involving conversion to or from organic carbon). Urbanski et al. (2007) found that 13 years of data allowed them to identify disturbance-related anomalies and their legacies and to measure underlying ecosystem trends toward greater rates of net carbon uptake, increased photosynthetic capacity, and higher rates of respiration—unexpected findings considering the age of the forest studied. The researchers demonstrated that long-term ecosystem flux measurements are absolutely essential for detecting interannual and decadal trends in response to climate and disturbance. They also showed how short-term data can lead to misinterpretation of results, even the trajectory of a particular ecosystem response. In contrast, alternative approaches producing carbon-flux estimates from remote sensing and models are inferential and do not capture the anomalies and trends of the features of complex systems. While these alternative approaches have merit for conducting desired continental-scale integration, remote sensing–derived products and data-assimilation approaches *must* be anchored with flux measurements, and model parameters should be shaped by continuous and long-term carbon-flux data across a spectrum of sites.

Disturbance and the Dynamics of Carbon Cycling and Biosequestration

Background

Variability of terrestrial net carbon flux at decadal and multidecadal time scales is strongly influenced by the frequency and intensity of disturbance (Irvine, Law, and Hibbard 2007; Bond-Lamberty, Wang, and Gower 2003; Law et al. 2003; Thornton et al. 2002). A common carbon-flux pattern emerges from both measurement and modeling studies investigating ecosystem response to disturbance. First, following a large carbon source associated directly with the disturbance process, an initial period of ecosystem recovery occurs during which source strength diminishes. This recovery is then followed by a period of increasing sink strength as vegetation structure is re-established. Next is a long "tail" phase during which sink strength declines gradually toward a neutral carbon flux; however, a new episodic disturbance can interrupt and reinitiate the pattern at any time. Thus over a long period of time, the emerging climatological mean of disturbance frequency and intensity plays a central role in establishing the mean carbon and nutrient stocks in vegetation, litter, and soil organic matter.

The carbon-flux response of a particular ecosystem to an individual disturbance event depends on a multitude of factors, including long-term mean carbon state, climate, existing community structure and its alteration during recovery, time since previous disturbance, physical properties such as topography and soil structure, disturbance type and magnitude, and climate variability during the postdisturbance period. Basic research needs are associated with each of these aspects of carbon cycle response to disturbance, and additional research requirements emerge when

Key Research Questions

Place-based observations have the potential to answer the following:

1. **What are the magnitudes of carbon storage and the exchanges of energy, CO_2, and water vapor in terrestrial systems? What are the spatial and temporal variabilities of these processes?**
2. **How are these variabilities influenced by vegetation type, phenology, soils and microbial populations, changes in land use and management, and disturbance history, and what is the relative effect of these factors?**
3. **What is the causal link between climate and the exchanges of energy, CO_2, and water vapor for major vegetation types? How do seasonal and interannual climate variabilities and anomalies influence fluxes by their effects on plants and associated microbial symbionts?**
4. **How do boundary-layer CO_2 concentrations vary spatially and temporally, and how do these levels vary with topography, climatic zone, and vegetation?**

Box 5.1

Partial List of Disturbance Types

Classifying disturbances as related either to climatic or anthropogenic factors is useful. The fact that these categories overlap underscores an area of pressing importance for new research and understanding.

Climate-Driven Disturbance

- Wildland fire
- Extreme events or severe weather (e.g, hurricanes and floods)
- Insects and disease
- Drought

Anthropogenic Disturbance

- Conversion of forest and grassland to agriculture
- Burning of agricultural waste products
- Implementation of biofuel or carbon biosequestration strategies
- Wood harvesting (e.g., for products or fuels)
- Urbanization
- Human-modulated burning of forest and grassland for establishment of new agriculture and grazing (Such activity is an important overlap with climate-driven fire disturbance.)

Key Research Questions

1. **What are the vulnerabilities of carbon sinks to natural and anthropogenic episodic disturbances?**
2. **How will these vulnerabilities change if disturbance frequency and intensity change?**
3. **How does ecosystem recovery following disturbance depend on atmospheric and climatic change (e.g., rising atmospheric pCO_2, warming, and nitrogen deposition)?**
4. **How do climate and carbon–nutrient cycle feedbacks impact potential carbon biosequestration strategies?**

considering the interactions of disturbance dynamics and carbon biosequestration strategies and practices (see Table 5.1. Research Needs for Carbon Cycle Consequences of Disturbance, p. 77). For example, details of stand structure, such as variation in tree spacing, were important determinants of Hurricane Katrina impacts on carbon stocks in Gulf Coast forests (Chambers et al. 2007).

Trajectories of change in net biome productivity and carbon stocks can vary greatly depending on severity, frequency, and type of disturbance. Prognostic models thus require *a priori* knowledge of carbon transformations (e.g., amounts moving from live to dead pools) and combustion efficiencies of different carbon pools (i.e., effects of various fire intensities and vegetation types). Such knowledge is critical for determining how much carbon is combusted in wildfires and how much remains to decompose over years to decades (Campbell et al. 2007). Data on such transformations are lacking, however, and related defaults used in some models result in gross overestimates of carbon combustion and respiration after fire. Also lacking are carbon-budget observations at different stages after disturbance—measurements critical for evaluating and improving models. Thus, more field observations are needed to inform models and develop remote-sensing techniques for mapping carbon pools and fluxes after disturbances (see Box 5.1, Partial List of Disturbance Types, this page). Table 5.1, p. 77, and Box 5.2, Observation Strategy for Long-Term Data to Improve, Modify, Parameterize, and Test Models of Terrestrial Carbon Processes, p. 78, list types of disturbances and outline research needed to understand their effects on carbon cycling.

Table 5.1. Research Needs for Carbon Cycle Consequences of Disturbance

Topic	Research Needs
Historical patterns of disturbance	• Represent current carbon stocks consistent with historical patterns of disturbance intensity and frequency.
Climate change impacts on frequency and intensity	• Progress from diagnostic to mechanistic to prognostic capabilities of disturbance patterns.
Multiple spatial and temporal scales	• Characterize disturbances by episodic nature in space and time. • Relate statistical mean, variability, and high-order moments of disturbances and carbon stocks. • Use variability as a scale-of-analysis function. Relate properties on coarse climate grids.
Fire	• Develop a globally gridded representation of current natural and anthropogenic spatial and temporal fire patterns. • Determine combustion efficiencies, total emissions, and speciated emissions of CO_2, CO, black carbon, aerosols, and reactive nitrogen. • Gain a mechanistic understanding of the relationships among climate drivers, vegetation community structure, and human influence. • Base predictions on climate and ecosystem-level drivers as well as interactions with human populations, land-use practices, and changing land cover. • Determine timing of energy balance. Study partitioning at site and pyrogenic (soot) deposition on snow. • Understand carbon cycle consequences of fire recovery and associated mechanics of climate and nutrient impacts. • Conduct on-site assessments of remaining carbon and fire effects on heterotrophic respiration and nutrient dynamics during recovery.
Insects and disease	• Understand the mechanistic relationships among climate, insect and disease outbreaks, and the carbon cycle. • Determine the consequences for carbon, nutrient, water, and energy cycling. • Increase predictive capability for insects and disease under future climate change scenarios. • Develop carbon biosequestration strategies to improve resilience. • Study historical examples of recovery dynamics and carbon cycle consequences.
Drought	• Determine controls of drought-induced carbon fluxes at the level of plant physiology and soil microbial functioning. • Understand impacts of climate and ecosystems on resilience factors. • Understand carbon cycle consequences of changed communities and behaviors within them.
Extreme weather (e.g., hurricanes, floods, and freeze-thaw dynamics)	• Conduct long-term studies on spatial and temporal patterns and vulnerabilities relative to carbon biosequestration. • Assess effects of climate change and related factors (e.g., CO_2, methane, flooding and N_2O, and freeze-thaw dynamics).
Changing allocation patterns	• Understand the influence of disturbance over time and its impacts on carbon pools. • Investigate carbon flux and partitioning of GPP to plant components within and among plant functional types under a range of climatic conditions and following disturbances.
Threshold behavior in climate change trends	• Elucidate mechanisms whereby ecosystems cross vulnerability thresholds as they develop.
Carbon cycle consequences of anthropogenic nitrogen deposition	• Quantify effects of a range of modest levels of nitrogen deposition on canopy and soil processes across various biomes, forest ages, and water availabilities. Also assess effects of such deposition on carbon pools, respiration, and nitrogen balance.
Disturbance–climate system feedbacks	• Understand carbon loss followed by carbon uptake. Determine ecosystem transformations resulting from climate and albedo shifts.
Technologies, theories, experiments, and observations	• Develop approaches for chronosequences and quantification of variables, carbon budgets and allocations, respiration, nutrients and water, and new agent-based dynamic vegetation models linking biogeochemistry and vegetation change.

Box 5.2

Observation Strategy for Long-Term Data to Improve, Modify, Parameterize, and Test Models of Terrestrial Carbon Processes

(Examples: Dynamic Global Vegetation Models and Coupled Climate–Carbon Cycle Models)

- Long-term observations are needed to better understand fundamental controls on terrestrial carbon accumulation rates and effects of climate and disturbance variations on carbon, nutrient, water, and energy exchange with the atmosphere. Uniformly and appropriately applying a range of new tools (e.g., isotopic methods coupled with genomics and molecular markers) will enable analysis of such controls and variations at all spatial and temporal scales.
- Momentum is building for future studies aimed at continental integration of carbon cycling research via data assimilation. As one component of an integrated North American carbon cycle research program, data assimilation will enhance this initiative's diagnostic, explanatory, and predictive capabilities. Success of assimilation depends on a continuous flow of high-quality carbon-flux measurements and meteorological, ecological, soil, and physiological data from a wide spectrum of climate zones, biomes, and disturbance classes. For accurate regional and continental analyses, the modeling community has stressed the value of data from AmeriFlux—a network providing continuous ecosystem-level measurements of, for example, CO_2, water, and energy exchanges from sites in North, Central, and South America. The importance of such observations has pushed the network to deliver high-quality data to a public archive at an unprecedented rate.
- Carbon-flux data from long-term site studies are invaluable for detecting trends in terrestrial ecosystem responses. Consequently, such data are becoming increasingly valuable as sites operate longer and increase in number and density (see section, Interannual Variability: Episodic Stress, p. 74).
- Maintaining carbon-flux data from long-term sites is imperative as the transition period from historic climate norms to perturbed and warming conditions continues. Ongoing measurements could help produce within the next 10 to 20 years an observation record by which society will be able to assess global warming's effect on the health and function of the biosphere. Ecosystem flux data will be crucial in developing coupled climate–carbon cycle models to interpret and predict the impact of future fossil fuel–consumption scenarios.
- Coordinated design and implementation of long-term observation strategies and methods will be critical to understanding short- and long-term terrestrial carbon processes and feedbacks to climate. Observationalists, experimentalists, and theoreticians working together can improve fundamental understanding of the controls on carbon stocks, fluxes, and terrestrial feedbacks to climate and can devise ways to implement this knowledge in a new generation of models. Achieving this goal will require careful and coordinated site selection, measurement, and analysis. For example, model-data integration could be applied to examine how disturbance affects carbon stocks and fluxes across chronosequences of sites in major biomes and climate zones or to assess how interannual variation in precipitation or long-term drought impacts carbon fluxes in different biomes.

Biogeochemical Cycling of Carbon in Oceans and Climate Change

Covering more than 70% of Earth, the world's oceans cycle carbon rapidly and exert strong, complex feedbacks on both contemporary geochemistry and ongoing climate change. Understanding the global carbon cycle will require a more refined accounting of processes and interchanges between total marine biota and relevant biogeochemical processes.

In climate change scenarios, potential impacts on ocean processes include suppression of nutrient fluxes resulting from increased stratification of water layers; acidification of surface waters driven by rising atmospheric CO_2 levels; and perturbations in the availability of nutrients, such as phosphorus and iron, that limit growth of planktonic communities. To predict shifts in marine biogeochemical cycling and carbon biosequestration resulting from climate change variables, new methods and research are needed to inform oceanic components of various Earth System Models.

Oceanic systems and processes span the full spectrum of biological and physical scales, from genomes to biogeochemical cycling. However, most current efforts to model ecosystem-scale biogeochemical processes in oceans lack the necessary level of spatial or temporal resolution to represent potentially important factors, including (1) functional variation of different phytoplankton classes, (2) heterotrophic bacteria, (3) grazers and higher trophic levels, (4) the chemistry of trace elements and their oxidation states, and (5) points of integration with atmospheric processes. For each of these areas, new research approaches are needed to assess the functional capabilities of marine communities, connect these functions to biogeochemical processes, determine turnover and transformation rates of relevant nutrients, and integrate resulting information into models across multiple scales of resolution.

Marine Carbon "Pumps" and Potential Consequences of Climate Change

Oceans are massive reservoirs for inorganic carbon, containing about 50 times as much CO_2 as the atmosphere. Oceans absorb atmospheric CO_2 by two fundamental processes—the so-called biological and solubility pumps. The biological pump operates via the action of surface-water photosynthetic microbes that transform dissolved CO_2 into organic carbon. Some of this organic carbon subsequently sinks into deeper waters and is effectively sequestered from the active carbon cycle (see sidebar, Marine Food Web and the Carbon Cycle, pp. 80–81). In contrast, the solubility pump is driven by a combination of physical and chemical processes. CO_2 has increased solubility in cooler waters, which are denser than warmer waters. As ocean currents circulate tropical waters to higher latitudes, cooling CO_2-laden water sinks, resulting in a net transport of CO_2 into the deep ocean (see sidebar, CO_2 Absorption and Ocean Acidification, pp. 90–91).

The vertical gradient of nutrients and carbon observed in ocean waters is determined by the coupled action of the biological and solubility pumps, with *(text continued on p. 82)*

Marine Food Web and the Carbon Cycle

Microscopic plants and other photosynthetic organisms that drift with ocean currents lie at the heart of the marine carbon cycle (see figure, Oceanic Food Web, p. 81). Sunlit surface waters teem with phytoplankton that convert inorganic carbon dissolved in surface waters to organic carbon—which forms the basis of the marine food web—and account for about half of all primary production on Earth (Field et al. 1998; Falkowski, Barber, and Smetacek 1998). In contrast to terrestrial carbon-turnover times that may take months to years, carbon cycles rapidly in oceans, with the entire phytoplankton population in some environments replacing itself weekly (Falkowski 2002).

Major Primary Producers

Coccolithophores (5 to 10 µm in diameter), single-celled algae prevalent in tropical oceans.

Diatoms (about 30 µm in diameter), prevalent in temperate and polar oceans.

Dinoflagellates (30 to 2000 µm in diameter), prevalent in the subtropics and tropics, as well as in temperate oceans in late summer.

Cyanobacteria (about 1 µm in diameter), the world's most abundant phytoplankton.

Phytoplankton, such as those described above, are grazed upon by marine heterotrophs known as zooplankton. These grazer species range from microscopic protozoa and copepods to worms, krill, crabs, jellyfish, and the larvae of fish and other organisms. Comprising most of the animal mass in the ocean, zooplankton serve as the crucial link between primary producers and the rest of the marine food web. Viruses, which act as predators in oceanic food chains by infecting and lysing marine bacteria, also play an important but still poorly understood role in marine carbon turnover.

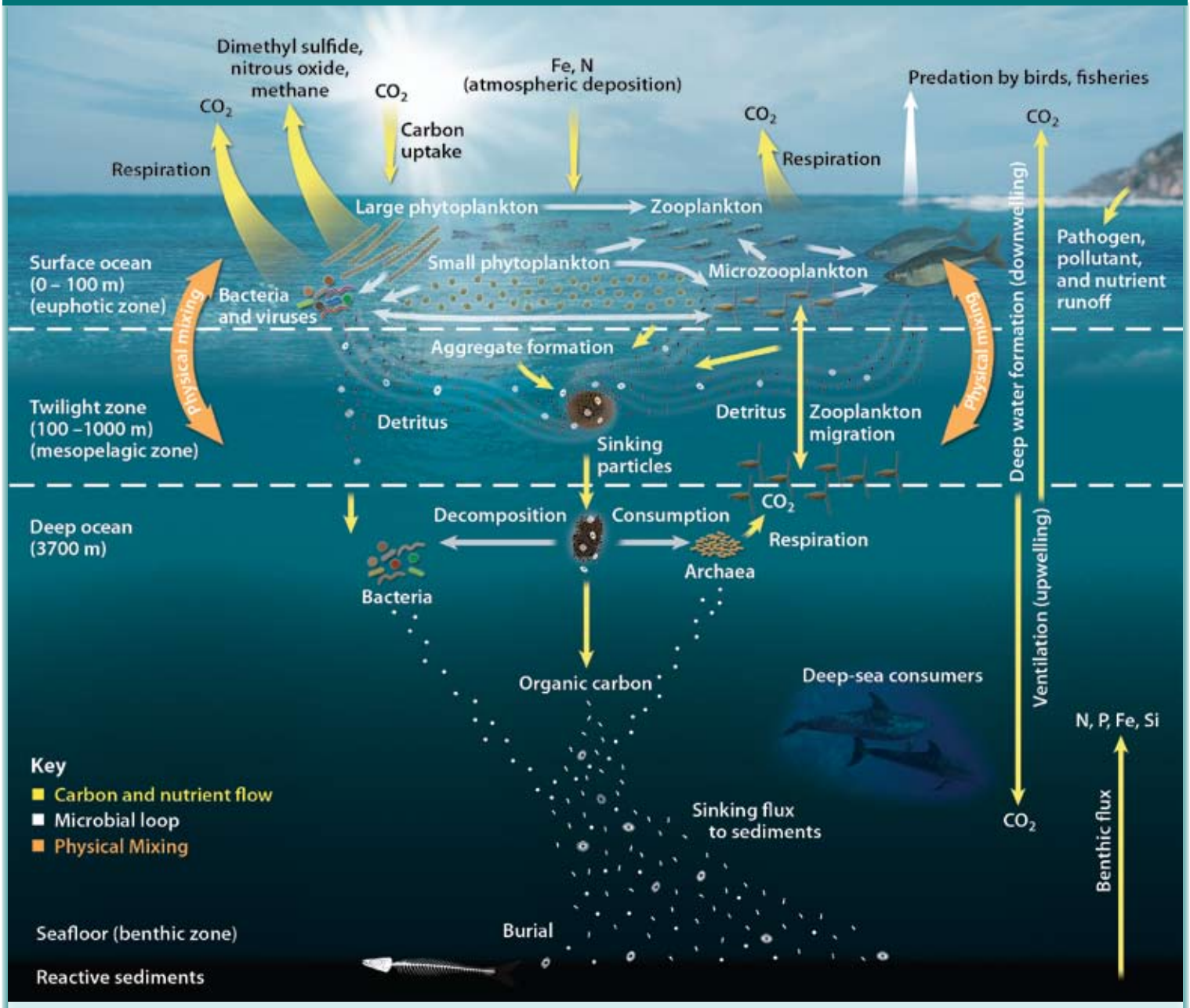
The overall efficiency with which organic carbon is exported to the deep ocean depends on the type of photoautotrophic cells that create the organic material and the efficiency with which heterotrophic organisms respire it.

