

Progressive Nitrogen Limitation of Ecosystem Responses to Rising Atmospheric Carbon Dioxide

YIQI LUO, BO SU, WILLIAM S. CURRIE, JEFFREY S. DUKES, ADRIEN FINZI, UELI HARTWIG, BRUCE HUNGATE, ROSS E. McMURTRIE, RAM OREN, WILLIAM J. PARTON, DIANE E. PATAKI, M. REBECCA SHAW, DONALD R. ZAK, AND CHRISTOPHER B. FIELD

A highly controversial issue in global biogeochemistry is the regulation of terrestrial carbon (C) sequestration by soil nitrogen (N) availability. This controversy translates into great uncertainty in predicting future global terrestrial C sequestration. We propose a new framework that centers on the concept of progressive N limitation (PNL) for studying the interactions between C and N in terrestrial ecosystems. In PNL, available soil N becomes increasingly limiting as C and N are sequestered in long-lived plant biomass and soil organic matter. Our analysis focuses on the role of PNL in regulating ecosystem responses to rising atmospheric carbon dioxide concentration, but the concept applies to any perturbation that initially causes C and N to accumulate in organic forms. This article examines conditions under which PNL may or may not constrain net primary production and C sequestration in terrestrial ecosystems. While the PNL-centered framework has the potential to explain diverse experimental results and to help researchers integrate models and data, direct tests of the PNL hypothesis remain a great challenge to the research community.

Keywords: biogeochemical cycles, carbon sequestration, climate change, ecosystem productivity, nutrient limitation

At the onset of the industrial revolution in the 18th century, global $p\text{CO}_2$, or partial pressure of atmospheric carbon dioxide (CO_2), began rising from approximately 27 pascals (Pa) to its current value of 37 Pa. The current $p\text{CO}_2$ is higher than at any time during the last 400,000 years. With the accelerating rate of increase of atmospheric CO_2 , we expect the global $p\text{CO}_2$ to reach 70 Pa by the end of the 21st century. According to predictions presented in the *Third Assessment Report of the Intergovernmental Panel on Climate Change* (IPCC 2001), this CO_2 increase alone could enhance the net primary production (NPP) of Earth's ecosystems enough to increase carbon (C) sequestration by 350 to 890 petagrams (Pg) C in the 21st century (1 Pg = 10^{15} grams [g]). To what extent soil nitrogen (N) availability will

constrain the predicted C sequestration, however, is still an open question. This question has ramifications for the future of terrestrial ecosystem productivity, atmospheric CO_2 concentration, and resulting feedbacks on climate (Hungate et al. 2003).

Experimental studies have examined N constraints on ecosystem C uptake in response to global change, primarily focusing on soil N availability and its regulation of photosynthesis and plant growth. Soil N availability has been found to decrease as a result of reduced decomposability of litter and to increase as a result of increased soil C substrate (Zak et al. 1993) and soil moisture (Hungate 1999) under elevated CO_2 . Measures that are often used to assess whether plants or whole ecosystems are limited by soil N availability include

Yiqi Luo (e-mail: yluo@ou.edu) is a professor, and Bo Su is a graduate student, in the Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019. William S. Currie is an assistant professor in the School of Natural Resources and Environment, and Donald R. Zak is a professor in the Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI 48109. Jeffrey S. Dukes is an assistant professor in the Department of Biology, University of Massachusetts, Boston, MA 02125. Adrien Finzi is an assistant professor in the Department of Biology, Boston University, Boston, MA 02215. Ueli Hartwig was a researcher at the Institute of Plant Sciences, ETH Zurich, Universitätsstr. 2, 8092 Zurich, Switzerland, when this article was being prepared. Bruce Hungate is an associate professor in the Department of Biological Sciences and the Merriam-Powell Center for Environmental Research, Northern Arizona University, Flagstaff, AZ 86011. Ross E. McMurtrie is an associate professor in the School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052, Australia. Ram Oren is a professor of ecology in the Nicholas School of the Environment and Earth Sciences, Duke University, Durham, NC 27708. William J. Parton is a senior research scientist and professor emeritus of rangeland and ecosystem science in the Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523. Diane E. Pataki is an assistant professor in the Department of Earth System Science and Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92697. M. Rebecca Shaw is a researcher in, and Christopher B. Field is the director of, the Department of Global Ecology, Carnegie Institution of Washington, Stanford, CA 94305. © 2004 American Institute of Biological Sciences.