Carbon Flow and Fate

Carbon fixed in phytoplankton eventually enters the water column as either particulate or dissolved organic carbon through direct exudation, consumption by grazing zooplankton, viral lysis, or cell death. Subsequently, most of this carbon material is degraded by heterotrophic bacteria, resulting in particulate solubilization and conversion of organic carbon back to CO_2. Some of the organic matter, however, sinks intact to the underlying twilight zone (the ocean's barely lit middle layer) and beyond, where lower temperatures, lack of oxygen, and other factors significantly slow degradation.

CO_2 fixed during photosynthesis by phytoplankton in the upper ocean can be transferred to the depths via three major processes: passive sinking of particles, physical mixing of particulates and dissolved organic matter through currents, and active transport by zooplankton migrating to deeper waters. Detrital particles and organic matter associated with mineral structures from phytoplankton, for example, may resist rapid microbial degradation and sift down as flakes, also called marine snow, becoming platforms for microbes to live on. As this particulate organic matter falls deeper, it can cluster with other small particles, such as zooplankton fecal pellets, molts, and larvacean houses, to form larger, heavier aggregates held together by a polysaccharide matrix. The carbon in these particles can be isolated from exchange with the atmosphere for centuries to millennia before upwelling currents return it and other nutrients from the deep ocean to warm surface waters. Some carbon is lost at each step of the way, however, as the organisms involved consume or degrade the organic carbon and remineralize it to CO_2 through respiration.

However, if climate change and ocean acidification significantly alter marine ecosystems' functions, the efficiency of this biologically mediated ocean carbon export may change, leading to an indirect effect on the net annual uptake of carbon.



Oceanic Food Web.

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Key Research Questions

1. **What are the potential impacts of climate change on carbon cycle pathways and fluxes mediated by microbes and the remainder of the marine food web?**
2. **What are the uncertainties associated with these predictions?**

biological processes carrying out more than 80% of the pumping (Gruber and Sarmiento 2002; Sarmiento and Gruber 2006). Atmospheric concentrations of CO_2 would be much higher in the absence of these ocean processes, and climate-driven changes in ocean circulation, chemical properties, or biological rates could thus result in strong feedbacks to the atmosphere. Moreover, records from ice cores indicate these processes are highly sensitive to shifts in atmospheric CO_2 concentration.

The biological pump is driven by phytoplankton inhabiting sunlit surface waters (see sidebar, Marine Food Web and the Carbon Cycle, with figure, Oceanic Food Web, pp. 80–81), and shifts in microbial community composition and function can have large impacts on the ultimate fate of carbon. Most organic matter produced by photosynthesis in surface waters is consumed for respiratory processes (either by phytoplankton or other organisms) and is returned to the atmosphere as CO_2. However, some fraction of this material sinks to the deep ocean. The ratio of export to primary production depends upon many factors, particularly the physiology and composition of phytoplankton that dominate surface waters, rates of mortality resulting from grazing and predation, and the efficiency of microbial processes that degrade dissolved and particulate organic material. Unlike terrestrial systems, which have a large living biomass with resultant inherent stability amid climate variability, ocean systems have very low resident biomass. The rapid rates and high efficiencies of carbon turnover in marine systems thus make biological processes in the surface ocean highly susceptible to shifting environmental variables. Predicting these potential effects on a global scale requires a better understanding of microbial community structure, function, and dynamics that will lead to more robust and predictive models of biogeochemical cycles in marine systems.

Nitrification and denitrification, two key processes that set the pace of the nitrogen cycle, are carried out by microbes. Researchers estimate that half of all microbially mediated nitrogen fixation occurs in the oceans, with planktonic archaea potentially playing a primary role in subeuphotic zone nitrification (Karl et al. in press). Additionally, greater amounts of wind-blown dust arising from drought-stricken areas are being deposited in oceans. Metals carried in this dust, including iron, are likely to affect marine microbial communities and the cycles they carry out. Marine microbes also may play a role in cloud formation by cycling compounds such as dimethyl sulfide into the atmosphere.

The oceans' future capacity as a carbon sink is uncertain because of potential (and currently uncharacterized) feedbacks among global climate change, ocean circulation, and the microbial communities that actively cycle carbon. These natural ocean carbon biosequestration processes are affected by the amount and availability of organic and inorganic pools of nitrogen, phosphorus, oxygen, and many other chemical species. A better understanding of the mechanisms and pathways governing these biogeochemical processes is critical for determining the magnitude of the oceans' capacity to mitigate changes in atmospheric CO_2 concentrations. Integrating genomics, transcriptomics, and proteomics with ecological, biophysical, and chemical techniques is necessary for delineating fundamental physiological processes, understanding their regulation, and determining how they relate to biogeochemical cycles.

Linking Microbial Community Structure to Biogeochemical Cycling of Carbon in Marine Systems

Many critical biological processes directing oceanic carbon cycling remain poorly understood. Particularly unclear is how these processes contribute to the formation of organic carbon compounds, their chemical character, and the biological and environmental factors governing their subsequent fate. Small perturbations in biological processes controlling the production or consumption of dissolved organic carbon pools in oceans could strongly affect the biological pump's functioning and thus the balance between oceanic and atmospheric CO_2. The uncertainty associated with these disruptions severely complicates efforts to represent many key microbially mediated processes in models of oceanic biogeochemical cycling and to predict potential impacts of climate change.

The phylogenetic composition of marine microbial communities plays an important role in the eventual fate of fixed organic carbon. For example, diatoms and coccolithophores are associated with distinct mineral structures (silicate and calcium carbonate, respectively) that affect the rate at which carbon fixed by these organisms is exported to deeper waters. The detritus from communities dominated by these algal phytoplankton types is expected to sink more rapidly than purely organic particles. Moreover, the mineral matrix of this detritus protects a fraction of the associated organic matter from heterotrophic respiration as the material sinks. Studies of material from sediment traps indicate a stronger association between calcium carbonate and organic matter below 1000 m than between silicate and organic matter at such depths. These findings suggest coccolithophore communities' importance in driving a more efficient biological carbon pump relative to diatoms or purely organic organisms (Klaas and Archer 2002). More research is needed to fully understand how community composition of primary producers influences the relative rates of carbon export from surface waters.

Mortality of primary producers, which may arise from viral lysis, grazers, predation, or simple aggregation and sinking, strongly influences the flow of carbon through the marine food chain. Developing a predictive understanding of these processes is essential for understanding the marine carbon cycle and anticipating potential impacts of climate change. Research has highlighted the specificity of interactions between microbial populations and forces driving mortality, but its magnitude and drivers in the natural environment are poorly understood. In particular, uncertainty surrounds the role of viruses as predators in marine food chains. Very little is known about the rates at which populations are infected, transformed, and lysed by viruses in the natural environment or the effective "epidemiology" behind such events. Combining targeted metagenomics with proteomics-based activity measurements ultimately can reveal the mechanisms directing prey selection or susceptibility to viral mortality.

Bacterial heterotrophs also significantly influence oceanic carbon cycling. These microbes largely govern the final fate of fixed carbon in marine systems and are responsible for most organic-matter transformation, solubilization, and subsequent remineralization occurring in the water column. Despite the crucial role of bacterial heterotrophs in mediating these processes, little is known about

Key Research Questions

1. **How are microbial community metabolic processes in marine habitats linked to the global carbon cycle, and how are these processes integrated across genetic, organismal, community, and ecosystem scales?**
2. **How is the structure of heterotrophic microbial communities in marine systems determined by dissolved organic matter composition and nutrient limitations?**
3. **How do environmental, ecological, and physiological factors interact to dictate pathways and regulate the flows of carbon and other elements through upper-ocean ecosystems?**

their identities, the key genes and proteins involved in organic-matter degradation, relative degradation rates of various types of compounds, and which factors control partitioning of carbon between particulate matter and dissolved organic carbon. The latter is particularly critical because, in some situations, dissolved organic carbon may be exported more efficiently to the deep ocean than particulate matter (Hopkinson and Vallino 2005).

Interactions Between the Marine Carbon Cycle and Other Relevant Biogeochemical Cycles (Nitrogen, Phosphorus, Iron, and Sulfur)

Although primary producers in ocean surface waters have plentiful supplies of water and light, these organisms' growth is limited by the relative scarcity of inorganic nutrients such as nitrate, phosphorus, and iron (see sidebar, Marine Nutrient Cycling, p. 92). Such nutrients become available to surface planktonic communities almost exclusively through upwellings of deeper, nutrient-laden waters or by transfer from terrestrial landmasses via, for example, runoff and atmospheric aerosols. A fundamental discovery in oceanography related to nutrient availability is the relatively constant ratio between carbon, nitrogen, and phosphorus in bulk oceanic particulate matter. This proportion, called the Redfield ratio, is 106C:16N:1P. Dissolved concentrations of these elements exhibit a similar but more variable ratio. Current biogeochemical models tend to use data from one element (typically nitrogen) to determine carbon pools using the Redfield ratio. Very small changes in the Redfield ratio of sinking particulate or dissolved organic matter can have potentially large impacts on estimates of global carbon flux. However, the particular processes and pools that might be most affected by these shifts remain unclear.

Unlike nitrogen and phosphorus, iron is not found in a constant ratio to carbon. In fact, ratios in phytoplankton range from 30,000C:1Fe to 500,000C:1Fe, possibly reflecting the importance of iron as a critical nutrient limiting primary productivity. Determining the factors regulating carbon-iron ratios in planktonic communities under different environmental conditions is essential for understanding carbon flow through these communities. Furthermore, iron availability in the open ocean potentially could undergo significant shifts as a result of global climate change or altered human activities. For example, increasingly arid continental interiors could cause greater quantities of iron to enter the atmosphere as dust aerosol and subsequently be redistributed to ocean surface waters. Such alterations could have profound effects on marine primary productivity and thus require further research to improve predictive capabilities.

In addition to their direct impact on carbon flow and fate, the iron and nitrogen biogeochemical cycles are interlinked with that of sulfur in ways having difficult-to-predict effects on nutrient availability and climate drivers. For example, nitrogen and sulfur may help facilitate the transformation of atmospheric iron into a form readily available to primary producers. Before phytoplankton can use iron within incoming dust aerosol, the element must be shifted from the Fe(III) to Fe(II) form through a combination of complexation and photoreduction chemistry. Mechanisms driving these reactions are poorly understood but seem to be favored under lower-pH, or acidic, conditions. The flow from sea to air of dimethyl sulfide

(DMS) and ammonia (NH_3)—the volatile, reduced gaseous forms of sulfur and nitrogen—controls the acidity of hydrometeors (i.e., atmospheric water particles) over much of the ocean surface and thus influences iron reduction. Shifts in the magnitude of ecological processing resulting from climate change may alter flows of both DMS and NH_3, impacting iron bioavailability from the bottom up.

Understanding the critical couplings between these cycles requires studying carbon cycle interrelationships with nitrogen, phosphorus, iron, and sulfur in the context of coupled physical-biological models. After exploring and refining the contrasting hypotheses in models, results must be used to design more insightful field experiments and observational strategies for marine ecosystems.

Omic and Systems Biology Approaches to Understanding the Marine Carbon Cycle

Integrated understanding of biological processes relevant to marine carbon cycling requires ecological approaches to study relationships of both individual organisms and whole communities in an environment. A major limitation in understanding biogeochemical cycling in oceans, as in most environments, is the inability to cultivate microorganisms of interest and study them under laboratory conditions. Even in cases in which physiological processes in cells under isolated conditions can be measured, extrapolation of laboratory results to natural environments can be misleading because of these systems' highly variable physiochemical conditions and complex webs of community interactions. Fortunately, DNA fragments from mixed microbial communities can now be extracted and characterized directly from environmental samples. This approach has provided novel insights into the ecology, evolution, and metabolism of uncultured microorganisms in nature (see sidebar, Marine Metagenomic Studies, p. 93).

Metagenomics, metatranscriptomics, and metaproteomics (collectively referred to as metaomic approaches) provide information on the identity, abundance, and physiology of marine microbes carrying out carbon fixation or degradation processes (see sidebar, Marine Metagenomics and the Discovery of Proteorhodopsin, p. 94). Such approaches also enable linked studies of community structure and function. Moreover, previous research has demonstrated reproducible patterns in marine microbial community structure that is predictive of physical and chemical conditions in the oceans (Morris et al. 2005; Fuhrman et al. 2006). By combining information from both isolated and environmental omic studies, scientists can begin to characterize mechanisms of carbon assimilation and transformation and develop biological indicators to measure these activities in situ.

The necessary integrated research approach for achieving such advances requires continued identification of model organisms that can be cultured and used for whole-genome sequencing; laboratory-based experimentation; and discovery of key genes, proteins, and pathways. Specifically, such studies could reveal which compounds an organism or group of organisms has the potential to use and which ones are being exploited under a defined set of conditions. The effectiveness of these approaches can be enhanced further when combined with rate studies, stable-isotope techniques, and pulse-labeling to measure species- or

Key Research Questions

1. **What are the effects of the availability of nitrogen, phosphorus, iron, and other micronutrients on biogeochemical cycling and biosequestration of carbon by microbial communities?**
2. **How does chemical processing in the troposphere link and affect terrestrial and ocean systems? How do these systems in turn impact tropospheric activity, with the air pollution–iron mobilization interaction as a primary example?**

lineage-specific contributions to ecosystem function. Identifying target genes, proteins, and pathways can catalyze development of high-throughput omic approaches for new sensors and field experiments. In situ omic information on microbial function is crucial for providing more-accurate data to inform modeling of marine biogeochemical carbon cycling. However, these approaches require improved analytical techniques, such as combining flow cytometric sorting and mass spectrometry of proteins and developing automated devices that can collect high-resolution environmental measurements on microbial community composition and function. By focusing on biogeochemical processes at ocean interfaces and along existing gradients, scientists can use variability within the system to inform experiments and predict ecosystem response to future perturbations.

Integration of Experiments, Observations, and Modeling Efforts

Emerging views of carbon cycling in upper-ocean ecosystems continue to affirm the importance of diverse and multifaceted interactions among marine biota and their environment across all levels of biological, spatial, and temporal complexity. However, characterizing these relationships with traditional approaches is extremely challenging. For example, the spatial scales of interaction between diverse biota and environmental conditions range from molecular to global. This variation demands creative, coordinated approaches to synergistic observational and modeling activities emphasizing the links between models and data derived from environmental observations and experiments (see Fig. 6.1. Modeling Marine Ecosystems: Genomes to Biogeochemical Cycles, p. 88).

Significant observational challenges hamper sustainable long-term monitoring of the genetic, biochemical, and ecological diversity of planktonic communities and their associated rates of carbon and energy transfer. Overcoming these challenges requires development of observational capabilities in conjunction with modeling frameworks readily capable of leveraging or assimilating data to identify strategic ocean and coastal sites for study. These efforts should be coupled with controlled experiments to study links among genetic, physiological, biochemical, and ecological information of important organisms that can be incorporated into models and investigated with hypothesis-driven approaches in natural environments. Ocean observatory facilities will provide critical infrastructure for such investigations. Emerging methods are enabling assessment of genetic and taxonomic diversity at the scales of relevant physical and geochemical forcing—or shifting of the climate system. New omic approaches must be adapted to provide critical links between environmental forcing and ecosystem structure and function. However, efforts to establish such links are in their infancy, requiring further research to connect microbial genetic characterizations, as well as genomic and metagenomic data, to questions on global biogeochemical cycles and climate change. Furthermore, global-scale ecodynamic models, which focus on primary producers, should include more-robust descriptions of marine heterotrophic processes, including predators and heterotrophic microbes, that currently are poorly represented.

New individual channels for modeling geochemical data are required to represent global change feedbacks on key marine microbial processes, including organic-compound recycling in the central ocean, nitrogen cycling by chemoautotrophs,

and biogeochemical functioning of abundant but understudied classes of organisms such as marine crenarchaea. Also needed are systems biology laboratory studies of model organisms and consortia that can improve understanding of environmentally important functional capabilities, resource allocation, and metabolic tradeoffs. Such studies can be followed by various metaomic approaches to characterize distributions, abundances, and in situ activities of related classes of organisms in the environment. Resulting information could then lead to process parameterizations for large-scale climate and biogeochemistry models incorporating stochastic, self-determining ecosystems.

Models representing climate change and carbon cycling rely heavily on observed ecological-response data. These observational data are gathered, processed, and parameterized into new models by various means. The amount and diversity of such data are exploding because of increasingly sophisticated metagenomic studies and development of new sensor technologies, remote-sensing methods, and ever-expanding numbers of networked sensors operating in real time (see Fig. 6.2. Oceanic Measurement Technologies, p. 89). This data explosion extends beyond traditional definitions of ecological observations by including quantitative measurements of how individual and communities of organisms carry out carbon cycling processes in biological and geobiological systems. Incorporating more experimental and observational data into predictive climate change and carbon cycling models will require assimilating information from not only traditional ecological measurements but also those derived from high-throughput biological observations and investigations.

Key Research Questions

1. **What are the physical and biological scales for biota-environment interactions most critical in regulating changes in ocean carbon fluxes over seasonal, interannual, and decadal time frames?**
2. **Which scales must be resolved in space and time and with respect to ecological diversity to better constrain predictions of changes in carbon flux?**
3. **How do we organize the explosion of data from metagenomic studies, place-based and remote-sensing efforts, and other data-intensive investigations? How can this information be used to enhance fidelity and efficiency in large-scale ecodynamic models? To what degree can the data-assimilation process be automated?**

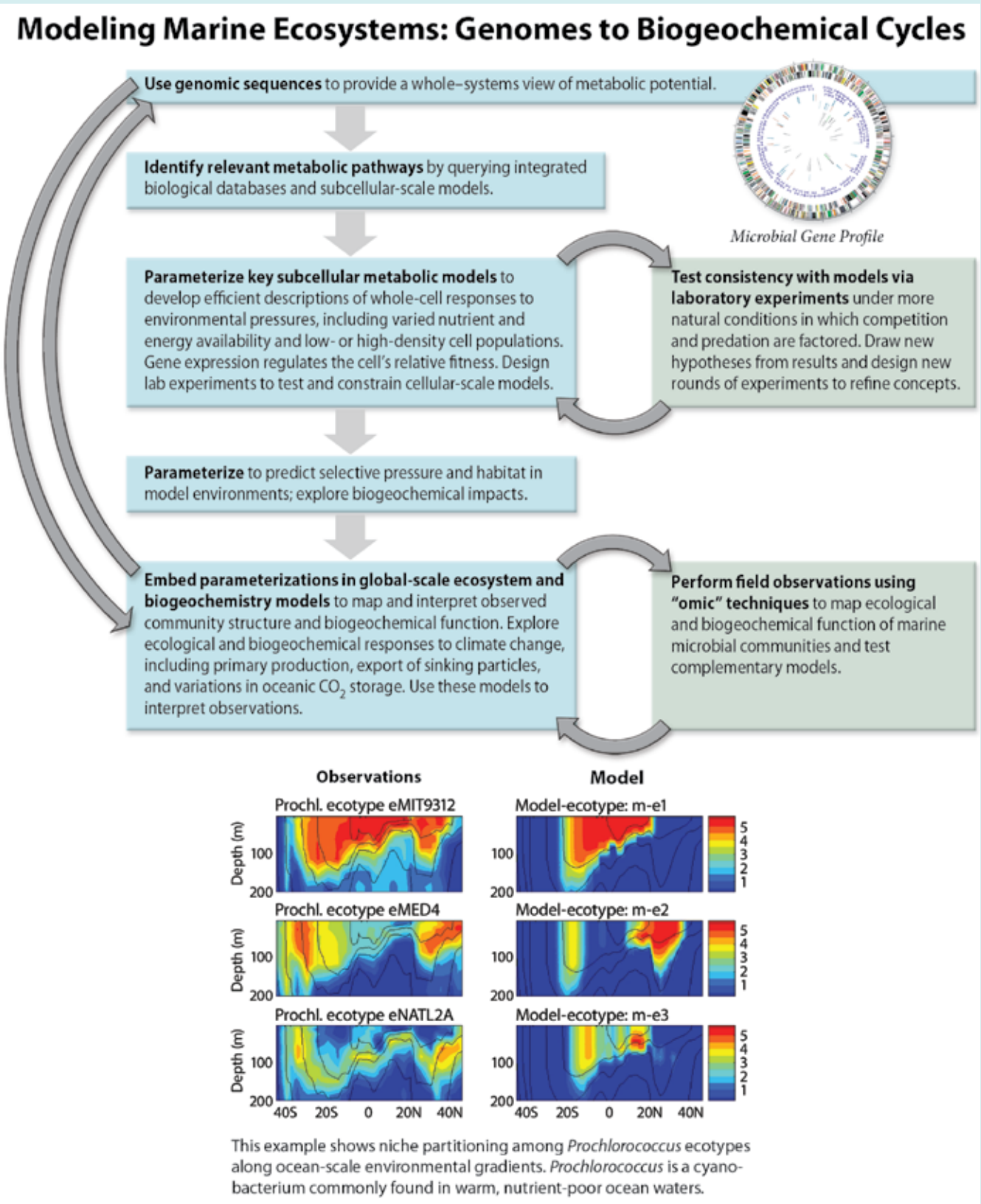


Fig. 6.1. Modeling Marine Ecosystems: Genomes to Biogeochemical Cycles. Depicted are observed and modeled distributions of ecotypes of *Prochlorococcus* [log (cells ml^{-1})] along a meridional transect in the Atlantic Ocean. Black lines indicate isotherms. Observations are from Johnson et al. (2006). Model ecotypes that qualitatively reflected real-world counterparts in terms of *Prochlorococcus* geographic habitat, ranking of abundance, and physiological specialism were emergent in the self-assembling model of global phytoplankton communities (Follows et al. 2007). [Source of Observations and Model graphs: Reprinted with permission from *Science* and AAAS.]

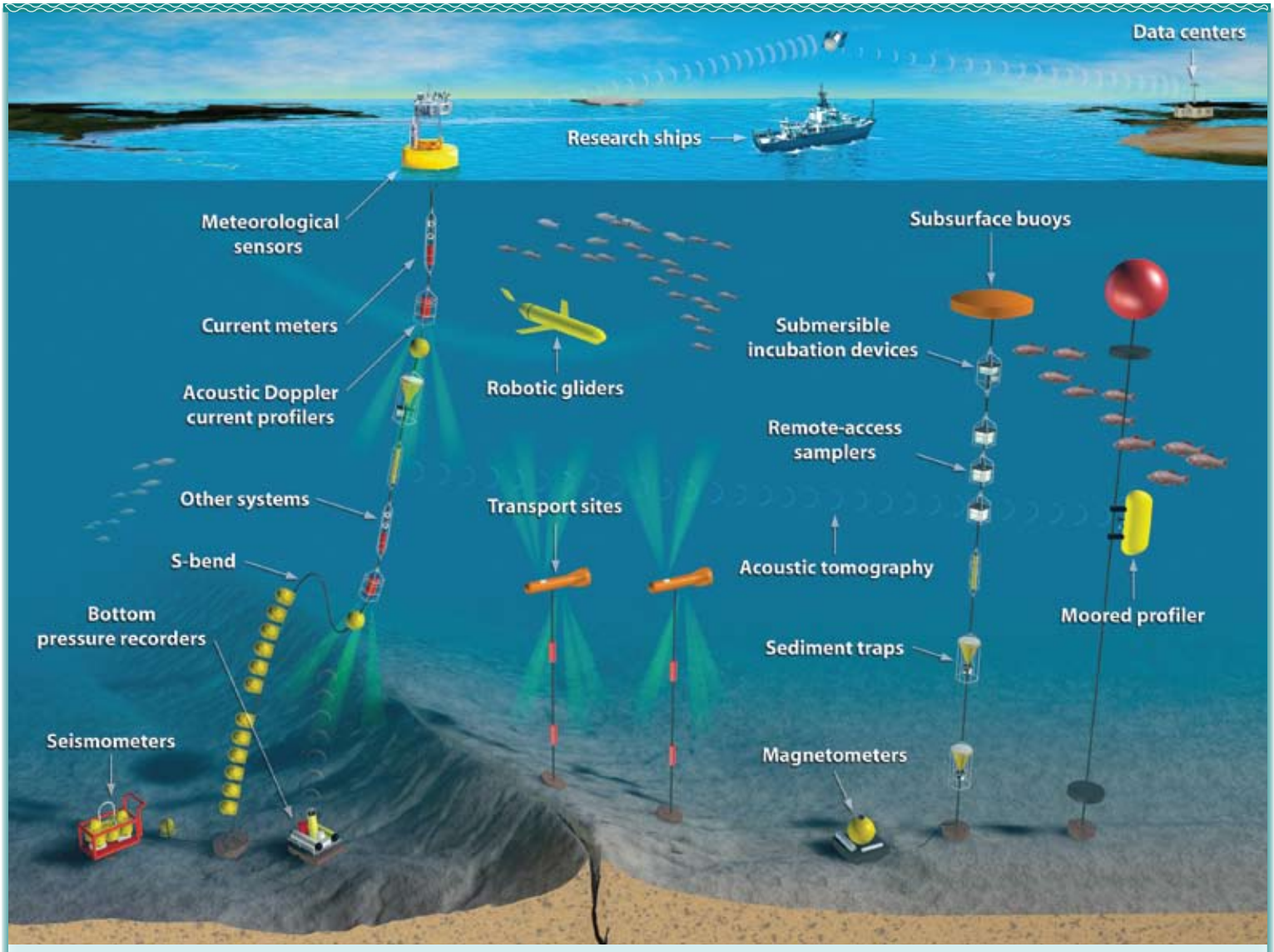


Fig. 6.2. Oceanic Measurement Technologies. A range of different sensors are used to measure meteorology, climate, physical oceanography, water transport, biogeochemistry, carbon cycle, biology, and geophysics. [Source: Cooperative Institute for Climate and Ocean Research, Woods Hole Oceanographic Institution. 2006. "OceanSITES: Taking the Pulse of the Global Ocean," http://www.oceansites.org/documents/oceanSITESbrochure.pdf. Illustration by Jack Cook, Woods Hole Oceanographic Institution.]

Meteorological sensors atop a surface buoy provide data for calculating heat, water, and momentum exchange between air and ocean. The self-reliant buoys carry batteries, solar panels, two satellite transmitters (in case one fails), and a GPS locator. Instruments in the hull record sea temperature, salinity, oxygen content, and carbon dioxide.

Current meters record current speed, direction, temperature, and salinity to produce a motion picture of flow and mixing in the water column.

Acoustic Doppler current profilers emit high-pitched pings and measure their echoes to calculate current speed at regular intervals in the water column.

Other systems record dissolved oxygen, light levels, photosynthetic activity, and nutrients like nitrogen, phosphorus, and silica.

Engineers build an **S-bend** in the mooring line to reduce the tension between anchor and buoy during heavy seas.

Bottom pressure recorders can sense the pressure from a passing tsunami wave, then beam a warning to a surface buoy.

Seismometers measure earthquakes in the seafloor.

Robotic gliders monitor precise locations on the fly, without requiring ship time or mooring hardware.

Transport sites measure water moving in important ocean currents. Rows of buoys placed in the deep ocean measure currents, temperature, and salinity. The data help scientists calculate how much water is moving from one ocean basin to another.

Subsurface buoys are good choices for studying the deep ocean. These moorings are not exposed to surface waves, so they get much less wear and tear than surface buoys.

Submersible incubation devices incubate seawater samples to measure phytoplankton productivity.

Remote-access samplers automatically do routine prep work, like filtering seawater, and then store the samples in individual jars to be analysed for nutrients, phytoplankton, or zooplankton.

Acoustic tomography sends sound waves long distances to calculate temperature and track warming across entire ocean basins.

Sediment traps collect falling "marine snow" (dead organic matter). They provide key data on how carbon cycles in the ocean.

Magnetometers measure changes in the Earth's magnetic field during earthquakes.

Subsurface moorings often are deployed in pairs. One line supports a **moored profiler**, which crawls up and down the cable measuring temperature, salinity, and currents. Instruments on the sister mooring measure different variables at fixed depths.

Research ships sample water for in-depth biogeochemical analyses.

Data centers receive buoy data, check quality, and serve calibrated data to the Internet.

CO_2 Absorption and Ocean Acidification

Rising atmospheric CO_2 concentrations are altering the chemical makeup of ocean waters, making them more acidic. Over the last 200 years, nearly half the CO_2 emitted from the burning of fossil fuels—about 525 billion tons—has dissolved into ocean surface waters (Sabine et al. 2004), lowering their pH by 0.1 units. The figure below shows the water column inventory of anthropogenic CO_2 in the oceans.

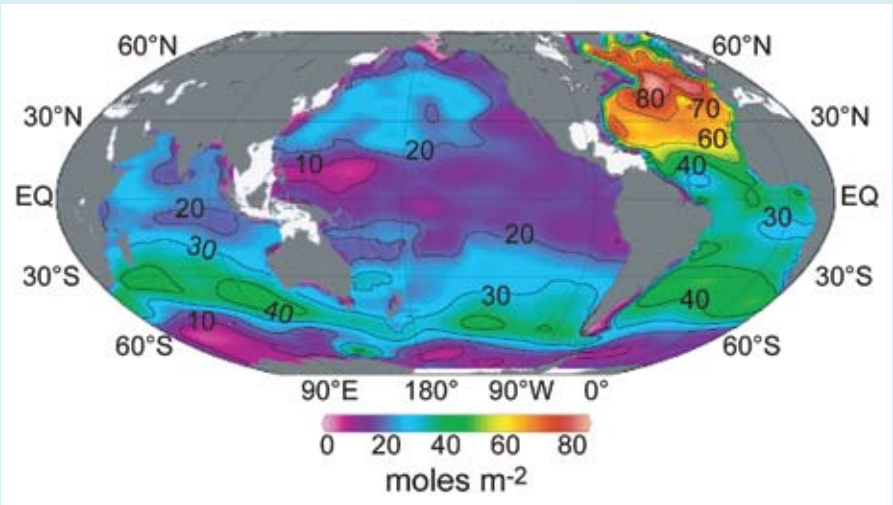
If CO_2 emissions keep rising at current rates, the average surface seawater pH level, which typically ranges from 7.8 to 8.2, could decline another 0.3 to 0.4 units by 2100 (IPCC 2007). Such a level would represent the lowest pH of the upper ocean in many millions of years and would constitute a rate of change 100 times greater than at any time spanning this period (Caldeira and Wickett 2003). The pH drop of 0.1 units observed to date is equivalent to a 30% increase in surface-water acidity. A further decrease of 0.3 to 0.4 pH units would translate to a 100% to 150% increase in acidity.

Oceans remove roughly 30% of the CO_2 emitted annually to the atmosphere. The resulting increasingly acidic waters could threaten a wide range of marine organisms—from microscopic phytoplankton and shellfish to massive coral reefs—as well as the food webs depending on them (see sidebar, Marine Food Web and the Carbon Cycle, pp. 80–81). Consequently, the oceans' capacity to absorb excess CO_2 could decline with alteration in trophic cascades, reducing the oceans' ability to mitigate global warming.

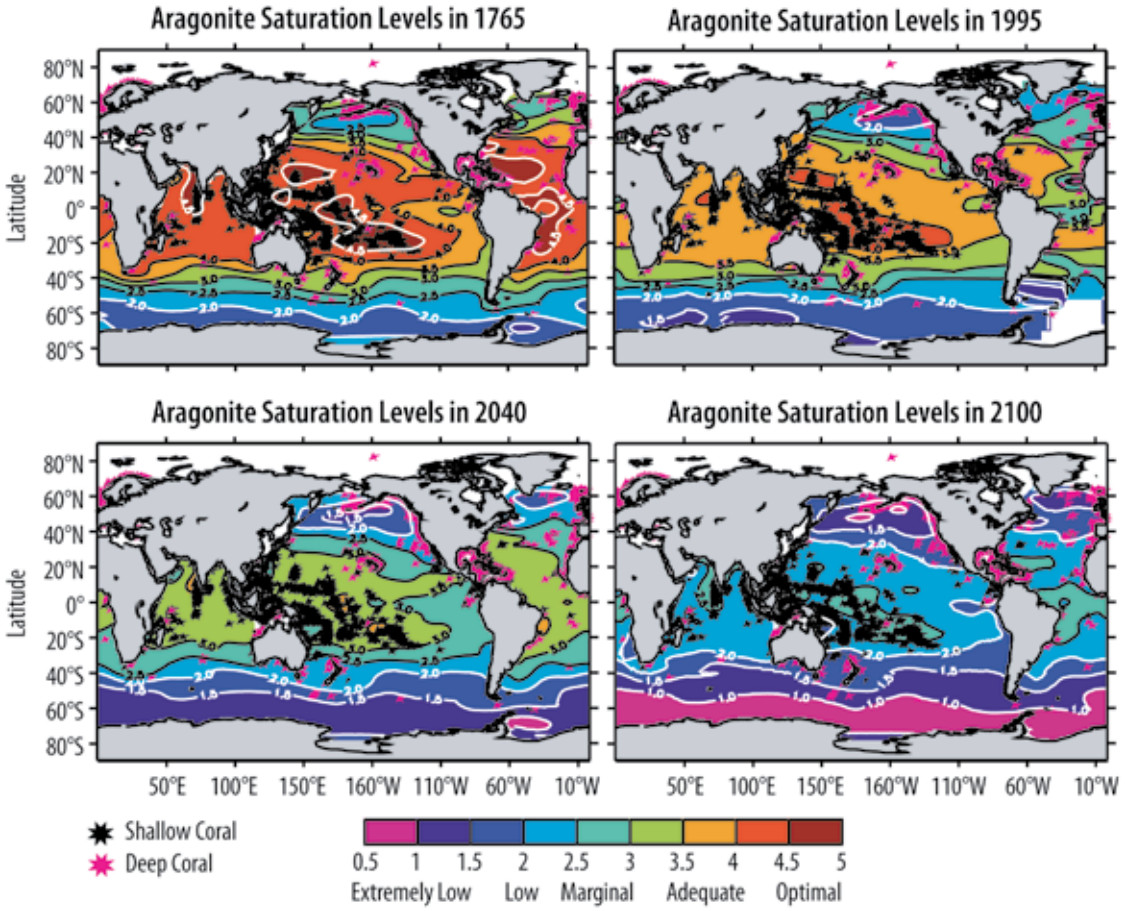
The Nature of Possible Biological Impacts

Calcium carbonate structures important for microorganism formation have two distinct mineral forms—calcite and aragonite. Each form has a different solubility, or tendency to dissolve in seawater, measured by what is known as the saturation rate. This rate, in turn, depends on the oceanic concentration of calcium, carbonate, and depth or pressure. Marine calcium concentrations are relatively constant; thus shifting carbonate concentrations determine rates of calcium carbonate formation.

If the saturation horizon moves closer to the surface, already-formed calcium carbonate could start to dissolve, decreasing concentrations of compounds marine organisms need to maintain shells or build new ones. Especially vulnerable are calcifying organisms that construct aragonite structures [e.g., corals and pteropods (tiny planktonic marine snails)] because this form of calcium carbonate is more soluble than calcite (Orr et al. 2005). Other organisms, including coccolithophores (microscopic algae) and foraminifera (microscopic protozoans), build skeletal structures of the more-resistant calcite. The figure, next page, shows the shifting aragonite saturation levels in the global oceans and the impact on coral formation.



Water Column Inventory of Anthropogenic CO_2 in the Ocean (mol m^{-2}). High inventories of anthropogenic CO_2 are associated with deep-water formation in the North Atlantic and intermediate and mode water formation between 30° and 50°S. Total inventory of shaded regions is 106 ± 17 Pg C for 1994. (Red and green indicate high amounts of carbon storage; purple and blue areas contain less carbon.) [Source: Sabine, C. L., et al. 2004. "The Oceanic Sink for Anthropogenic CO_2," *Science* **305**, 367–71. Reprinted with permission from AAAS.]



Aragonite (Form of Calcium Carbonate) Saturation Levels Shown from Before the Industrial Revolution to 2100, and How These Saturation Levels Affect the Growth of Both Shallow and Deep Corals. Before the Industrial Revolution, large bands of the tropical ocean were optimal for growth. By 2040, these same bands are projected to be only adequate, and by 2100 (in the IS92 business-as-usual scenario, Orr et al. 2005), most areas are only marginal at best. [Source: Ocean Acidification Network FAQs at http://ioc3.unesco.org/oanet/FAQacidity.html. From Feely, R. A., et al. In press. "Present and Future Changes in Seawater Chemistry due to Ocean Acidification," AGU Monograph on *The Science and Technology of Carbon Sequestration*. Eds. B. J. McPherson and E. T. Sundquist.]

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Marine Nutrient Cycling

In the ocean, nutrients fuel the production of organic matter and the sinking of carbon. Microscopic marine phytoplankton transform nitrogen-, iron-, phosphorus-, and sulfur-containing compounds in ways affecting these nutrients' availability for biological production and, consequently, influence on the global climate. Of these four nutrient cycles, nitrogen is the most complex, given the diversity of nitrogen metabolism and its existence in numerous inorganic and organic forms and oxidation states.

Nitrogen cycling consists of five main processes whose descriptions follow.

- **Fixation** through metabolic processes, whereby microbes transform atmospheric nitrogen (N_2) into ammonium, a form useful to organisms.
- **Uptake** via the growth of organisms that assimilate the element into organic matter.
- **Mineralization** or decay, by which much of the nitrogen within dead organisms is converted back to ammonium for use by plants or for further transformation into nitrate via **nitrification**.
- **Denitrification**, which returns nitrate to the atmosphere as N_2 and nitrous oxide, a volatile and highly potent greenhouse gas. This process results in the loss of biologically available nitrogen from the ocean system.

Iron, a scarce micronutrient, limits both primary production (Coale et al. 1996) and nitrogen fixation in many areas of the ocean (Falkowski 1997). Iron is highly reactive and quickly removed from the water column by biological uptake as well as scavenging and desorption onto sinking particles. Inputs from the atmosphere are an important source of iron for marine systems, and some open-ocean regions exhibit enhanced productivity following remote dust events.

Phosphorus is cycled as either inorganic or organic phosphate—with no major gaseous intermediate—making it distinct from other nutrient cycles. During this cycling, the only existing form of inorganic phosphate is transformed into an organic compound and back again. Under natural conditions, phosphorus is the slowest nutrient cycle because of the gradual rate at which phosphate salts are released from rocks and soils through weathering. Consequently, phosphorus often is a limiting agent in plant and algae growth, particularly in freshwater systems. In oceans, phosphorus concentrations can vary significantly with depth, with biologically productive surface layers generally containing less phosphorus than deeper waters.