CO₂-induced changes in leaf N concentration (Peterson et al. 1999), down-regulation of photosynthetic capacity (Luo et al. 1994), reduction in plant growth rates (Oren et al. 2001), and decreases in the rate of N mineralization (the conversion of organic N to the plant-available mineral form) (Gill et al. 2002). Most of the studies on C-N interactions in the past two decades were conducted primarily from a plantcentric perspective. This perspective focuses on plant growth as regulated by N (through short-term N turnover, soil mineral N availability, plant physiological adjustments, and reallocation of N from soil to plants). However, the plantcentric perspective is not fully compatible with ecosystem biogeochemical models, which consider the dynamics of both plant and soil pools and the C and N fluxes among them. This perspective also does not readily explain the contradictory results (e.g., responsiveness versus nonresponsiveness, increased versus decreased soil N availability, and N limitation versus nonlimitation in ecosystems) obtained from various CO₂ experiments.

To understand the C-N interactions with respect to ecosystem C sequestration under elevated CO₂, it is necessary to examine C and N dynamics in both soil and plant pools (Field 1999). Elevated CO₂ stimulates C input into ecosystems and potentially results in sequestration of C and N in soil organic matter (SOM) and long-lived plant biomass. Similarly, during forest development, C and N are tied up in plant biomass and SOM (Lichter 1998, Richter et al. 2000). This C and N sequestration induces changes in many other processes, including N competition between microbes and plants, litter decomposition, and mineralization. Understanding these aspects of ecosystem C and N dynamics requires data from long-term experiments, which are now beginning to become available. Many field CO₂ experiments (e.g., in Chesapeake Bay salt marsh, Florida oak woodland, North Carolina pine forest, Tennessee sweet gum forest, Wisconsin birch-aspen forest, California grassland, and the Mojave Desert) have been under way for a decade or so. Now that more data sets are available to shed light on C-N interactions under elevated CO₂, an ecosystem perspective is urgently needed to guide future experimental studies and advance predictive understanding of how N regulates C sequestration.

In this article, we develop a conceptual framework for an ecosystem perspective that can be used to study C-N interactions and evaluate this framework using data sets from various field experiments on the effects of elevated CO₂. Specifically, we consider that an increase in C influx into an ecosystem under elevated CO₂ stimulates two processes that are critical in regulating long-term ecosystem N dynamics. These processes are (1) increased demand for N to support stimulated plant growth and (2) enhanced sequestration of N into long-lived plant biomass and SOM pools. The latter can decrease soil N availability for plant growth and serves as the core mechanism driving progressive N limitation (PNL), the central concept developed in this article. The increase in N demand can also induce changes in short-, medium-, and long-term supply mechanisms that determine whether or not PNL may occur. After examining those mechanisms, we

review experimental evidence to illustrate the diversity of C and N interactions under elevated CO₂ among different ecosystems. To facilitate future research on PNL, we recommend a suite of measurements that could help probe mechanisms and detect patterns of PNL, and we present future research needs for experimental and modeling studies. This article is restricted to N limitation, partly because most experiments have data on this nutrient, but the PNL concept (with substitutes for N) may also apply in ecosystems where nutrients other than N are more limiting.

Progressive nitrogen limitation: Definition

When an ecosystem is exposed to elevated atmospheric CO₂, photosynthesis is stimulated because of the enhanced efficiency of C-fixing enzymes. The additional C that flows into the ecosystem at elevated levels of CO₂ is used for production of plant biomass, stored in SOM, and returned to the atmosphere through autotrophic and heterotrophic respiration. The additional growth of long-lived plant biomass (e.g., wood in forests) and the increased C storage in soil cause N to be sequestered in organic matter (van Groenigen and van Kessel 2002), progressively decreasing the mineral N available for plant uptake in the long term (figure 1). Thus, PNL describes the idea that, without new N input or decreases in N losses, the availability of mineral N declines over time at elevated CO₂ levels in comparison with its availability at ambient CO₂ levels. The long-term dynamics of PNL could be obscured by short-term processes, such as changes in N-use efficiency (C fixed per unit N) or transfers of N from pools with a low C:N ratio (e.g., SOM) to pools with a high C:N ratio (e.g., woody biomass). PNL could also be avoided if increased N fixation and decreased N losses under elevated CO₂ result in long-term ecosystem N gains. If PNL does occur, the CO₂-induced increment in ecosystem C storage declines over time.

The PNL concept is built on the biological principle that the formation of organic matter requires a certain amount of N and other nutrients in a relatively fixed ratio with C, in accordance with biochemical stoichiometry (Sturner and Elser 2002). Different forms of organic matter, however, vary in N concentration. For example, proteins average 17% N in dry weight, whereas plant cell wall materials have 0.2% N (Sturner and Elser 2002). When different organic chemical compounds are combined to form plant tissues and SOM, N requirements differ. Woody tissues in trees consist primarily of cellulose and lignin. Their C:N ratios are very high, ranging from 200 to more than 1000 (Levi and Cowling 1969). SOM, which is partly composed of dead soil microbes and partly formed by condensation reactions that chemically bind inorganic N into complex, long-lived organic molecules, has very low C:N ratios (Baisden et al. 2002). Because these pools differ greatly in C:N ratios, redistribution of N among them could lead to increased C sequestration per unit of N already present in an ecosystem under elevated CO₂.

The concept of PNL has a time component that stems primarily from gradual changes in pool sizes of C and N in ecosystems. Photosynthesis is a rate variable and can instan-

taneously respond to a change in the atmospheric CO_2 concentration. However, the changes in C and N pools are cumulative. The rate of C accumulation in an ecosystem is the net ecosystem productivity, equal to the difference between inflow of C from photosynthesis and outflow from respiration. Even if the photosynthetic stimulation under elevated CO_2 is constant, the ecosystem C accumulation rate declines over time because of gradually increasing respiration (Luo and Reynolds 1999). Respiration is usually proportional to C pool sizes. In pools with a fast turnover rate (e.g., microbial mass and fine roots), it takes a short time to accumulate C before the pool size reaches a new equilibrium. These pools are usually small. In pools with a long residence time (e.g., woody biomass and old humus in soil), the cumulative change in C and N can take from decades to thousands of years before the pool size reaches a new equilibrium. Pools with long residence times generally are large but change very slowly, making it difficult to detect statistically significant changes in soil C pools by conducting short-term CO_2 experiments (Hungate et al. 1996, Schlesinger and Lichter 2001). Like C, N accumulates slowly in large pools with long residence times and rapidly in small pools with short residence times. Thus, the development and onset of PNL depend on pool sizes and their residence times (McMurtrie and Comins 1996).

The PNL concept is distinct from the interactive effects of elevated CO_2 and N supply. Whereas soil fertility can set the initial responsiveness of ecosystems to elevated CO_2 (Oren et al. 2001), PNL expresses the concept of diminishing N availability under elevated CO_2 without additional N influx or reduced N losses. As shown in modeling studies (VEMAP 1995, Rastetter et al. 1997), N availability decreases with elevated CO_2 as a consequence of increased C storage, regardless of initial N availability. It is the progressive decrease in N availability, instead of the initial N level, that constrains the long-term responses of NPP and C storage (Field 1999).

Overall, PNL develops only if elevated CO_2 causes long-lived plant biomass and SOM to accumulate, sequestering substantial amounts of both C and N into long-term pools. The larger the initial enhancement of C and N accumulation is, the more likely the subsequent PNL is to occur. By contrast, negligible growth responses to elevated CO_2 , and low levels of C and N accumulation in organic forms, will not elicit PNL (figure 2). If N sequestration in long-lived plant biomass and SOM is compensated by additional N supply, it is possible that N will not limit C accumulation at all. Thus, it is critical to examine various N supply mechanisms.