Sulfur cycling largely parallels the nitrogen cycle, with the exception of sulfur fixation from the atmosphere to land or water. Marine phytoplankton affect the sulfur cycle by producing dimethylsulfoniopropionate (DMSP), a precursor to dimethyl sulfide (DMS) that, when oxidized, becomes sulfate. The flux of DMS from ocean surface waters is the predominant source of sulfur to the atmosphere (Kettle and Andreae 2000). Once there, DMS-derived sulfate aerosol particles may cool the Earth system by reflecting solar radiation back into space and by promoting cloud formation or modifying cloud properties. Additionally, atmospheric sulfur deposition can lead to surface-water acidification, impairing the uptake of other nutrients, especially phosphate.

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Marine Metagenomic Studies

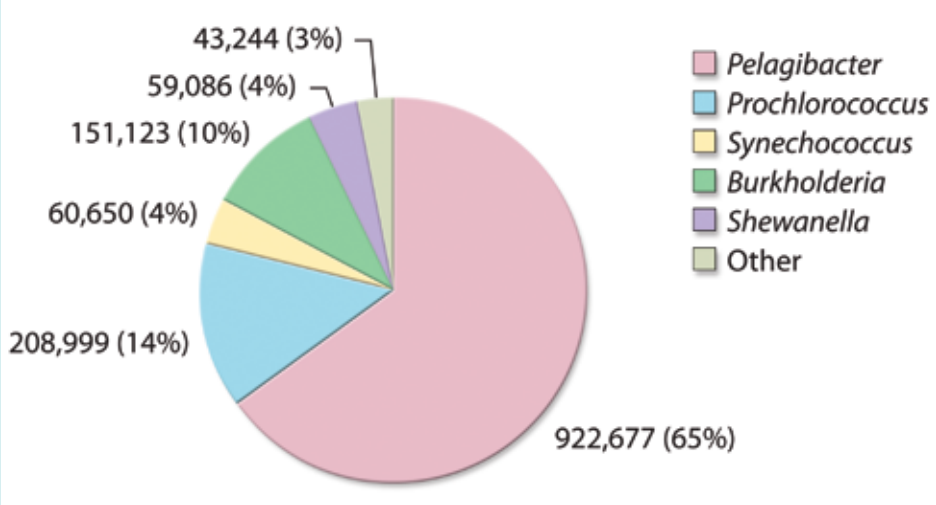
In the largest metagenomic survey to date—the Global Ocean Sampling (GOS) expedition—an enormous amount of genomic diversity was found among upper-ocean microbial communities. High-throughput DNA sequencing and computational genomics produced a massive dataset of more than 6 million new genes and thousands of new protein families that include a wide range of novel metabolic pathways.

This gene catalogue includes and extends the results of an earlier metagenomic pilot project conducted in 2003 to study the genomic diversity of microbes collected from the nutrient-poor Sargasso Sea near Bermuda. That project alone led to the discovery of more than a million new genes and the identification of more than 1800 species in an area thought to be low in diversity. DNA shotgun sequencing also verified both the abundance and variety of a new class of light-harvesting proteins, suggesting they play a potentially important role in energy metabolism under low-nutrient conditions (see sidebar, Marine Metagenomics and the Discovery of Proteorhodopsin, p. 94).

To study the global extent of this genomic diversity, as well as how different environmental pressures might be reflected in organisms and communities residing in heterogeneous ocean biomes, the GOS study covered an 8000-km transect extending from the North Atlantic through the Panama Canal and ending in the South Pacific. Forty-one different samples were collected from a wide variety of surface waters (mostly marine). Major differences were found at almost every site, and researchers could determine from where in the ocean a sample was derived by its DNA sequence alone.

Surprisingly, despite the wealth of diversity and variation found at the gene and protein levels, only five bacterial genera dominate the GOS sequence data: *Pelagibacter*, *Prochlorococcus*, *Synechococcus*, *Burkholderia*, and *Shewanella* (see figure below).

Further studies are expected to help elucidate key biological processes that eventually could offer new solutions to address climate change and other environmental issues.



Dominant Bacterial Genera and Global Ocean Survey Sequences Aligning to Them. Indicated are the numbers and percentages of sequence reads that aligned with the reference genomes from five dominant bacterial genera and all other reference genomes. [Source: Figure adapted by permission from Macmillan Publishers Ltd. From Koonin, E.V. 2007. "Metagenomic Sorcery and the Expanding Protein Universe," *Nature Biotechnology* **25**(5), 540–41.]

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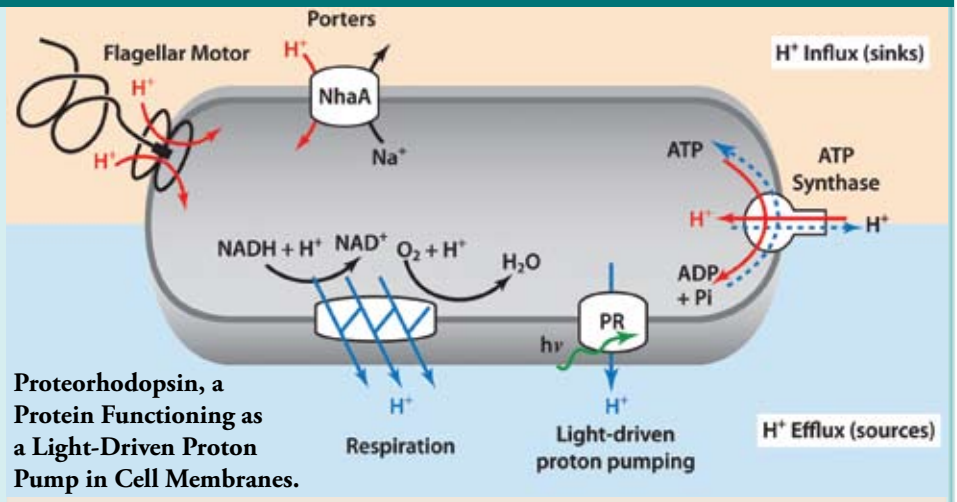
Marine Metagenomics and the Discovery of Proteorhodopsin

Although surface waters in the open ocean receive ample sunlight to fuel photosynthetic growth, concentrations of dissolved organic carbon, a photosynthetic byproduct, typically are very low (less than 200 µmol C per liter). Heterotrophic members of the bacterioplankton community thus are forced to contend with severe carbon substrate limitation, yet the specific metabolic strategies employed by these marine oligotrophs to grow under these conditions are just being discovered. As in many environments, the lack of insight arises primarily from difficulties inherent in cultivating relevant organisms in a laboratory setting. New metagenomic approaches, however, are overcoming this challenge by characterizing microbial DNA directly from the environment.



Proteorhodopsin, a Protein Functioning as a Light-Driven Proton Pump in Cell Membranes.

[Source: Lawrence Berkeley National Laboratory. 2007. "Research News: Shedding New Light on Proteorhodopsin," http://www.lbl.gov/Science-Articles/Archive/PBD-proteorhodopsin.html. Image used with permission and adapted from original figure in Walter, J. M., et al. 2007. "Light-Powering *Escherichia Coli* with Proteorhodopsin," *Proceedings of the National Academy of Sciences of the USA* **104**(7), 2408–12. doi: 10.1073/pnas.0611035104. Copyright (2007) National Academy of Sciences, USA.]

Metagenomic sampling of microbial communities in the open ocean has revealed a surprising new pathway for energy conservation by marine heterotrophs. Proteorhodopsin, a protein functioning as a light-driven proton pump in cell membranes, has been detected in a wide range of ocean habitats. Previously thought to exist only in archaeal extremophiles living in salt ponds, genes encoding these proteins are ubiquitous in marine bacterioplankton such as the SAR cluster, which was originally isolated from samples taken in the Sargasso Sea. In those samples alone, more than 782 rhodopsin-like photoreceptors were identified (Venter et al. 2004). The common occurrence of bacterioplankton harboring this protein in surface waters worldwide suggests a potential mechanism for widespread mixotrophic energy conservation in marine environments. Mixotrophy is a form of growth in which two methods of energy generation are used simultaneously. In this form, bacteria would augment energy derived from the consumption of organic substrates and conserve carbon resources by creating an additional proton gradient using light energy to drive synthesis of ATP, a multifunctional nucleotide responsible for cellular energy transfer and storage (see figure above).

The initial observation of proteorhodopsin in metagenomic samples sparked a series of experiments to test the mixotrophic-growth hypothesis. Expression of the proteorhodopsin gene in *Escherichia coli* confirmed the protein was involved in light-dependent ATP formation. Preparations of bacterial cell membranes collected from ocean surface waters reveal not only high levels of proteorhodopsin, but various types of the protein tuned to absorb different wavelengths of light. This variation suggests ecological specialization for different niches and depths in the water column. Furthermore, experiments using recently cultivated marine heterotrophs equipped with proteorhodopsins have shown that at least some types grow more efficiently under substrate-limited conditions when exposed to light.

Though further field studies are needed to assess the role of proteorhodopsin in marine ecosystems, initial results suggest this novel mode of growth could represent an important new pathway affecting carbon flow and energy conservation in ocean-surface habitats. Proteorhodopsin discovery also represents an important early success story for using metagenomic approaches to detect previously untapped metabolic capabilities, facilitate development of new hypotheses and experiments, and reveal significant components of the global carbon cycle.

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Integrating Biology and Climate Through Systems Science

Genomics and Systems Biology

Genome Analyses Related to Climate Response of Ecosystems

The genomic and postgenomic eras have provided unprecedented potential to understand plants', microbes', and even entire communities' molecular and cellular responses to global change. Early efforts to apply genomic technologies and concepts to climate change research are already improving our capability to predict organism response to such change. For example, genome-wide analyses of soybean and poplar have revealed that elevated CO_2 down-regulates key genes in the octadecanoid pathway—a biosynthetic process producing an important defense hormone. Such findings may explain why ecosystems dominated by these plants show increased susceptibility to insect herbivory and delayed canopy senescence. Genomic technologies are now tractable in a wide range of organisms, including important agronomic (e.g., maize and rice) and forest (pine) species. Incorporating these high-throughput "omic" tools into current and emerging global change experiments will accelerate discovery and strengthen the predictive power of this research.

A main goal of such studies is determining whether individual genes or small groups of genes play keystone roles in controlling ecosystem capacity to store atmospheric CO_2. Related to such research is investigation into whether the similarity or dissimilarity in different ecosystems' reactions to global change is explained by coordinated and synergistic genetic responses across taxa.

Metaomics

Microbial communities inhabiting soils, oceans, and other types of terrestrial and aquatic environments play crucial roles in the global carbon cycle, yet these organisms and the processes they catalyze remain poorly understood. Although hundreds of terrestrial and aquatic microbial genomes have been completely sequenced (and even fewer have been studied in sufficient detail to develop robust models of metabolism and regulation), they represent only a small fraction of the total diversity of microorganisms, most of which defy laboratory cultivation. New and emerging technologies in metagenomics, metatranscriptomics, and metaproteomics, which can probe whole communities, offer insight into the metabolisms and lifestyles of diverse microbes, including those that remain uncultivated. The daunting complexity of most terrestrial and aquatic communities and the inability to directly translate gene sequence into potential biological function thus far have limited our ability to extract detailed insights into functionality.

Overcoming these obstacles will require, in part, developing and pursuing techniques that enable targeted metagenomic (or other omic) research. Using narrowed, highly specific approaches makes study of a microbial community manageable and progressively helps unravel the complexity of the overall system. Specifically, methods such as stable-isotope probing or metabolic labeling with bromodeoxyuridine will allow scientists to effectively target important segments

of a microbial community without cultivation and thus begin to understand these particular segments' functional roles. Metatranscriptomics and metaproteomics, which by nature primarily target the metabolically "active" microbial community and its expressed macromolecules, will provide real-time insight into actively occurring processes. Single-cell genomics, using cells obtained via flow sorting or micromanipulation, offers the potential for even more targeted analyses of microbial community members, further reducing the challenges arising from the incredible complexity within microbial communities.

These developing techniques and other similar approaches can begin to surmount some of the technical complications related to studying complex and heterogeneous soil and marine microbial communities. Equally important is an overall understanding of entire communities associated with key environments. Such an understanding would serve as an invaluable baseline from which to view future metaomic studies relevant to carbon cycling. As DNA sequencing becomes increasingly accessible and less expensive, a human genome–type project would target the microbiome in a spectrum of representative habitats, as suggested in a recent report by the National Academies Press (http://www.nap.edu/openbook.php?record_id=11902&page=R1). Serving as models for this type of large-scale endeavor are the National Institutes of Health human microbiome project (http://nihroadmap.nih.gov/hmp/) and the Global Ocean Sampling survey (http://collections.plos.org/plosbiology/gos-2007.php). The latter, which resulted in a massive metagenomic dataset, also is a useful resource for data-mining information relevant to carbon cycling and for applying complementary omic methods for testing hypotheses regarding community function. The Department of Energy's Joint Genome Institute, also a valuable resource in this regard, has begun sequencing numerous ecologically relevant organisms and communities, including those inhabiting soils, plant biomes, and oceanic environments (http://www.jgi.doe.gov/).

Systems Biology

Achieving a predictive, systems-level understanding of carbon processing by plants, microbes, and biological communities will require integration of fundamental science and technology. A key emphasis should be developing and employing genomic and systems biology approaches to model, for example, the regulatory networks that control carbon flow and fate, from assimilation by phototrophs to processing of organic matter by heterotrophs. Succeeding in this endeavor will allow scientists to predict molecular-network states under untested conditions, such as in anticipated climate change scenarios or gene modifications. The power of systems biology to accomplish such goals is illustrated by the recent success in predicting gene regulatory networks that control the physiology of a free-living bacterial cell in response to genetic and environmental perturbations (Bonneau et al. 2007). A central goal of systems biology approaches in this context is to develop models of metabolic and regulatory networks in keystone soil and marine microbes, plants, and biological communities that ultimately will inform climate and biogeochemical models (see sidebar, Systems Biology, p. 97).

Systems Biology

Systems biology can be defined as "the exercise of integrating the existing knowledge about biological components, building a model of the system as a whole, and extracting the unifying organizational principles that explain the form and function of living organisms" (von Bertalanffy 1968). Genome-scale analyses in microbes and plants have the potential to provide the necessary data to understand on a systems level how an entire organism works. In a practical sense, a systems approach to understanding biology can be described as an iterative process including (1) collection and integration of all available data (ideally for all of an organism's components and their relationships), (2) system modeling, (3) experimentation at a global level, and (4) generation and testing of new hypotheses (Ideker et al. 2001). The ultimate goal of a systems approach is not to describe and model what is known, but to predict how a system will react under untested conditions or in response to perturbation. Only then can researchers use systems-based models in a predictive fashion to manipulate biological systems for optimizing a specific process or function.

Ecologists and physiologists for many years have used systems biology to study organisms, yet applying this approach to examine molecules is only now feasible with the advent of genomics-inspired technologies able to supply a sufficient volume of information at many levels of organization. Thus, the postgenomic era offers the prospect of integrating knowledge across different levels of biological organization and anchoring this insight at the molecular level.

Connecting Omics to Biochemical Function

A major DOE objective is developing methods that use knowledge of genome-based microbial ecophysiology (i.e., functionality) to ultimately assess global carbon biosequestration strategies and climate impacts and feedbacks. The challenge of this objective can be stated simply as the need to advance scientific understanding from "sequence to physiology to activities." Accomplishing these goals requires a clear strategy for selecting which processes and systems are most important for developing a predictive understanding of carbon cycling and biosequestration (see Chapter 2, Technical Strategy, including Fig. 2.1. Scales and Processes of the Global Carbon Cycle, p. 16).

Identification of these elements could be aided greatly by an approach centering on the concept of intensive characterization of keystone genes and organisms. This method, for example, first could involve genomic and systems biology laboratory studies of relevant, experimentally tractable organisms or communities. Research then would progress to field experiments to answer fundamental questions such as which genes are functioning under various environmental conditions. The latter studies must include sensitive high-throughput methods not requiring large concentrations of biomass. Genomic and functional genomic approaches also can be used to reveal organismal processes and characteristics important in the environment and thus necessary for incorporation into models.

Critical for global-scale climate and biogeochemical models are accurate estimates of process rate constants, which influence biochemical functionality in organisms (see Fig. 2.2b. Knowledge Integration and Synthesis, p. 20, in Chapter 2, Technical Strategy). This functionality (V_{max}, rate per unit biomass) generally is defined as catalytic property plus rate constant, which can be incorporated into system models operating at larger scales. A crucial enabling research need is the ability to use omic information to provide estimates of catalytic rates and identify the types of processes and mechanisms occurring in organisms. Currently, genomic, transcriptomic, and proteomic measurements can give, at best,

relative abundances of functional molecules whose activity is inferred largely from sequence homology. Thus, more precise assessments of biochemical function are needed and will require concerted, extensive research as well as new and innovative approaches and technologies. Achieving this level of functionality understanding has the potential to tremendously advance not only carbon cycling objectives, but all DOE science missions, including those related to environmental remediation, bioenergy, and beyond.

Another difficulty in progressing to a genome-to-activity understanding involves challenges associated with annotation—predicting protein function from DNA sequence and homology. In some cases, defining a specific protein's general functional class, such as an amino acid transporter, is relatively easy. However, identifying its substrate range (i.e., which amino acids it transports) can be extremely difficult, yet doing so can help answer important ecophysiology questions and determine the function of these molecules within metabolic networks. A potentially powerful approach for determining gene function and ultimately improving predictive capabilities combines comparative genomics with experimental techniques such as those used by Yang et al. (2006) to characterize the *N*-acetylglucosamine utilization pathway in *Shewanella*. The study identified genes involved in this particular metabolic pathway. A complementary research method would target specific enzyme systems that process important extracellular compounds key to carbon cycling in terrestrial and marine systems.

Connections to Phylogeny

An important functionality question relating to variability in V_{max} is the extent of sequence divergence in orthologs (i.e., similar genes or gene segments appearing in different species and arising from a common ancestor). Studying this divergence, in extracellular hydrolases for example, can provide useful insight into how phylogenetic information [structure of bacterial small-subunit ribosomal RNA (abbreviated as ssu rRNA) and multilocus sequence typing (MLST)] relates to functionality, both substrate catalysis and environmental-stress responses.

Stable-isotope probing offers one approach to further advance these studies to determine biochemical function. For example, labeling key organic substrates with ^{13}C could help identify important taxa (phylogenetic designations) that function in the carbon metabolic process. This labeling could be conducted in representative habitats worldwide. Resulting phylogenetic information would then be used to isolate representative microbes from taxa carrying out the functional processes important to carbon cycling. (Amann, Ludwig, and Schleifer 1995; Madsen 2005). Next, a set of microbes covering the phylogenetic breadth of a key taxon would be identified, and functional process rates (V_{max}) under optimal conditions or functional response to environmental stress would be measured. Variance in these properties then could be determined across the taxon's phylogenetic breadth, and if little or none is observed, phylogeny can directly inform functionality. In summary, these three steps connect omic approaches to biochemical function:

- Obtain relevant sequence information (using MLST for phylogenetic placement and DNA sequences for specific functional genes).
- Apply analysis of variance techniques to determine if functional rate is predictable from gene-sequence information.

- Conduct comparative genomic investigations of strains within a species to provide an estimate of core capabilities of a specific taxon.