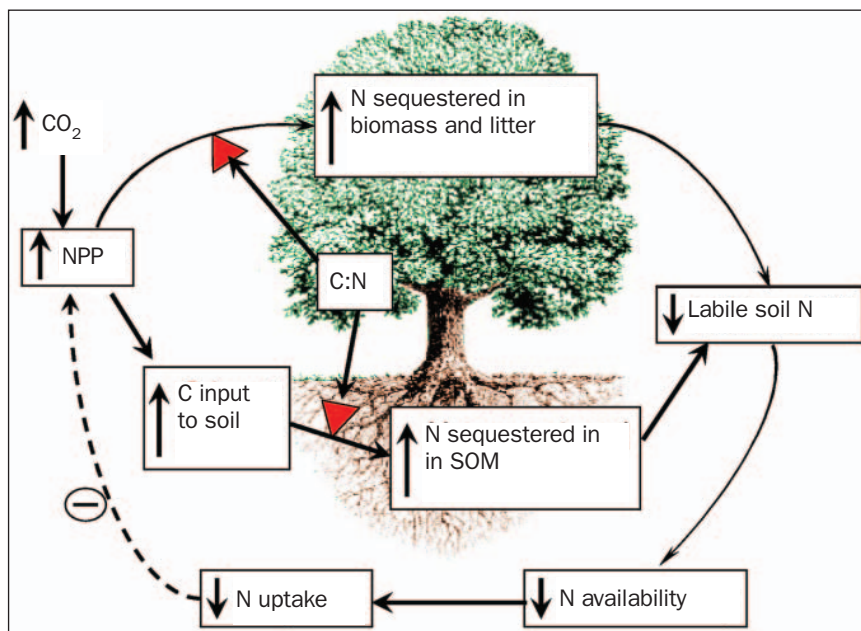


Figure 1. Two sets of feedback processes to elevated carbon dioxide (CO_2) leading to progressive nitrogen (N) limitation. In the upper pathway, the initial productivity response to elevated CO_2 results in N sequestration in plant biomass and litter pools, slowing N release to labile soil N. In the lower pathway, increased net primary production (NPP) in response to elevated CO_2 increases carbon (C) input to soil (increased exudation, root growth and death, and aboveground litter-fall), leading to increased N sequestration in soil organic matter (SOM). This N sequestration reduces N availability to plants and subsequent plant N uptake. Flexible C:N ratios in ecosystem organic matter pools can modify both pathways. Flexible soil C:N pools could allow increased C storage without restricting short- to medium-term N availability. A flexible plant C:N ratio could temporarily decrease N demand.

Mechanisms of nitrogen supply

When plants and ecosystems are subject to N stress under elevated CO_2 , a suite of short- and long-term mechanisms can act to prevent or alleviate PNL. The short-term mechanisms include reallocation of N among pools, increases in C:N ratios in plant tissues and SOM, and increased soil exploration by fine roots and mycorrhizae. The long-term mechanisms, which affect overall N supply, include increases in biological N fixation, decreases in N leaching and gaseous N loss from the soil, and enhanced retention of deposited N from the atmosphere.

Transfer of N from pools with a low C:N ratio (e.g., SOM) to pools with a high C:N ratio (e.g., woody biomass) enables ecosystems to sequester C over the short term under elevated CO_2 without additional N increases. Such a response has been observed in a ponderosa pine experiment in California (Johnson et al. 1997), a scrub oak woodland in Florida (Johnson et al. 2003), a pine forest in North Carolina (Finzi and Schlesinger 2003), a tallgrass prairie in Kansas (Jastrow et al. 2000), and a grassland ecosystem in Texas (Gill et al. 2002). The N transfer usually occurs through increased N uptake by plants at elevated levels of CO_2 . As a consequence,