Tracking carbon via ^{13}C labeling and determining rate constants for carbon processing through different ecosystems could become a very important tool with direct linkages to larger, perhaps even global, scales of the carbon cycle. Moreover, this approach offers the additional virtue of obtaining phylogenetically informative tagged macromolecules.

Value and Challenges of Visualization Tools and Modeling

Linking genomics-based information to function requires both genome-scale data generation and systems biology tool development. Generation and collection of transcriptomic, proteomic, and metabolomic data require critical parameters that must be assayed and quantified. Computational requirements include development of visualization and other types of tools to integrate genome-scale data over various time scales of experimentation. Also critically needed are predictive modeling tools.

The value of visualization tools in showing genomic relationships is illustrated by the use of multinetworks to graphically display information about the manifold connections among genes, proteins, and molecules—all generically referred to as "nodes" in a network (see Fig. 7.1. Multinetwork Analysis of *Arabidopsis* Genome, p. 100). Node-linking "edges" are drawn based on experimental evidence or predictive algorithms. For example, protein:protein and protein:DNA edges could be determined experimentally but also might be predicted based on, for the former, two homologous proteins interacting in a different species or, for the latter, the presence of a transcription-factor binding site in the promoter of a gene. Another experimentally derived edge, for instance, could originate by determining that a gene encoding a certain enzyme uses a particular metabolite in a nonreversible catalytic reaction. Thus, a gene-encoding enzyme:metabolite edge would represent this interaction. An edge connection between genes also could be drawn based on transcriptional activation of a target gene by a transcription factor, depicted as a protein:DNA interaction edge. The latter two examples include nodes connected by "directed edges" (e.g., the transcription factor regulates the target gene, not vice versa, and thus is represented by a directional arrow). Alternatively, an edge might be nondirected, as is the case for those representing a protein:protein interaction.

A great obstacle to connecting genomic data to biological function centers on incorporating this information into models that can be tested dynamically. For example, researchers are faced with mathematical and computational challenges—automating and integrating into models the massive volumes of high-throughput data from experimental systems biology as well as that from ecological observations. Generation of these omic data should be motivated by the specific need to build larger-scale models rather than indiscriminate collection of information. In turn, the larger scale will drive data development to populate these models, thus enhancing their predictive capabilities.

Fostering communication between modelers and metaomic researchers is a first step in identifying the data most important for improved models. One way to do so is mutual list building and intercomparison of such lists between the two groups. For example, biologists would itemize the level of metabolic and biogeochemical

information they currently (or in the near future) can provide to large-scale modelers. Meanwhile, computational scientists studying global change would identify their metabiogeochemical data needs. Comparisons between the groups would identify key areas of overlap and facilitate concept development and expansion of intersecting research.

Simple list comparisons also can be valuable in helping modelers and metaomic researchers readily identify—and thus ultimately connect—experimentally observed enzymatic or protein functions and associated gene sequences. Making these connections involves leveraging gene expression to determine the sequence underlying a metabolic pathway of particular importance to modeling. This process can be considered classical annotation run both forward (using DNA sequence to determine protein function) and backward (using observed protein function to sequence DNA). For example, within sequences derived from metagenomic surveys, the mapping of genes to enzymes remains largely incomplete.

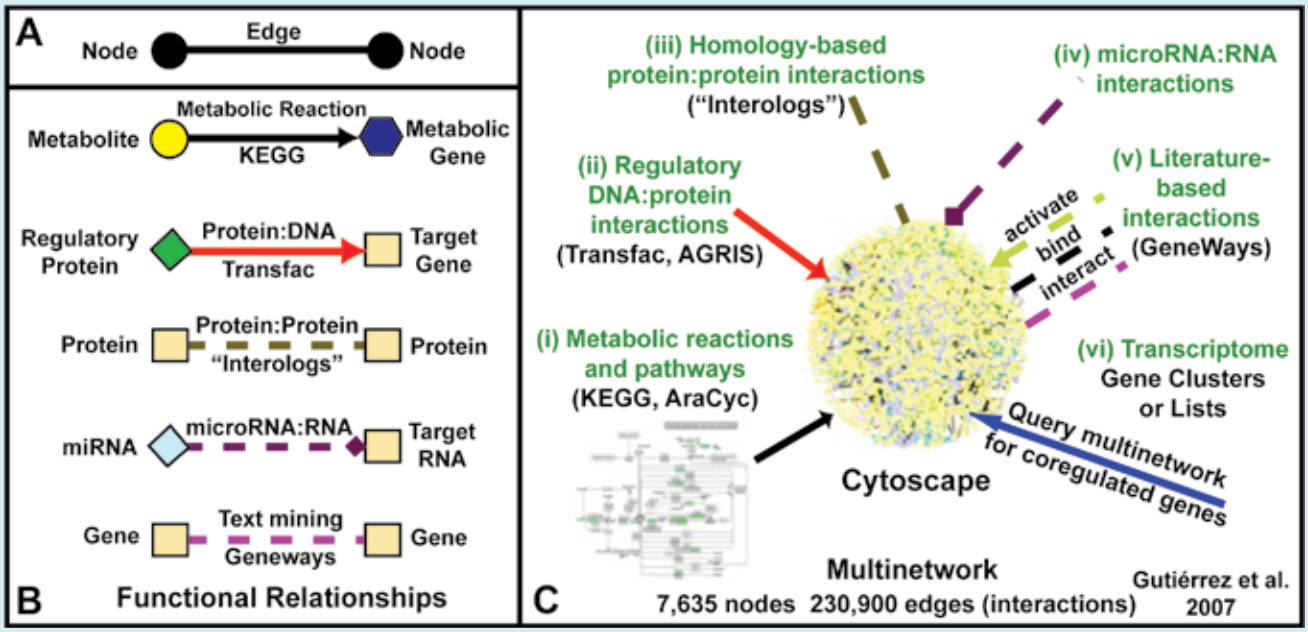

Fig. 7.1. Multinetwork Analysis of *Arabidopsis* Genome. (A) Networks in their simplest forms are made up of "nodes" and the "edges" that connect them. (B) Various types of node connections can be displayed using colors and shapes that indicate different types of molecules and the relationships between them. (C) Multinetwork representation of *Arabidopsis* metabolic, regulatory, and predicted regulatory connections between genes, proteins, and metabolites (Gutiérrez et al. 2007; Gifford et al. 2006). Specifically, the data used to draw edges comes from (i) information about metabolic reactions and pathways from the KEGG and AraCyc databases; (ii) known DNA:protein regulatory interactions from the Transfac and AGRIS databases; (iii) predicted protein:protein interactions based on homology to experimentally verified protein:protein interactions in *Saccharomyces cerevisiae*, *Drosophila melanogaster*, and *Caenorhabditis elegans* using the "Interolog" approach (Gutiérrez et al. 2007); (iv) predicted relationships between microRNAs and their targets (provided by Pam Green and Blake Meyers, Delaware Biotechnology Institute, University of Delaware, USA); and (v) known interactions between genes or proteins gleaned from published literature using the text-mining tool GeneWays (A. Rhetzsky, University of Chicago). The Cytoscape software is used to visualize the multinetwork in an interactive way (Shannon et al. 2003); (vi) the multinetwork can then be queried to find regulatory subnetworks and interactions between a subset of genes.

[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 L. Taiz and E. Zeiger. Sinauer Associates, Inc., Publishers, Sunderland, Mass. (see also http://www.virtualplant.org and Gutiérrez et al. 2007).]

However, laboratory experiments with relatively simple, defined model systems can demonstrate at the metabolic level the activity of certain key enzymatic processes whose genetic controls may be unknown. (Such experiments have been used to study marine organisms, including cyanobacteria, diatoms, and other eukaryotes along with certain classes of heterotrophs.) These observed metabolic pathways, if not apparent from initial genomic analyses, can be mapped in reverse to determine the gene sequence directing them. Reverse mapping thus identifies a subgenome containing a piece of critical biogeochemical information. List comparisons between modelers and experimentalists can accelerate this process by pinpointing important pathways whose genetic bases can be determined by quick laboratory and field studies.

The entire progression of data processing—from genome sequences to biogeochemical function—may be viewed as a unified (or potentially unifiable) information-sciences challenge. Many of the individual steps spanning this progression already are automated. For example, genome sequencing (molecular-level data) has driven development of databases that now feature modular ecosystem (global-scale) information. In the near future, research must attempt to automate intermediate data collection, including information on a system's full complement of RNA transcripts (transcriptome), expressed proteins (proteome), and metabolites (metabolome). Useful to automation efforts is viewing the genome and transcriptome as vectors of the most fundamental biogeochemical data, the proteome as an amino acid matrix, and the metabolome as a multidimensional space containing stoichiometries and process rates. Integrating model assembly to higher scales then becomes a matter of mathematically manipulating the resulting datasets from each of these stages of biogeochemical function. Data may be configured in a relational manner. Standard matrix algebra is then applied to yield biogeochemical source-sink relationships. In fact, data arrays and their mathematical relationships constitute the most concise possible theoretical representation of global biotic systems.

Knowledge Integration and Synthesis with Biogeochemical Models

Accurately quantifying contemporary terrestrial carbon sinks and projecting their future stability require continuous improvement of models via integration and synthesis of various datasets and greater understanding of key mechanisms.

Scientists have developed various terrestrial biogeochemical models that simulate ecosystem carbon processes (e.g., Parton et al. 1987; Luo and Reynolds 1999; Cramer et al. 2001; McGuire et al. 2001). These models generally incorporate current understanding of ecosystem activity and use carbon-process data for parameterization and validation. In fact, qualitative knowledge of major carbon-transfer processes within ecosystems is fairly well developed. For example, as discussed in Chapter 3, Carbon Flows in Ecosystems—Ecosystem Processes, p. 27, scientists have established that (1) a portion of photosynthetically fixed carbon is used for plant growth, and some is released via plant respiration; (2) plants store carbon in live structures for periods ranging from several months to hundreds of years; and (3) dead plant materials (i.e., litter) are partially incorporated into soil organic matter (SOM), which can sequester carbon in soil for centuries and longer before it is broken down into CO_2. Knowledge of carbon-transfer processes has been incorporated into a common structure shared by most biogeochemical

models. This modeling structure partitions photosynthetically fixed carbon into several pools (Rastetter et al. 1997; Luo et al. 2001), with transfers among pools controlled by the carbon-donor pool (Luo and Reynolds 1999).

Critical for effective climate–carbon cycle models is robust representation of nitrogen, whose availability strongly regulates carbon biosequestration amid rising atmospheric CO_2 concentrations**.** More and more, carbon cycling models are incorporating nitrogen processes according to stoichiometric relationships observed between carbon and nitrogen in all plant and soil pools. However, little is known about shifts in ecosystem nitrogen availability in response to global change. Alterations in the nutrient's total amount in an ecosystem are related to microbially mediated nitrogen fixation and processes resulting in nitrogen loss (see Fig. 3.5. Nitrogen Cycle, p. 41, in Chapter 3, Carbon Flows in Ecosystems—Ecosystem Processes). Understanding these shifts thus requires more research on nitrogen fixation in natural ecosystems under steady state and in response to elevated CO_2 and other climatic changes and disturbances. Also needed is greater insight into how denitrification, leachage, volatilization, and other nitrogen-loss processes respond to increased atmospheric CO_2 and global change.

Climate warming affects almost all physical, chemical, and biological processes. Experimental studies have identified several key regulatory mechanisms underlying ecosystem responses to warming. Such responses include acclimation of photosynthesis and respiration, shifts in phenology and nutrient dynamics, and ecohydrological regulation (Luo 2007). Most models, however, still are incapable of quantitatively representing how climate change alters basic ecosystem processes.

Carbon allocation and partitioning among plant parts and autotrophic respiration and among different soil pools are not well understood or represented in models. Rising atmospheric CO_2 concentration, climate warming, altered precipitation, and nitrogen deposition likely change trophic cascades from plant to litter to soil organic matter, resulting in shifts in concomitant community structures of plants and microbes. Critical to predicting the implications of such shifts are improved models, particularly Dynamic Global Vegetation Models (DGVM) used to study how plant functional types respond to disturbances and other factors. Improving DGVMs requires enhancing model-response functions that link alterations in community structure to global change factors at different time scales.

Several **requirements** are necessary to enhance carbon cycle modeling capability, including the following.

1. **Model Structure.** Terrestrial carbon cycling models require multiple carbon pools with different accumulation and residence times.
2. **Initial Value Problems.** Models should accurately quantify contemporary carbon sinks; attribute them to different historical causes, such as disturbances and climate change; and relate sink state to age.
3. **Response Functions.** Models should represent ecosystem-response functions—as they relate to major carbon processes—to environmental variables of global change. Key areas include:
    a. Nitrogen fixation, nitrogen loss, and nutrient limitations for plant and heterotrophic processes in response to rising atmospheric CO_2 concentration and climate change.

b. Climate change effects on basic biological, chemical, and physical processes represented in models (e.g., acclimation of photosynthesis and respiration at the enzyme level and shifts in phenology).

c. Alterations in carbon allocation and partitioning, including autotrophic and heterotrophic respiration response to elevated CO_2 and other global changes.

d. Trophic-cascade (plant → litter → SOM) sensitivity to environmental factors.

e. Carbon-nitrogen-phosphorus-water interactions coupled to nitrogen fixation.

f. Climate-induced shifts in plant and microbial community structure. (DGVMs must link these alterations to global change at various time scales).

g. Hydrological controls.

Parameter Values and Their Variability. Better integration of field data into models is needed to improve predictions of terrestrial carbon biosequestration and feedbacks to climate. Field data are used to constrain model parameters, characterize dynamic disequilibrium of carbon cycling, and quantify carbon biosequestration over space and time (from years to centuries). Acquiring these valuable data requires careful experimental design to optimize sampling.

Spatial Patterns of Carbon Sinks. Scientific knowledge and data on carbon cycling and biosequestration largely are derived from research in temperate climates. Broadening our understanding of the global carbon cycle thus requires more information from several understudied areas such as tropical and high-latitude zones.

Data-Assimilation Techniques. Further development is required to improve integration of information with models. Such techniques are new to ecology but are well established within the climate research community. Early data-assimilation papers (e.g., Williams et al. 2004; Braswell et al. 2005) state that for model-data integration to advance, consistent information is vitally needed on long- and short-term processes across biomes, climate zones, and disturbance classes. Furthermore, measurements of long-term ecosystem fluxes of carbon, nutrients, water, and energy are essential to develop, test, and apply carbon cycling models.

Development and Refinement of Spatial-Temporal Carbon Cycling Models across Scales

Critical to climate-mitigation and carbon biosequestration strategies is the ability to conduct predictive modeling. Needed are models that anticipate how a system will react under specific conditions rather than those that simply reproduce results already established through experimentation or observations. Achieving this predictive capability requires equipping models with increasing levels of detail over different space and time scales (see Fig. 2.1. Scales and Processes of the Global Carbon Cycle, p. 16, in Chapter 2, Technical Strategy). However, representing key processes at the necessary scales is a central challenge of global carbon cycle research. Part of the complication arises from the disconnect in information from scientists working at different spatial and temporal scales. For example, environmental scientists can measure ecosystem functions and phenomena but have difficulty relating results to higher and lower scales and in extrapolating behavior outside the range of observations. Understanding and effectively modeling carbon processes thus require data from scientific investigation across all scales. For instance, researchers examining system attributes at lower scales can capture

important details, while those working at higher scales can provide data needed for model parameterization. Moreover, since most climate effects on carbon cycling are manifest at the macroscale, efforts should be made to generate data relatable to higher scales.

Current climate change models rely on geophysical data obtained at widely varying scales. Much of this data is from well-characterized sources with longstanding methods of incorporating such information into climate change models. However, researchers envision a continuous progression of modeling science characterized by increasingly accurate predictions and critical new capabilities to ask and answer "what if" questions concerning climate change. Attaining the desired level of predictability will require models dramatically more detailed and mechanistically based. Advanced climate change models also must span ever-increasing lengths and time scales and draw upon more precise and quantitative data on all ecosystem processes relevant to carbon cycling.

The lengths and time scales of carbon cycling processes represented in future climate change models likely will range from microscopic to aggregate (mm to cm) to field and beyond. In particular, model development that includes carbon processing across scales will generate data yielding fundamental understanding of complex biological systems—from single cells to microbial communities to organisms with multiple cells and tissues to diverse ecosystems with many species. Furthermore, these data also will aid development of parameterized dynamic models capable of quantitatively predicting ecosystem response to climate change and disruptions. Such model development in some cases will require measuring and quantitatively characterizing carbon cycling processes specifically for model parameterization and validation as opposed to meeting needs of general scientific interests. Other development requirements include new methods for coupling parameterized models of system response at various levels of complexity and scales to informatics data derived from ecological observations or high-throughput systems biology studies of cellular processes. In particular, improved model scalability and coupling of mathematically heterogeneous representations are necessary for developing increasingly sophisticated and detailed models that include complex processes contributing to and ultimately governing carbon cycling. Moreover, current climate change models have "hooks" to incorporate parameterized versions using more detailed carbon cycling data, but next-generation models probably will require new methods for submodel parameterization and coupling.

Integration of Different Types of Data into Models

The science of carbon cycling and biosequestration across hierarchical levels from genomics to ecosystems requires integration of measurements and models using systems approaches. First, conceptual frameworks must be developed to guide the integration of theoretical understanding, knowledge of carbon processes, data, and quantitative relationships. Such frameworks also would enable scientists to connect nodes—relationships among genes, proteins, and molecules—at different hierarchical levels and evaluate scalable variables. Thus, development of quantitative models should be based on these conceptual frameworks.

Although existing carbon cycle models can connect information from leaf-level photosynthesis to global flows of carbon, advanced models are needed to link

knowledge, data, theory, and quantitative relationships from genomic studies to subcellular and cellular processes and eventually to those occurring at organismal and ecosystem scales. Such model enhancement can be aided by recently developed data-assimilation techniques integrating observational data into ecological models with rigorous statistical and mathematical approaches. Data assimilation is a valuable tool to improve model parameterization, choose between alternative model structures, design better sensor networks and experiments for data collection, and analyze uncertainty of model predictions. The ecology research community recently explored, examined, and developed various data-assimilation techniques (e.g., inverse analysis, hierarchical Bayesian analysis, model-selection approaches, and state-space modeling) to analyze multiscale ecological data in space and time.

Uncertainty in Model Projections

Although carbon cycling models have been used extensively to predict carbon biosequestration in terrestrial ecosystems, uncertainty associated with model parameters and predictions has not been analyzed carefully. If such uncertainty is inadequately assessed, carbon sink potentials cannot be understood fully. In fact, some carbon sinks may be underestimated, while others overestimated, even to the extent resulting in contradictory source-sink designations. In such situations, policies to stabilize CO_2 concentrations based on current understanding will fall short in meeting environmental-mitigation targets.