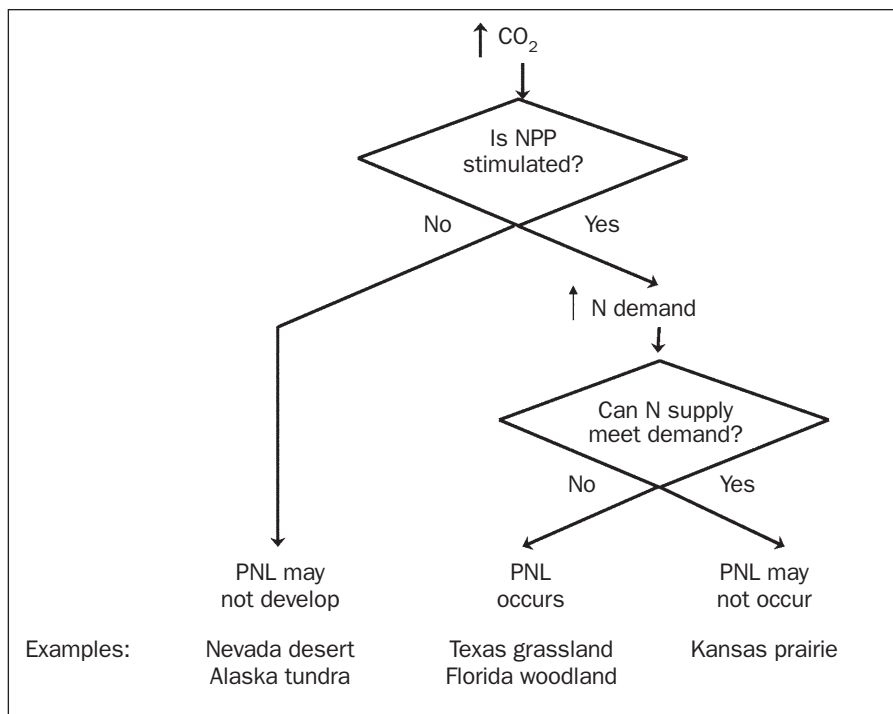


Figure 2. Schematic diagram of the progressive nitrogen limitation (PNL)-centered framework for identifying patterns of interactions between carbon (C) and nitrogen (N) under elevated carbon dioxide (CO_2). If elevated CO_2 does not stimulate enough biomass growth and C accumulation (net primary production, or NPP) in soil during the initial phase of CO_2 experiments, as in Nevada's Mojave Desert and Alaska's tussock tundra, N sequestration in the long-lived plant biomass and soil organic matter will not be substantial enough to affect soil N availability. In this case, PNL may not develop. If the initial stimulation of C sequestration under elevated CO_2 is high, N demand will build up to balance the C influx. If this extra N demand can be met through short-, medium-, or long-term supply mechanisms, as in Kansas tallgrass prairie, PNL may not occur. However, if N supplies cannot satisfy the N demand, as in Texas grassland and Florida scrub oak woodland, PNL may occur.

N-use efficiency generally increases, in a result similar to that observed in N-stressed environments. N can also be transferred from senescent leaves to young leaves (Billings et al. 2003). This N transfer has not been significantly affected by elevated CO_2 in many ecosystems and appears to play a minor role in regulating C processes in response to rising CO_2 (Norby et al. 2001).

Increases in plant C:N ratios have been often observed in CO_2 experiments (Pregitzer et al. 1995, Johnson et al. 1997), and increases in soil C:N ratios have occasionally been found as well (Gill et al. 2002). For example, the C:N ratio of SOM increased from approximately 18 to 22 as CO_2 increased from 21 to 55 Pa in the CO_2 -gradient tunnel study in Texas (Gill et al. 2002). The C:N ratio of leaves usually increases more than that of litter in response to elevated CO_2 (Norby et al. 2001, Billings et al. 2003). Even without additional N increases, the increases in plant or soil C:N ratios at elevated levels of CO_2 result in higher N-use efficiency than at ambient CO_2 levels, increasing the capability of plants or soil to sequester C over the short term.

Plants could also alleviate N demands at elevated levels of CO_2 by increasing their exploration of the soil for available N. There are two ways by which plants can explore for soil N: (1) increases in fine-root production and (2) increases in mycorrhizal infection (Zak et al. 2000, Hu et al. 2001). While there is little evidence that elevated CO_2 directly stimulates arbuscular mycorrhizal fungi (Staddon et al. 2002), it does appear to increase the percentage of ectomycorrhizal infection (Rygielgicz et al. 1997). Fine-root biomass often increases at elevated CO_2 (Norby et al. 2002). For example, Matamala and Schlesinger (2000) observed a 78% greater fine-root biomass in Duke Forest, North Carolina, at elevated CO_2 levels than at ambient levels. Fine-root biomass in an aspen and paper birch plantation near Rhinelander, Wisconsin, increased by 96% at elevated levels of CO_2 (King et al. 2001).