Considering the importance of uncertainty analysis to policymaking, the research community investigating global climate change recently directed considerable attention to studying the stochasticity and uncertainty in ecosystem processes and how various sources of randomness affect prediction of ecosystem changes (Murphy et al. 2004; Dose and Menzel 2004; Forest et al. 2002; Wang et al. 2001). Expert-specified probability density function [(PDF) e.g., Murphy et al. 2004] has been used to quantify key uncertain properties of climate change simulations. Researchers have introduced the Bayesian paradigm to incorporate *a priori* PDFs with measurements to generate *a posteriori* PDFs for parameters of ecosystem models (Braswell et al. 2005; Knorr and Kattge 2005). With a probabilistic approach, Mastrandrea and Schneider (2004) presented a cumulative probability function (CDF) to assess dangerous anthropogenic interference and showed CDF utility by applying it to analysis of uncertainty in model predictions of future changes. On a global scale, the Bayesian approach has been applied to constrain parameters in biosphere models against atmospheric CO_2 concentration data and to assess biosphere carbon fluxes and uncertainties (Kaminski et al. 2002; Rayner et al. 2005). A probabilistic inversion within a Bayesian framework was conducted by Xu et al. (2006), who used six datasets and a terrestrial ecosystem model to evaluate uncertainty in parameter estimation and projected carbon sinks. In this analysis, measurements were treated as random variables with certain probability distributions. A joint PDF was constructed for model parameters to analyze information within observed datasets. Samples were taken from the joint PDF using a Markov chain Monte Carlo technique appropriate for sampling high-dimensional PDFs of model parameters and widely used in inverse problems in engineering and geosciences (e.g., Dosso and Wilmut 2002; Oh and Kwon 2001; Geman and Geman 1984). The samples were used to construct marginal distributions for model parameters, calculate parameter correlations, and make CDFs for simulated pool sizes in forward modeling.

Regional and Geographic Issues

Both on land and in the ocean, certain regional-scale ecosystems require special treatment in Earth System Models because they are either sensitive to climate change or poorly understood. Terrestrial examples include tundra—which already may be transitioning to shrub lands—and tropical and boreal forests. In the ocean, distinctive areas include (1) coastlines subject to rapid nutrient recycling from river inputs and proximate, underlying sediment; (2) the continental shelf, which is considerably more complex than the pelagic zone and can be defined in such a way that it influences a large fraction of planetary geocycling surface area; and (3) the poles, further distinguishable by Arctic and Antarctic regions. The former consists of an enclosed peripheral sea surrounded by landmasses while the latter consists of the opposite. Although climate change will affect polar biota hardest and fastest, Arctic and Antarctic organisms may react very differently to induced stresses.

In some cases, critical geochemical processes occur at the intersection of special terrestrial and oceanic ecosystems. Methane clathrates, for example, form preferentially on continental shelves below the coldest and most productive waters. These clathrates harbor carbon stocks comparable to those of known global coal reserves. If even a tiny fraction of the clathrates is destabilized, the implications for further climate change would be huge and carbon biosequestration efforts overwhelmed.

Visualization Tools

Various visualization tools are excellent catalysts for discovery and communication across disciplines and at multiple scales. Moreover, applying systems biology approaches to carbon cycling and biosequestration research will require development of these and other tools and methods for integrating disparate data types across different scales. In general, new tools are needed for informatics, imaging, math, and statistics to enable dynamic modeling and visualization of processes ranging from molecular networks in cells to populations in ecosystems. Integrating these tools in a common platform is a key goal that will facilitate a better understanding of how internal and external perturbations affect processes, pathways, and networks controlling organism growth and development and how these disruptions impact ecosystem "nodes."

The visualization aspect of research often is underappreciated but can catalyze cutting-edge research. Furthermore, visualization tools and approaches can greatly enhance communication and information sharing across scientific disciplines. In particular, the genomics–to–cell–to–global ecology concepts of interest to the carbon cycling research community hold rich potential for detailed, color-enhanced visual representations. Multilevel biological networks, three-dimensional biochemistry, and large-scale biogeography can be displayed and animated simultaneously. For ocean studies, the interaction of turbulent fluid flow with ecodynamics can be simulated and graphically displayed along time coordinates. Advances in visualization technologies have made associated tools extremely useful and, in some cases, critical to research. As scientists reach milestones in network and geochemistry mapping, workshops should be held for collective examination of these advances. Detailed, mobile data fields associated with such examinations tend to stimulate new directions of analysis and cross-disciplinary discussion.

Crosscutting Issues, Measurement Methods, and Strategies

State-of-the-Art Instrumentation and Methods

Imaging and Microspectroscopy

A major crosscutting technology need identified at the DOE Carbon Cycling and Biosequestration Workshop is development of advanced imaging and microspectroscopy tools. In particular, new imaging technologies are required to analyze at appropriate scales key ecosystem components, processes, and properties, including macromolecular complexes, microbes, plant-root cells, and soil microaggregates. For ocean systems, there are parallel needs to characterize phytoplankton and marine snow as it forms and decomposes. These analyses demand new and sensitive approaches (e.g., sensors, probes, and stable isotopes) for measuring and monitoring biological activities and physical and chemical processes in situ. Such approaches could give valuable insight into extracellular enzymes and their activities, which are particularly important in organic carbon processing in both soil and marine environments. For example, many of the polymers produced by phototrophs are too large to be transported into microbial cells and require depolymerization by extracellular enzymes. Relatively little is known, however, about the nature of these proteins and their in situ catalytic activities, requiring further study aided by new imaging tools. Technologies also are needed for measuring at high resolution macro- and micronutrient concentrations and chemical forms in situ. Such information is critical for providing the context for biological properties and processes and assessing how they influence carbon cycling. This type of environmental characterization data is critical for determining how organisms respond to changes in their surroundings and, when coupled with genomic and other omic data, is particularly effective for understanding these responses. Similarly, more insight also is needed into how organism responses alter the environment. Numerous in situ observing systems are beginning to provide long-term data on how organisms' environments respond to such shifts. Fully exploiting this detailed information requires improvements in measurement technologies, such as automated soil moisture profiling, precipitation sensors, and tools to assess soil enzyme activities.

Understanding the fundamental mechanisms that control the biogeochemical cycling of carbon requires analyzing the physical and chemical micro- and macroenvironments at soil-water-microbe-plant-fungi interfaces. The physical and chemical microenviroments at these interfaces potentially are some of the more critical controls on biogeochemical cycling of elements, yet characterizing them is difficult. Overcoming this challenge will require high-throughput imaging and chemical and structural analysis of bacteria, roots, and soil aggregates coupled with investigations of microbial communities and metabolic expression and activity.

To accomplish the desired level of analysis, new facilities and high-throughput instrumentation—used in parallel with omic approaches—should be developed for physical and chemical characterization of environmental systems at many scales. These facilities and techniques must be available to the entire scientific community. Some will require additional technical experts who must be knowledgeable about carbon cycling and carbon biosequestration. Furthermore, an integrated use of standard and exotic techniques should be employed often to enable new insights.

Key Research Question

1. **How can we assess uncertainty in model estimates of ecosystem carbon sinks and improve confidence in these projections?**

In addition to studies of natural materials, integrated approaches must be used to investigate defined yet representative systems in the laboratory. Some technologies important or potentially important to understanding carbon cycling and biosequestration include (1) microelectrodes; (2) focused ion beam; (3) secondary ion mass spectrometry (nano-SIMS); (4) time-of-flight (TOF) SIMS; (5) nuclear magnetic resonance (NMR); (6) synchrotron-based approaches; (7) electron microscopies (e.g., transmission electron microscopes and conventional and environmental scanning electron microscopes); (8) atomic force microscopies; and (9) gas chromatography, liquid chromatography, and mass-spectrometry approaches.

Synchrotron-Based Approaches

Some synchrotron-based instrumentation already is available and being used to measure the chemical and physical characteristics of biological and environmental samples relevant to carbon cycling and biosequestration. These techniques include (1) protein crystallography (for determining protein structure); (2) small-angle X-ray scattering (for measuring the size distribution of solution-phase submicron particles); (3) X-ray tomography (for three-dimensional characterization of soil porosity and connectivity and for measuring organic carbon, water, and mineral distributions within soil microaggregates, aggregates, and microcosms); (4) hard and soft X-ray fluorescence and transmission microscopies [for providing suboptical spatial resolution information, such as size and chemical speciation of organic matter, micronutrients, and macronutrients (see, for example, Fig. 3.7a–b. Distribution of Micronutrients in Plant Roots and Associated Fungal Hyphae, p. 45, in Chapter 3, Carbon Flows in Ecosystems—Ecosystem Processes)]; (5) soft X-ray spectroscopy (for chemical speciation analysis of inorganic and organic carbon); and (6) hard X-ray spectroscopy (for chemical speciation analysis of macro- and micronutrients and identifying the valence state of redox-active elements). Although powerful, many of these techniques need to be made more readily available to novice users of synchrotron radiation. Several of the most useful and effective techniques still are "tour de force" measurements, underscoring the need for increased availability of these tools and approaches. Similarly, because researchers must characterize numerous biological and environmental samples to obtain statistically significant results, further development of present synchrotron-based techniques is greatly needed to enable standardized, user-friendly, and high-throughput measurements.

Isotope Techniques

Isotope-based technologies are particularly promising for measuring carbon cycling processes and linking such processes to the organisms and metabolic pathways that catalyze them. Isotope ratios of organic matter and CO_2 also provide powerful tools for understanding and tracing carbon flux and storage, from cellular to global scales. Stable isotopes of a given element differ in the number of neutrons they contain. For example, about 99% of carbon on Earth is ^{12}C, which has 6 protons and 6 neutrons ($6 + 6 = 12$). However, the other 1% is ^{13}C, which contains 7 neutrons. Both are stable isotopes, meaning they do not decay. Stable carbon and oxygen isotope ratios (defined as $^{13}C{:}^{12}C$ and $^{18}O{:}^{16}O$, respectively, and represented as $\delta^{13}C$ and $\delta^{18}O$) have been used successfully for cellular forensics and tracing carbon flow at cellular, tissue, organismal, ecosystem, regional, and global scales.

Also valuable for carbon studies is the radioisotope ^{14}C, which can be used to quantify the residence time and age of carbon in organic matter. Known as radiocarbon, ^{14}C—with its radiodecay and use as an isotope tracer—also can provide information on the time scales of carbon exchange with the atmosphere. Relatively recent technological developments have dramatically improved the usefulness and cost-efficiency of isotopic analyses, making previously inconceivable experiments now possible. As such, a significant opportunity exists to use isotopic techniques to understand the carbon cycle at cellular to global scales and in frameworks relevant to various environments and biological processes.

Isotopic analyses are valuable to multiscale studies of carbon biosequestration and the carbon cycle because stable isotopes integrate—over a temporal period—significant processes leading to biomass or CO_2 formation. Isotopes indicate key mechanisms resulting in carbon storage and fluxes because these processes often fractionate, or change the isotopic ratio, between source and product. Furthermore, isotopes record these same processes in organic, inorganic, or gaseous forms, such as in cell walls and tree rings. Stable isotopes also can be used to trace origins of carbon fluxes and pools. These powerful, multifaceted uses of isotopes make them a critical tool for future carbon cycling and biosequestration research (West et al. 2006; Dawson and Siegwolf 2007).

The $\delta^{13}C$ in organic matter is driven in part by isotopic fractionation that occurs during photosynthesis, which strongly depends on water stress, biochemical CO_2-uptake capacity, and type of carbon-fixation pathway used [e.g., C_3 or C_4 (Ehleringer et al. 1993)]. The isotopic signature of carbohydrate resulting from photosynthesis is used for organic-matter production and metabolic respiration for all autotrophic and heterotophic organisms within an ecosystem, thus providing both an organic record and a CO_2 tracer of photosynthetic processes at large scales.

The $\delta^{18}O$ of organic matter and ecosystem-respired CO_2 is driven largely by the $\delta^{18}O$ of water in the major water pools of ecosystems, the canopy, and soil. The $\delta^{18}O$ in this ecosystem water is in turn controlled by the regionally unique signature of incoming precipitation and is modified primarily by evaporative enrichment during dry periods. As such, evolved $\delta^{18}O$ in CO_2 carries a tracer of the ecosystem of origin and of drought. Furthermore, oceanic exchange of CO_2 with the atmosphere has only very small isotopic fractionations, thus atmospheric CO_2 contains a signature of terrestrial carbon cycle processes. Models of terrestrial carbon flows therefore can be uniquely constrained by measurements either of atmospheric CO_2 or of that respired from ecosystems, as has been done over the past few decades using flask-sampling techniques.

Radiocarbon produced cosmogenically is a valuable time-dependent tracer of carbon cycle processes and carbon biosequestration on time scales of centuries and beyond because of its half-life of 5730 years. Since 1964, however, radiocarbon injected into the atmosphere by aboveground nuclear testing has provided a global isotope tracer for the carbon cycle. In the past two decades, development of accelerator mass spectrometry, which measures ^{14}C atoms individually rather than waiting for them to decay, has increased sample throughput and decreased sample size dramatically.

Isotopes provide the means—unavailable via other methods—to understand the carbon cycle at scales ranging from the genome to globe. Observational, manipu-

lative, and pulse-label experimental approaches using isotopic techniques will facilitate achieving the following advances.

- Determining how different carbon substrates contribute to biomolecule synthesis versus metabolic respiration (e.g., microbial efficiency).
- Tracing plant allocation and, in particular, relating changes in it to shifts in gene expression [e.g., using ^{11}C, ^{13}C, or ^{14}C to do so (Schwachtje et al. 2006; Carbone et al. 2007)].
- Using new technology to differentiate between autotrophic and heterotrophic respiration in soils (Trumbore 2006).
- Tracing carbon flow from plants to soils for a range of time scales [e.g., using ^{13}C pulse label for days to weeks (Bowling et al. 2002) or ^{14}C pulse label for years to decades]. For example, researchers could measure tracer concentrations in biomarkers to ascertain the timing of photosynthetic-product transfer to a microbial community.
- Using new tools such as nano-SIMS to allow isotope-tracer mapping at subcellular levels.
- Using natural-abundance ^{14}C to identify components of soil carbon that represent long-term stores and determining how this residence time responds to management strategies or climate change.
- Testing and constraining models of carbon cycling within microbial communities, soils, foliage, ecosystems, and ultimately the globe (Barbour et al. 2007; McDowell et al. 2008).
- Improving the deconvolution of global records of CO_2, $\delta^{13}C$, $\delta^{18}O$, and ^{14}C to assess the importance of particular regions as net carbon sources and sinks.

Although the value of isotopic measurements is widely accepted, such techniques historically have been time consuming and expensive, greatly limiting researchers' ability to capitalize on these powerful tools. Recent and continuing advances, however, are yielding breakthroughs in measurement frequency and cost, allowing previously unfeasible integration of isotopic measurements into field and laboratory experiments. For example, laser-based measurements of $\delta^{13}C$ and $\delta^{18}O$ in CO_2 have increased sampling frequency significantly—from once a week using field sampling and tedious laboratory analyses to once every couple of minutes using measurements taken directly in the field (Bowling et al. 2005; Griffis 2005; Barbour et al. 2007; McDowell et al. 2008). Furthermore, sampling improvements now allow measurements at the sub-Hertz time scale for ecosystem foliage and atmospheric fluxes. Proven applications of laser-based isotopic measurements include (1) assessing mesophyll conductance (Flexas et al. 2006), (2) testing photosynthetic models (Bickford et al., in review), (3) observing for the first time the $\delta^{13}C$ and $\delta^{18}O$ signature of light-enhanced dark respiration (Barbour et al. 2007), (4) examining the transient response of soil-respired $\delta^{13}C$ to precipitation (Powers et al., in review), (5) measuring ecosystem-scale partitioning of photosynthesis and respiration, (6) investigating ecosystem carbon cycle dependency on climate (Bowling et al. 2005), and (7) testing ecosystem carbon cycle models (McDowell et al. 2008).

Potential future applications of stable-isotope techniques are vast, offering tremendous opportunities for scientific creativity. Such laser-based systems are appropriate for cellular- to regional-scale monitoring, pulse-labeling experiments, and long-term observations. Moreover, within only the past year, new isotopic systems have emerged that are more portable and consume less energy and other resources, making these techniques further amenable to long-term field monitoring at remote locations. Additionally, accelerator mass spectrometry for ^{14}C analyses is becoming more economical, dramatically increasing the number of analyses that can be conducted to trace carbon flow and residence time in ecological systems. Smaller and easier-to-maintain accelerator mass spectrometers are being produced, including, for example, the new system at the University of California-Irvine, which is the first ^{14}C accelerator available exclusively for studying the carbon cycle. The significant increase in analytical capacity has accelerated carbon cycle research science and demonstrated that such facilities can be operated at lower per-sample costs. However, many facilities already have reached their analytical capacity because of existing needs (Trumbore 2006). Thus, extending the availability of such instrumentation is a critical requirement for better understanding the carbon cycle and devising strategies to enhance carbon biosequestration.

Organic-Matter Biogeochemistry and Analytics

A critical crosscutting need in carbon cycling and biosequestration science is characterization of natural organic matter, including its biochemical processing, changes in structure and physical biochemistry, and interactions with nonbiotic environmental factors. New, robust analytical and characterization methods are needed to measure compositional changes in organic material during its transport and degradation in both terrestrial and ocean environments. Challenges associated with such measurements in many ways are parallel to problems faced by scientists investigating biomass synthesis and deconstruction.

Previous characterization of organic matter in soils and oceans has been based largely on operational definitions, certain size fractions, and extraction with specific chemicals. Despite significant improvements in analytical methods for studying organic molecules (e.g., mass spectrometry, NMR, and synchrotron-based X-ray spectroscopy), few advanced technologies have been directed toward characterization of natural organic matter. Consequently, relatively little is known about the chemical composition and biotic and abiotic reactions involved in the biochemical degradation or alteration of organic matter. With their extensive infrastructure and capabilities in these innovative technologies (e.g., light sources and the Environmental Molecular Sciences Laboratory), DOE national laboratories and associated user facilities have the potential to fill in significant gaps in this knowledge.

A predictive understanding of the global carbon cycle requires linking theory, observations, experiments, and models because no single approach is sufficient. As noted, observations and experiments are profoundly valuable for informing researchers on how the carbon cycle works. However, implementation and interaction of empirical datasets from such studies must be done in conjunction with models so results can be integrated into a framework capable of forecasting future climate impacts on the carbon cycle.

Transport Between Reservoirs and Phases

Global climate change research communities have tended to focus their thinking and efforts within conceptual or geographical reservoirs distributed through the geochemosphere. Examples include land plants as a geochemical pool and processing unit; the soil ecosystem supporting them; the atmosphere as a medium of material transport from land-based continental systems to the ocean; and within the sea, the euphotic and twilight zones as well as central oceanic layers. The means of carbon transport among these subunits within the global system may determine rates of flow and introduce critical roadblocks. For example, the transition from litter to soil organic material is mediated in part by microfauna and links additionally to the hydrological cycle. Long-range transport of dust from terrestrial sources such as deserts carries iron to the remote ocean, but this nutrient becomes bioavailable only under appropriate hydrometeoric pH and photochemical conditions. Aerosol and cloud acidity in turn are determined by the flux of reduced sulfur and nitrogen from the ocean surface. Air-pollution sources also constitute critical modulators of such acidity in and of themselves. Furthermore, Asian economic growth is expected to trigger increases in acidity over the North Pacific even as North America and Europe cease pollution of the Atlantic atmosphere. The consequences of such increases remain underexplored but could result in significant feedbacks to carbon biosequestration strategies and climate change.