The increases in N transfer, C:N ratios, and soil exploration by root systems can support a positive NPP response to elevated CO_2 in the short term, even if the total amount of ecosystem N remains unchanged. However, these changes potentially trigger two negative feedback mechanisms over time. Increased production of plant tissues with a high C:N ratio, or increased C:N ratio in existing plant materials, yields more litter of low quality (i.e., low N content) at elevated

CO_2 levels than at ambient levels. Low-quality litter generally releases N slowly during decomposition, potentially leading to a negative N feedback on plant growth. Similarly, increased C:N ratios of SOM result in less N mineralization (Gill et al. 2002), decreasing the supply of soil N for plant uptake and leading to PNL. Modeling studies suggest that (a) adjustments in plant C:N ratio and increased soil exploration regulate C sequestration on a time scale of a few years, (b) transfers of N from SOM to plants influence C storage in biomass on a time scale of decades, and (c) long-term alleviation of PNL depends on net accumulation of N through reduced N losses or increased N gains (Rastetter et al. 1997).

Natural ecosystems gain N primarily through atmospheric deposition and biological fixation. Atmospheric N deposition adds as much as 2 g N per square meter per year to ecosystems in industrial and urbanized areas (Holland et al. 1999), but it has considerable spatial variation between regions. The extent to which N deposition is actually available for plant uptake and C sequestration is unclear. No field experiments have been performed to quantify the effects of CO_2 on

efficiency of use of deposited N. Assuming that 5% to 10% of deposited N can support C storage, Hungate and colleagues (2003) concluded that atmospheric N deposition is not adequate to meet the demand for C sequestration in response to gradually rising atmospheric CO₂ concentration. Biological N fixation occurs in symbiotic and free-living bacteria in natural ecosystems. Elevated CO₂ can increase both symbiotic and free-living N fixation, particularly in laboratory and agricultural studies (Tissue et al. 1997, Cheng et al. 2001), but results from experiments in native ecosystems are less clear (Billings et al. 2002). Since enhanced N fixation does not appear to be a universal response to elevated CO₂, we cannot predict how much it will help sustain long-term stimulation of NPP and C sequestration caused by elevated CO₂.

Nitrogen losses affect the accumulation of N in terrestrial ecosystems and hence the ability of terrestrial ecosystems to sequester C under elevated CO₂. Nitrogen is lost from an ecosystem in both gaseous and aqueous forms. Ammonia volatilization, nitrification, and denitrification account for most of the gaseous loss from ecosystems. Nitrate and dissolved organic N are the major aqueous forms of N lost from ecosystems (Perakis and Hedin 2002). Elevated CO₂ usually increases plant N uptake and decreases the standing pools of inorganic N. Since nitrification and denitrification are substrate dependent, ammonia volatilization and nitrate leaching decrease at elevated levels of CO₂ (Johnson et al. 2001, Reich et al. 2001, Mosier et al. 2002). However, elevated CO₂ may decrease water loss and increase soil water content (Hungate et al. 2002, Schäfer et al. 2002), favoring denitrification and N leaching (Arnone and Bohlen 1998). Overall, if N losses are reduced and N input is increased under elevated CO₂, PNL may be avoided.

Experimental evidence

A comprehensive test of the PNL hypothesis requires long-term field experiments with repeated measurements of C and N supply and demand under ambient and elevated CO₂ over time. Few studies have all the data necessary to test the components of the PNL hypothesis. In this section, we present a synthesis of published data to illustrate diverse patterns of C and N interactions among ecosystems.

When the Alaska tussock tundra was exposed to elevated CO₂ for 3 weeks, the photosynthetic capacity of sheathed cottontsedge (*Eriophorum vaginatum*) was completely down-regulated (Tissue and Oechel 1987). Plants grown under elevated CO₂ had photosynthetic rates similar to those grown under ambient CO₂. In the free-air CO₂ enrichment (FACE) experiment in the Mojave Desert, Nevada, photosynthesis responded to elevated CO₂ predominantly in wet periods, which are usually of short duration in this environment (Smith et al. 2000). In those CO₂ experiments in which photosynthesis was substantially down-regulated or photosynthetic stimulation was small, so that little C and N were sequestered in long-term plant and soil pools, PNL was unlikely to develop (figure 2). In these conditions, it is usually

difficult to detect any significant differences in N processes between ambient and elevated CO₂ treatments (Billings et al. 2002).