Carbon Cycling and Biosequestration Workshop Participants and Agendas

March 2008

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DOE Carbon Cycling and Biosequestration Workshop

Agenda for March 4, 5, and (6), 2008

Hilton Executive Meeting Center and Hotel, Rockville, Maryland

Tuesday, March 4

8:00 a.m.	Welcome, Program Goals: Jerry Elwood
8:30 a.m.	Workshop Objective, Agenda, and Output: Sharlene Weatherwax, Joe Graber, Jeff Amthor, Roger Dahlman, Mike Knotek, Betty Mansfield
9:00 a.m.	Plenary Presentations:
	Jae Edmonds, Pacific Northwest National Laboratory "Biotechnology, Energy, and a Climate-Constrained World"
	Scott Denning, Colorado State University "Global Biogeochemical Cycles and the Climate of the 21st Century"
10:30 a.m.	Break
10:45 a.m.	Introduction of Working Groups' Scopes, Discussion Points:
	Working Group 1: Terrestrial Plant Productivity and Carbon Biosequestration Cochairs: Dan Bush and Stan Wullschleger
	Working Group 2: Biological Cycling of Carbon in Terrestrial Environments Cochairs: Mary Firestone and Don Zak
	Working Group 3: Biological Cycling of Carbon in Ocean Environments Cochair: Scott Elliott (Other Cochair, Ginger Armbrust, during March 17–18 workshop in Denver)
	Working Group 4: Effects of Climate Change on Carbon Cycling and Biosequestration Cochairs: Jim Ehleringer and Rich Norby
	Working Group 5: Crosscutting Science, Technology, and Infrastructure Cochairs: Jim Fredrickson and Scott Elliott
12:00 noon	Box Lunch Meeting with Assigned Working Groups
3:00 p.m.	Group Break
3:30 p.m.	Working Group Sessions Resume, Continue Discussion
6:00 p.m.	Group Dinner
7:00 p.m.	Working Group 5: Crosscutting Meeting; Working Groups 1, 2, and 4 Continue Independent Work

Wednesday, March 5

8:00 a.m.	Group Writing and Discussions of Basic Research Needs Plans and Transformational Challenges
12:00 noon	Working Lunch
1:00 p.m.	Plenary Outbriefs and Discussion
3:00 p.m.	Working Groups Reconvene; Final Discussions
4:00 p.m.	Main Meeting Adjourns

Thursday, March 6

8:00 a.m.	Working Group 5 and Cochairs and Writers for Working Groups 1, 2, and 4 Continue Discussions, Writing
12:00 noon	Workshop Adjourns

Detailed March 4–6 Agenda: General Process Flow for Working Group Sessions

Day 1, Tuesday, March 4		
Time Slot	**Activity**	**Product**
12:30 – 2:30	Frame topical area: Presentations and discussions	Consensus systems definition of problem area: System diagram
2:30 – 4:00	Create prospective list of Basic Research Need Plans (BRNPs)	Prospective list of items End-to-end systems analysis: Definition of critical-path biology items
4:00 – 6:00	Cull list and identify research requirements	Consensus list and completed BRNP forms ~5:00 p.m.: Each session provides topic list (electronic) for printing and intergroup comparison over dinner.
6:00 – 7:30: All groups together for dinner	Discuss BRNPs that transcend groups	Assignments of transcending BRNPs to specific groups
Evening	Continue defining research requirements and outlining thoughts for write-ups Crosscutting Working Group meets for discussions	Consensus items and research areas with prioritization Beginning definition of crosscutting portfolio
Day 2, Wednesday, March 5		
8:00 – 12:00	Group writing of BRNPs Group chairs audit process, discuss items as needed	Draft and refine BRNPs
12:00 – 1:00	Lunch	All files (including presentations) provided electronically to ORISE staff for printing
1:00 – 3:00	Plenary outbriefs and discussions	Presentations and comments
3:00 – 4:00	Working group sessions reconvene Final group discussions	Final modifications
4:00: Main workshop adjourns Working Group 5 and cochairs and writers from Working Groups 1, 2, and 4 continue working		Copies of all draft files, organized by group, provided by ORISE staff to participants. Electronic files can be loaded on thumb drives.
3:00 – 6:00: Remaining working group participants meet	Working Group 5: Discuss and coalesce common crosscutting capabilities, confirm with other groups as needed, assess gaps, and make writing assignments Working Groups 1, 2, and 4: Identify gaps, make writing assignments	Consensus output of targets; refined outlines
6:00 – 7:00: Dinner	Cochairs dine together	Identification of issues and problems
Evening	Work and discuss as appropriate; individual work time	Refined work products
Day 3, Thursday, March 6		
8:00 – 11:00	Working groups continue writing	Second drafts for comparison
11:00 – 12:00	Groups convene in plenary session for ad hoc presentations for intercomparison and review of output.	Strategy adjustments; new assignments
12:00	Workshop Adjourns	Electronic and hard-copy files shared among groups, with ORISE assistance Agreement as to additional items needed, follow-up process, and assignment schedules Final files emailed to all participants and available on website

DOE Carbon Cycling and Biosequestration Workshop: Biological Cycling of Carbon in Ocean Environments

Agenda for March 17–18, 2008

Courtyard Marriott Denver Airport, Denver, Colorado

Monday, March 17

8:00 a.m.	Welcome, Program Goals, Workshop Objectives: Joe Graber, Dan Drell, Mike Knotek, and Betty Mansfield
8:30 a.m	Summary of Previous Carbon Cycling and Biosequestration Workshop Sessions: Scott Elliott
9:00 a.m	Carbon Cycling in Ocean
12:00 noon	Lunch
1:00 p.m.	Reconvene; Continue Discussion
3:00 p.m.	Group Break
3:30 p.m.	Reconvene; Continue Discussion
6:00 p.m.	Main Workshop Adjourns
6:00 p.m.	Dinner for Cochairs, Writers, and DOE Staff

Tuesday, March 18

8:00 a.m.	Writing Group Convenes
12:00 noon	Workshop Adjourns

Detailed March 17–18 Agenda: General Process Flow

Day 1, Monday, March 17		
Time Slot	**Activity**	**Product**
9:00 – 12:00	Frame topical area: Discussion	Consensus definition of topical areas: System diagram
1:00 – 3:00	Create prospective list of Basic Research Need Plans (BRNPs)	Prospective list of items Definition of critical-path biology items
3:30 – 6:00	Group writing of BRNPs: Cull list and identify research requirements	Consensus list and completed BRNP forms
6:00 – 7:30: Dinner	Discuss writing assignment	Writing Assignments
Evening	Cochairs, writers work independently	Consolidation and prioritization of BRNPs (first drafts)
Day 2, Tuesday, March 18		
8:00 – 12:00	Group Writing: Compare first drafts, discuss, and adjust strategy as necessary	Consolidation of group output Development of additional materials
12:00	Workshop adjourns	Shared electronic and hard-copy files Agreement as to additional items needed; follow-up process, and assignment schedule Final files distributed to all participants via website

Glossary

adsorption: Accumulation of molecules or cells on the surface of a substance.

aerobic: Requiring oxygen.

aerosols: Airborne solid or liquid particles (typically no larger than a few micrometers) that can remain in the atmosphere for hours to days. Aerosols impact climate by scattering or absorbing radiation, initiating cloud formation, or altering the optical properties of clouds.

albedo: Proportion of light or radiation reflected by a surface.

alfisols: Fertile soils in temperate forests with an underlying clay horizon.

algae: Photosynthetic, aquatic, eukaryotic organisms that contain chlorophyll but lack terrestrial plant structures (e.g., roots, stems, and leaves). Algae can exist in many sizes ranging from single cells to giant kelps several feet long.

algorithm: Formal set of instructions that tells a computer how to solve a problem or execute a task. A computer program typically consists of several algorithms.

anaerobic: Lacking or not requiring oxygen.

andisols: Volcanic soils containing ash and volcanic glass.

anthropogenic: Resulting from human activity.

archaea: Single-celled prokaryotic microbes that are structurally and metabolically similar to bacteria but share some features of their molecular biology with eukaryotes.

aridisols: Dry desert soils with a prominent clay horizon.

atomic force microscopy: Technique that uses a mechanical probe to characterize and magnify surface features with atomic detail.

ATP (adenosine triphosphate): A multifunctional nucleotide responsible for cellular energy transfer and storage.

autotroph: An organism that biochemically synthesizes its own organic materials from inorganic compounds using light or chemical energy.

bacterioplankton: Bacteria that inhabit marine and freshwater environments.

Bayesian approach: Use of statistical methods that assign probabilities or distributions to future events based on knowledge of prior events.

biochar: Biomass-derived black carbon.

biogeochemical model: A type of ecosystem model used to represent biologically mediated transformations and flows of carbon and other materials within an environment.

biogeochemistry: Study of how interactions among biological and geochemical processes influence the global cycling of such essential elements as carbon, nitrogen, phosphorus, and sulfur.

biogeographical model: A type of ecosystem model used to determine how populations in a particular region change over long time scales.

bioinformatics: Science of managing and analyzing biological data using advanced computing techniques.

biological pump: Collection of biological ocean processes that regulate the uptake, storage, transformation, and release of carbon.

biome: A terrestrial region (e.g., grasslands, tropical forests) characterized by dominant vegetation and climate characteristics in terrestrial ecosystems. In aquatic environments, a biome is defined by a particular range of depths and biogeochemical properties.

biopolymer: A large biological molecule formed by the linking together of smaller subunit molecules.

biosequestration: Biologically mediated uptake and conversion of carbon dioxide to inert, long-lived, carbon-containing materials.

biosphere: All living organisms.

bole: Stem or trunk of a tree.

C_3 plant: Plants (e.g., soybean, wheat, and cotton) whose carbon-fixation products have three carbon atoms per molecule. Compared with C_4 plants, C_3 plants show a greater increase in photosynthesis with a doubling of CO_2 concentration and less decrease in stomatal conductance, which results in an increase in leaf-level water use efficiency.

C_4 plant: Plants (e.g., maize and sorghum) whose carbon fixation products have four carbon atoms per molecule. Compared with C_3 plants, C_4 plants show little photosynthetic response to increased CO_2 concentrations above 340 ppmv but show a decrease in stomatal conductance, which results in an increase in photosynthetic water use efficiency.

CO_2 fertilization: Increase in plant growth due to a higher-than-normal carbon dioxide concentration in the environment.

Calvin cycle: A series of photosynthetic chemical reactions that do not require light to occur. The Calvin cycle uses energy produced by light-dependent reactions of photosynthesis to incorporate carbon from carbon dioxide into organic compounds used to make sugars, starches, and other biological molecules.

carbon allocation: See *carbon partitioning.*

carbon cycle: The complex carbon flows and transformations among major Earth system components (atmosphere, oceans, and terrestrial systems). The global flow of carbon from one reservoir (carbon sink) to another. Each carbon exchange among reservoirs is mediated by a variety of physical, biogeochemical, and human activities.

carbon dioxide: Gas that is an important part of the global carbon cycle. CO_2 is emitted from a variety of processes (e.g., cellular respiration, biomass decomposition, fossil fuel use) and taken up primarily by the photosynthesis of plants and microorganisms. CO_2 is a greenhouse gas that absorbs infrared radiation and traps heat in the Earth's atmosphere.

carbon fixation: Conversion of inorganic carbon dioxide to organic compounds by photosynthesis.

carbon flux: Rate of carbon movement as it flows from one carbon reservoir to another in the global carbon cycle. For the global carbon budget, carbon flux is usually expressed in gigatons of carbon per year (GT C/yr).

carbon partitioning: Partitioning to different parts of a plant (e.g., leaf, stem, root, and seed) versus carbon allocation (partitioning between biomass and respiration).

carbon sequestration: Biological or physical process that captures carbon dioxide and converts it into inert, long-lived, carbon-containing materials.

carbon sink: A pool (reservoir) that absorbs or takes up released carbon from another part of the carbon cycle. For example, if the net exchange between the biosphere and the atmosphere is toward the atmosphere, the biosphere is the source, and the atmosphere is the sink.

carbon source: A pool (reservoir) that releases carbon to another part of the carbon cycle.

carbon use efficiency (CUE): Ratio of net primary productivity to gross primary productivity.

chemoautotroph: An organism that biochemically synthesizes its own organic materials from inorganic compounds using chemical energy.

chemostat: Apparatus for the continuous cultivation of bacteria. Chemostats keep bacterial cultures in an optimal growth state by continually adding media and removing old cells.

chlorophyll: A type of green pigment used to harness light energy in the chloroplasts of plants and other photosynthetic organisms.

chloroplast: An organelle in the cells of green plants. It contains chlorophyll and functions in photosynthesis and protein synthesis.

climate: Average weather conditions over a time period, usually several decades. Climate is largely determined by local geographical features, latitude, altitude, land- and sea-masses, and atmospheric circulation patterns.

climate model: Mathematical model used to understand, simulate, and predict climate trends by quantitatively analyzing interactions among Earth system components (e.g., land, ocean, atmosphere, and biosphere).

coccolithophore: A type of single-celled marine algae distinguished by its production of intricate, microscopic shells that are aggregates of calcium carbonate discs called coccoliths.

cofactor: An organic or inorganic substance required by an enzyme to function.

community: All the different species of organisms living together and interacting in a particular environment.

copepod: A type of microscopic marine and freshwater crustacean that has an elongated body and a forked tail.

crenarchaea: A phylum of archaea distinguished from other phyla based on rRNA sequence. Crenarchaea are the most abundant type of marine archaea.

cyanobacteria: Division of photosynthetic bacteria found in many environments, including oceans, fresh water, and soils. Cyanobacteria contain chlorophyll a and other photosynthetic pigments in an intracellular system of membranes called thylakoids. Many cyanobacterial species also are capable of nitrogen fixation.

cytoplasm: All cellular contents surrounding the nucleus of a membrane-bound eukaryotic cell.

denitrification: Anaerobic conversion of nitrate or nitrite to nitrogen gas (N_2) by denitrifying bacteria. A small portion of nitrate or nitrite may be converted to nitrous oxide (N_2O), a potent greenhouse gas.

desorption: Removal of a substance in the reverse of absorption or adsorbtion.

detritus: Remnants of biological material.

diatom: Type of microscopic, photosynthetic algae known for its intricately designed, silica-containing shell. Thousands of diatom species are known; most are unicellular, but some form colonies. Diatoms are responsible for a large portion of photosynthetic carbon assimilation in marine and freshwater environments.

dinoflagellate: Any of a group of eukaryotic microorganisms containing both plant-like and animal-like species that lives in marine and freshwater environments. These unicellular microorganisms use a pair of dissimilar cellular appendages called flagella for motility.

disturbance: Any abrupt event that drastically changes ecosystem characteristics such as population diversity, behavior, or climate response.

DNA (deoxyribonucleic acid): Molecule that encodes genetic information. DNA is a double-stranded molecule held together by weak bonds between base pairs of nucleotides. The four nucleotides in DNA contain the bases adenine (A), guanine (G), cytosine (C), and thymine (T).

dynamic global vegetation model (DGVM): Biogeographical model used to study how general categories of plant functional types are established and respond to competition, disturbances, and other factors.

Earth System Model (ESM): A type of complex, global model that combines physical climate models, global biological processes, and human activities.

ecophysiology: Study of the physiological functions of organisms as they pertain to their ecology or interactions with each other and their environment.

ecosystem: Set of living organisms (plants, animals, fungi, and microorganisms) and the physical and chemical factors that make up a particular environment.

ectomycorrhizae: A type of mycorrhizal fungus that surrounds a plant root tip but does not penetrate the cell walls of the root with its hyphae.

edaphic: Related to or determined by soil characteristics (e.g., soil texture, composition, drainage).

El Niño: An irregular variation of ocean current that flows off the west coast of South America, carrying warm, low-salinity, nutrient-poor water to the south. El Niño events, which occur every 4 to 12 years, can cause die-offs of plankton and fish and unusual weather patterns by altering jet stream winds and storm tracks.

electron acceptor: Substance that gains electrons from another substance in an oxidation-reduction reaction.

electron donor: Substance that loses electrons to another substance in an oxidation-reduction reaction.

endomycorrhizae: A type of mycorrhizal fungus that surrounds a plant root tip and uses its hyphae to penetrate the cell walls of the root. Endomycorrhizal fungi form vesicle-like structures at the root cell surface that enhance the transport of substances between a plant and fungus.

endophyte: Any organism (usually a fungus or microbe) that lives inside another organism and establishes a parasitic or mutualistic relationship with its host.

entisols: Undifferentiated soils of recent origin found in river valleys and deltas.

epiphyte: Any organism that grows upon or attaches to a living plant for physical support but not for nutrients.

eukaryote: A single-celled or multicellular organism (e.g., plant, animal, or fungi) with a cellular structure that includes a membrane-bound, structurally discrete nucleus and other well-developed subcellular compartments. See also *prokaryote*.

euphotic zone: The layer of a body of water that receives sufficient sunlight for photosynthesis. The depth of this layer, which is about 80 m, is determined by the water's extinction coefficient, its cloudiness, and the sunlight's angle of incidence.

extremophile: An organism that can survive in physically or chemically extreme conditions that are not livable to most other organisms.

exudates: See *root exudate*.

feedback: An interaction mechanism between processes in the Earth system that occurs when the result of an initial process triggers changes in a second process that in turn influences the initial one. A positive feedback intensifies the original process, and a negative feedback reduces it.

flow cytometry: A method for analyzing and separating cells or chromosomes based on light scattering and fluorescence. Also known as flow sorting.

gas chromatography: An automated method for separating a substance into its components. The substance is volatilized and carried by a stream of gas through a column containing an inert solid or liquid matrix that separates each component before reaching a detector device.

gelisols: Cold surface soils with underlying permafrost.

gene: Fundamental physical and functional unit of heredity. A gene is an ordered sequence of nucleotides, located in a particular position on a particular chromosome, that encodes a specific functional product (i.e., a protein or RNA molecule).

gene expression: Process by which a gene's coded information is converted into structures present and operating in the cell. Expressed genes include those transcribed into mRNA and then translated into proteins, as well as those transcribed into RNA but not translated into proteins [e.g., transfer (tRNA) and ribosomal RNA (rRNA)].

gene product: Biochemical material, either RNA or protein, resulting from expression of a gene. The amount of gene product is used to measure a gene's level of activity.

gene regulatory network: Intracellular network of regulatory proteins that control the expression of gene subsets involved in particular cellular functions. A simple network would consist of one or more input signaling pathways, regulatory proteins that integrate the input signals, several target genes (in bacteria a target operon), and the RNA and proteins produced from those target genes.

genera: A taxonomic category of organisms that ranks between family and species. Genera (singular: genus) for higher organisms generally consist of species with similar characteristics.

general circulation model (GCM): A class of computer-driven models (sometimes called global circulation models) that provide weather forecasts and climate projections. GCMs integrate a variety of fluid dynamical, chemical, and biological equations that represent processes in Earth system components (e.g., land, ocean, atmosphere, and biosphere).

genome: All the genetic material in the chromosomes of a particular organism. Most prokaryotes package their entire genome into a single chromosome, while eukaryotes have different numbers of chromosomes. Genome size generally is given as total number of base pairs.

genome sequence: Order of nucleotides within DNA molecules that make up an organism's entire genome.

genomics: The study of genes and their function.

genotype: An organism's genetic constitution, as distinguished from its physical characteristics (phenotype).

gigaton (GT): One billion metric tons; a metric ton is a unit of mass equal to 1000 kg (about 2200 lb).

greenhouse gas: Heat-trapping gas such as carbon dioxide, methane, nitrous oxide, or dimethyl sulfide released into the atmosphere as a result of human activities (primarily fossil fuel combustion) and natural processes (e.g., cellular respiration, biomass decomposition, volcanic activity).

gross primary productivity (GPP): Total amount of organic matter created by photosynthesis over a defined time period (total product of photosynthesis).

haplotype: A segment of DNA containing closely linked gene variations that are inherited as a unit.

hemicellulose: Any of several polysaccharides (e.g., xylans, mannans, and galactans) that cross-link and surround cellulose fibers in plant cell walls.

heterotroph: Organism that obtains organic carbon by consuming other organisms or the products of other organisms.

hexose: A type of sugar molecule that contains six carbon atoms (e.g., glucose, fructose).

high throughput: Done on a massive, automated scale.

histosols: Poorly drained soils in swamps and bogs that contain more than 20% organic matter.

homeostasis: Tendency of an organism or a cell to maintain its internal conditions regardless of external changing conditions.

homology: Similarity in DNA sequence or structure based on descent from a common ancestor.

humus: Long-lived mixture of organic compounds derived from the microbial decomposition of plant and animal matter in soils.

hydrometeoric: Relating to atmospheric phenomena that depend on water vapor.

hydrophilic: Having the ability to readily interact with water.

hydrophobic: Incapable of interacting with water.

hydrotropism: Ability of a plant to sense and grow toward water.

hyphae: Branching, threadlike filamentous cells of a fungus.

in silico: Using computers to simulate and investigate natural processes.

in situ: In a natural environment.

in vivo: Within a living organism.

inceptisols: Variable soils with horizon development in early stages.

interaction network: Diagram that shows numerous molecular interactions of a cell. Each point or node on the diagram represents a molecule (typically a protein), and each line connecting two nodes indicates that two molecules are capable of interacting.

interactome: Molecular interactions of a cell, typically used to describe all protein-protein interactions or those between proteins and other molecules.

isotope: Atom that has the same number of protons as another atom but a different number of neutrons and hence atomic mass. For example, ^{13}C is an isotope of carbon that has one more neutron than the most common isotope of carbon, ^{12}C.

La Niña: An irregular variation of ocean current that flows off the west coast of South America, carrying cool, nutrient-rich water to the surface. La Niña typically follows El Niño events, which occur every 4 to 12 years.

lateral gene transfer: Exchange of genetic material between two different organisms (typically different species of prokaryotes). This process gives prokaryotes the ability to obtain novel functionalities or cause dramatic changes in community structure over relatively short periods of time.

lignin: Complex, insoluble polymer whose structure, while not well understood, gives strength and rigidity to cellulose fibers in the cell walls of woody plants. Lignin makes up a significant portion of the mass of dry wood and, after cellulose, is the second most abundant form of organic carbon in the biosphere.

liquid chromatography: An automated method used to separate, identify, and quantify the components of a liquid solution. A sample is carried by a mobile liquid phase through a column packed with solid particles that separates each component before reaching a detector device.

loci: Chromosomal locations of genes or genetic markers. (singular: locus)

macroaggregates: Large (greater than 250 micrometers in size) mineral–organic matter complexes in soils that physically protect organic matter from degradation.

marine snow: Aggregates of mostly organic materials that sink to the ocean floor from the photosynthetically active surface layers.

mass spectrometry: Method involving specialized instruments for measuring the mass and abundance of molecules in a mixture and identifying mixture components by mass and charge.

membrane: Semipermeable biological barrier consisting of lipids, proteins, and small amounts of carbohydrate. Membranes control the flow of chemical substances (e.g., nutrients, protons, ions, and wastes) in and out of cells or cellular compartments. They also serve as structural supports for systems of membrane-embedded proteins that mediate important biological processes such as photosynthesis and cellular respiration.

mesofauna: Any animal of intermediate size (e.g., insects, earthworms).

mesophyll: Internal, irregularly-shaped, photosynthetic tissue within a leaf.

messenger RNA (mRNA): RNA that serves as a template for protein synthesis. See also *transcription* and *translation*.

metabolic flux analysis (MFA): Method for measuring all the metabolic fluxes of an organism's central metabolism; ^{13}C-labeled substrate is taken up by an organism, and the distribution of ^{13}C throughout the metabolic network enables the quantification of labeled metabolite pools.

metabolism: Collection of all biochemical reactions that an organism uses to obtain the energy and materials it needs to sustain life. An organism uses energy and common biochemical intermediates released from the breakdown of nutrients to drive the synthesis of biological molecules.

metabolites: Small molecules (<500 Da) that are the substrates, intermediates, and products of enzyme-catalyzed metabolic reactions.

metabolomics: Type of global molecular analysis that involves identifying and quantifying the metabolome—all metabolites present in a cell at a given time.

metadata: Data that describe specific characteristics and usage aspects (e.g., what data are about, when and how data were created, who can access the data, and available formats) of raw data generated from different analyses.

metagenomics: Study of the collective DNA isolated directly from a community of organisms living in a particular environment.

metaomics: High-throughput, global analysis of DNA, RNA, proteins, or metabolites isolated directly from a community of organisms living in a particular environment.

metaproteomics: High-throughput, global analysis of proteins isolated directly from a community of organisms living in a particular environment. Metaproteomics can reveal which genes are actively translated into functional proteins by a community.

metatranscriptomics: High-throughput, global analysis of RNA isolated directly from a community of organisms living in a particular environment. Metatranscriptomics can reveal which genes are actively expressed by a community.

methane clathrates: Ice crystals that contain large amounts of methane. Massive quantities of methane clathrates have been found under sediments in the ocean floor.

microaggregates: Small (50–250 micrometers in size) mineral–organic matter complexes in soils that physically protect organic matter from degradation.

microarray: Analytical technique used to measure the mRNA abundance (gene expression) of thousands of genes in one experiment. The most common type of microarray is a glass slide onto which DNA fragments are chemically attached in an ordered pattern. As fluorescently labeled nucleic acids from a sample are applied to the microarray, they bind the immobilized DNA fragments and generate a fluorescent signal indicating the relative abundance of each nucleic acid in the sample.

microbiome: A community of microorganisms that inhabit a particular environment. For example, a plant microbiome includes all the microorganisms that colonize a plant's surfaces and internal passages.

microorganism: Any unicellular prokaryotic or eukaryotic organism, sometimes called a microbe.

mixotrophic: Having both autotrophic and heterotrophic capabilities.

model: Mathematical representation used in computer simulations to calculate the evolving state of dynamic systems.

model ecosystem: A specific type of ecosystem that is widely studied in great detail by a community of researchers to provide insights into the processes controlling the behavior of other ecosystems.

model organism: Organism studied widely by a community of researchers. Biological understanding obtained from model-organism research is used to provide insights into the biological mechanisms of other organisms. Microbial model microorganisms include the bacteria *Escherichia coli*, the yeast *Saccharomyces cerevisiae*, and the mustard weed *Arabidopsis thaliana*.

modeling: Use of statistical and computational techniques to create working computer-based models of biological phenomena that can help to formulate hypotheses for experimentation and predict outcomes of research.

molecular machine: Highly organized assembly of proteins and other molecules that work together as a functional unit to carry out operational, structural, and regulatory activities in cells.

mollisols: Grassland soils with a thick, dark organic-surface horizon.

mycelium: Mass of hyphae that make up the body of a fungus.

mycorrhizae: Fungi that establish symbiotic relationships with plant roots.

nano-SIMS: Imaging technique that uses a nanoscale secondary ion mass spectrometer (nano-SIMS) and cells labeled with stable isotopes of carbon and/or nitrogen to identify areas of active growth and follow nutrient fluxes between cells.

net biome productivity (NBP): Amount of organic carbon that remains in a biome after accounting for carbon losses or gains from disturbances such as fire, disease, and human land use.

net ecosystem productivity (NEP): Amount of organic carbon (e.g., plant biomass, soil organic matter) that remains after respiration by photosynthetic organisms, heterotrophs, and decomposers.

net primary productivity (NPP): Fraction of photosynthetically fixed energy that remains after accounting for cellular respiration. NPP also is defined as the total amount of photosynthetic biomass created annually.

nitrification: Transformation of ammonium ions to nitrate by nitrifying bacteria.

nitrogenase: Enzyme that catalyzes the conversion of atmospheric nitrogen (N_2) to nitrate in nitrogen-fixing bacteria and archaea.

nitrogen fixation: Process carried out by certain species of bacteria and archaea in which atmospheric nitrogen (N_2) is converted to organic nitrogen-containing compounds that can be used by other organisms.

nuclear magnetic resonance (NMR): Technique used to study molecular structure by analyzing the absorption of electromagnetic resonance at a specific frequency in atoms subjected to strong magnetic field.

oligotrophic: Term used to describe lakes or other bodies of water that lack nutrients and plant life and have high concentrations of dissolved oxygen.

omics: Collective term for a range of new high-throughput biological research methods (e.g.,transcriptomics, proteomics, and metabolomics) that systematically investigate entire networks of genes, proteins, and metabolites within cells.

orthologs: Similar gene or gene segments appearing in the genomes of different species but resulting from speciation and mutation.

oxidation: Loss of one or more electrons from a chemical substance.

oxisols: Tropical soils rich in iron and aluminum oxides.

pathway: Series of molecular interactions that occur in a specific sequence to carry out a particular cellular process (e.g., sense a signal from the environment, convert sunlight to chemical energy, break down or harvest energy from a carbohydrate, synthesize ATP, or construct a molecular machine).

PCR (polymerase chain reaction): Rapid technique for generating millions or billions of copies of any piece of DNA. PCR also can be used to detect the existence of a particular sequence in a DNA sample.

pelagic zone: Open ocean that is not near the coast or ocean floor.

perennial: Plant that lives from year to year.

pH: Scale used to specify acidity or alkalinity. The hydrogen ion (H^+) concentration of a sample determines its pH (pH = –log10 [H^+]); the higher the H^+ concentration, the lower the pH. A solution with a pH value of 7 is neutral; less than 7 is acidic; and greater than 7 is alkaline or basic.

phenology: Study of recurring biological phenomena.

phenomics: Collective study of multiple phenotypes (e.g., all phenotypes associated with a particular biological function).

phenotype: Physical characteristics of an organism.

phloem: Vascular tissue that distributes sugars and nutrients throughout a plant.

photosynthate: Organic carbon produced by photosynthesis.

photosynthesis: Process by which plants, algae, and certain types of prokaryotic organisms capture light energy and use it to drive the transfer of electrons from inorganic donors (e.g., water) to carbon dioxide to produce energy-rich carbohydrates.

photosystem: Large, membrane-bound molecular complex consisting of multiple proteins containing pigment molecules (e.g., chlorophylls) that absorb light at a particular wavelength and transfer the energy from the absorbed photon to a reaction center that initiates a series of electron-transport reactions.

phototroph: Organism capable of photosynthesis.

phylogeny: Evolutionary history that traces the development of a species or taxonomic group over time.

physicochemical: Relating to both physical and chemical properties.

physiology: Study of the functions of living organisms and the factors that influence those functions.

phytoplankton: Free-floating, microscopic photosynthetic organisms (e.g., algae, cyanobacteria, dinoflagellates) found in the surface layers of marine and freshwater environments.

phytosiderophores: Chemical compounds released from the roots of certain plants (e.g., grasses) to sequester iron from the environment.

polymer: A large molecule formed by the linking together of smaller subunit molecules.

population: Collection of organisms of the same species living together in a given area. A community comprises several different populations.

primary production: Production of organic compounds from carbon dioxide, primarily through photosynthesis.

prokaryote: Single-celled organism lacking a membrane-bound, structurally discrete nucleus and other subcellular compartments. Bacteria and archaea are prokaryotes. See also *eukaryote*.

promoter: DNA site to which RNA polymerase will bind and initiate transcription.

protein: Large molecule composed of one or more chains of amino acids in a specific order; the order is determined by the base sequence of nucleotides in the gene that codes for the protein. Proteins maintain distinct cell structure, function, and regulation.

protein complex: Aggregate structure consisting of multiple protein molecules.

proteome: Collection of proteins expressed by a cell at a particular time and under specific conditions.

proteomics: Large-scale analysis of the proteome to identify which proteins are expressed by an organism under certain conditions. Proteomics provides insights into protein function, modification, regulation, and interaction.

proteorhodopsin: Light-dependent proton pumps in marine bacteria.

protozoa: Single-celled, eukaryotic microorganisms that use cellular appendages called flagella to propel them through their environments.

radioisotope: Unstable isotope of an element that releases radiation as it decays to a stable form.

Redfield ratio: Optimal ratio of carbon, nitrogen, and phosphorus for phytoplankton growth (106C:16N:1P) based on molecular concentrations.

reduction: Electron-transfer reaction in which a substance gains one or more electrons.

regulatory elements: Segments of the genome (e.g., regulatory regions, genes that encode regulatory proteins, or small RNAs) involved in controlling gene expression.

regulatory region or sequence: Segment of DNA sequence to which a regulatory protein binds to control the expression of a gene or group of genes that are expressed together.

respiration: Series of biochemical redox reactions in which the energy released from the oxidation of organic or inorganic compounds is used to generate cellular energy in the form of ATP.

rhizosphere: Narrow zone of soil surrounding a plant root.

ribosomal RNA (rRNA): Specialized RNA found in the catalytic core of the ribosome, a molecular machine that synthesizes proteins in all living organisms.

RNA (ribonucleic acid): Molecule that plays an important role in protein synthesis and other chemical activities of the cell. RNA's structure is similar to that of DNA. Classes of RNA molecules include messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA), and other small RNAs, each serving a different purpose.

root exudate: Chemical substance released from the root of a plant.

RuBisCo (Ribulose-1,5-bisphosphate carboxylase/oxygenase): Enzyme that catalyzes the first major step of photosynthetic carbon fixation by adding a molecule of carbon dioxide to a short 5-carbon sugar called ribulose bisphosphate. The resulting 6-carbon sugar is split into two 3-carbon molecules that can be used to build larger sugar molecules. RuBisCo also catalyzes photorespiration, which releases CO_2.

senescence: Process of aging.

simulation: Combination of multiple models into a meaningful representation of a whole system that can be used to predict how the system will behave under various conditions. Simulations can be used to run in silico experiments to gain first insights, form hypotheses, and predict outcomes before conducting more expensive physical experiments.

solubility pump: System of physical processes [e.g., changes in water temperature, ocean circulation, and gradient of carbon dioxide (CO_2) spanning the ocean depth] that influences the ocean's uptake of CO_2 from the atmosphere. In combination with ocean circulation, the solubility pump results in net CO_2 emissions at the equator and net CO_2 drawdown at high latitudes.

species: Taxonomic group of closely related organisms sharing structural and physiological features that distinguish them from individuals belonging to other species. In organisms capable of sexual reproduction, individuals of the same species can interbreed and generate fertile offspring. For microorganisms, a species is a collection of closely related strains.

spodosols: Acidic soils—typically found in coniferous forests—containing organic matter, aluminum oxides, and iron oxides.

stable isotope: Isotope that does not undergo radioactive decay.

stochastic: Relating to a series of random events.

stoichiometry: Ratio of molecules in a structural complex or chemical reaction.

superoxide dismutase: Enzyme that protects cells from oxidative damage by catalyzing the transformation of superoxide (a harmful species of oxygen) into oxygen and hydrogen peroxide.

symbiosis: Ecological relationship between two organisms in which both parties benefit.

synchrotron: Research facility that accelerates charged particles and uses an increasing magnetic field to keep the particles in a circular path. Electromagnetic radiation emitted by the high-energy, accelerated particles can be used in a variety of scientific applications.

systems biology: Use of global molecular analyses (e.g., measurements of all genes and proteins expressed in a cell at a particular time) and advanced computational methods to study how networks of interacting biological components determine the properties and activities of living systems.

taxa: Categories (e.g., phylum, order, family, genus, or species) used to classify animals and plants (singular: taxon).

taxonomy: Hierarchical classification system for naming and grouping organisms based on evolutionary relationships.

transcript: RNA molecule (messenger RNA, or mRNA) generated from a gene's DNA sequence during transcription.

transcription: Synthesis of an RNA copy of a gene's DNA sequence; the first step in gene expression. See also *translation*.

transcription factor: Protein that binds to regulatory regions in the genome and helps control gene expression.

transcriptomics: Global analysis of expression levels of all RNA transcripts present in a cell at a given time.

translation: Process in which the genetic code carried by mRNA directs the synthesis of proteins from amino acids. See also *transcription*.

troposphere: Region of the atmosphere closest to the Earth's surface.

tussock: A tuft or clump of grass or other vegetation.

ultisols: Acidic, clay-containing soils with strong horizons found in temperate humid and tropical regions.

Van der Waals bonds: Weak intermolecular bonds resulting from the attraction between electron-rich regions of one molecule and electron-poor regions of another.

vertisols: Seasonally dry soils with a high clay content that swell when moist and then crack when dry.

virus: Noncellular biological entity that can replicate only by infecting a host cell and using its reproductive capabilities.

windthrow: Trees uprooted by wind.

zooplankton: Free-floating, microscopic animals that drift with water currents.

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Acronyms and Abbreviations

Al aluminum
ATP adenosine triphosphate
BNF biological nitrogen fixation
CAMERA Community Cyberinfrastructure for Advanced Marine Microbial Ecology Research and Analysis
CDF cumulative distribution function
CO carbon monoxide
CO_2 carbon dioxide
CUE carbon use efficiency
CWD coarse woody debris
DGVM dynamic global vegetation model
DMS dimethyl sulfide
DNA deoxyribonucleic acid
DOC dissolved organic carbon
DOE Department of Energy
EMS ethyl methane sulfonate
ESM Earth System Model
FACE Free-Air CO_2 Enrichment program
Fe iron
GOS Global Ocean Sampling
GPP gross primary productivity
GT gigaton
GtC/y gigatons of carbon per year
GTL Department of Energy's Genomics:GTL program (formerly Genomes to Life)
HI harvest index
HRM Hierarchical Response Model
IAM Integrated Assessment Model
IMG/M JGI's Integrated Microbial Genome/Metagenome database
IPCC Intergovernmental Panel on Climate Change
JGI Department of Energy's Joint Genome Institute
KIS knowledge integration and synthesis
LPJ-GUESS Lund-Potsdam-Jena General Ecosystem Simulator
Mg magnesium
MLST multilocus sequence typing
Mn manganese
MOC mineral-associated organic carbon
N nitrogen
N_2 molecular nitrogen
N_2O nitrous oxide
NBP net biome productivity
NEE net ecosystem exchange
NEP net ecosystem productivity
NH_3 ammonia
NMR nuclear magnetic resonance
NPP net primary productivity
NUE nutrient use efficiency
OBER Office of Biological and Environmental Research
P phosphorus
pCO_2 partial pressure of carbon dioxide
PCR polymerase chain reaction
PDF probability density function
PFT plant functional type
Pg petagram
POC particulate organic carbon
ppmv parts per million by volume
Ra autotrophic respiration
Rd respiration by decomposers
Rh heterotrophic respiration
Rs respiration by plant roots and soil heterotrophs
RI recombinant inbred
rRNA ribosomal RNA
RNA ribonucleic acid
ROS reactive oxygen species
S sulfur
Si silicon
SOC soil organic carbon
SOD superoxide dismutase
SOM soil organic matter
ssu rRNA small subunit ribosomal RNA
TDF transcript-derived fragments
T-DNA transfer DNA
TMS theory, modeling, and simulation
TOF time of flight
uv ultraviolet
V_{max} maximum enzyme velocity
WUE water use efficiency

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