During an open-top chamber (OTC) experiment from 1989 to 1997 in a Kansas tallgrass prairie, final soil analyses indicated that a considerable amount of additional C and N accumulated in the ecosystem during 8 years of elevated CO₂. The initial CO₂ stimulation of plant growth was maintained in roots and increased over time for aboveground biomass (Owensby et al. 1999). The C:N ratio did not change much for either plant tissues or different classes of SOM; both C and N levels in soil were significantly higher at elevated CO₂ levels than at ambient levels (Jastrow et al. 2000, Williams et al. 2000). In addition, average inorganic N in soil, N mineralization, and turnover of microbial N were higher at elevated than at ambient levels of CO₂ (Williams et al. 2000, 2001). An isotope-labeling study showed that elevated CO₂ was associated with significantly greater increases in relative ¹⁵N recovery in SOM than in roots and rhizomes (Williams et al. 2001), suggesting that elevated CO₂ stimulated N sequestration in SOM.

Progressive N limitation was observed in the 4th year of CO₂ enrichment at the prototype plot of the Duke Forest FACE experiments in a loblolly pine forest; PNL was reversed after half the plot received N amendment (Oren et al. 2001). These results have not been observed during the first 4 years of the replicated experiment in the same stand. However, it appears that mechanisms underlying PNL are in operation at the Duke Forest site. The productivity of this ecosystem might decline as rapidly cycling N pools in soil are reduced at elevated levels of CO₂. During the first 4 years of fumigation, canopy photosynthesis increased by 40% (Luo et al. 2001), NPP by 20% to 32%, woody biomass by 4% (Finzi et al. 2002, Hamilton et al. 2002), and the C content of the organic horizon and top 30 centimeters of mineral soil by 24% (Schlesinger and Lichter 2001). The substantial increases in C sequestration in long-lived plant biomass and SOM pools have increased N uptake and sequestration in these same pools (Schlesinger and Lichter 2001, Finzi et al. 2002). Woody biomass and organic-horizon pools combined contained 6.3 g more N per square meter under elevated than under ambient CO₂ after 4 years of the treatment. This amount is greater than the 2.8 g N per square meter estimated to have been added to the site in atmospheric deposition and N fixation during the same period of time, indicating a redistribution of N from mineral soil horizons. Similar responses have been observed in other loblolly pine forests during stand development (Richter et al. 2000). Nitrogen immobilization in biomass and organic-horizon pools is assumed to drive these forests to a state of acute N limitation. In addition, there are no differences in the rate of N inputs or outputs under ambient and elevated CO₂ at the Duke Forest FACE site. Therefore, long-term mechanisms of N supply may not be in place to prevent PNL from happening at Duke Forest.

During the first 3 years of a tunnel experiment with a gradient of CO₂ concentration from 21 to 55 Pa in the Texas

grassland, the C:N ratios of plant tissues (leaves, roots, crowns) and SOM all increased linearly with CO₂ concentration (Gill et al. 2002). Without changes in the total amount of N in the ecosystem, the increased C:N ratio at high CO₂ levels enhanced C storage. However, the greatest increment in C accumulation occurred between preindustrial and present-day CO₂ concentrations, with substantially less enhancement between present-day and future levels. PNL was implicated as the mechanism responsible for this declining response. As the soil C:N ratio increased, N mineralization declined, releasing less inorganic N to the available N pool and thereby reducing C accumulation in response to elevated CO₂.

PNL was also apparent in experiments examining the CO₂ responses of a calcareous grassland in Switzerland and of a scrub oak woodland in Florida. Elevated CO₂ stimulated plant biomass growth (Leadley et al. 1999) and increased plant and soil C:N ratios (Niklaus et al. 1998) in the calcareous grassland. Nitrogen stocks in living plants and surface litter increased at elevated levels of CO₂ compared with those at ambient levels, but the soil N content did not change (Niklaus et al. 2003). In the scrub oak woodland, elevated CO₂ stimulated photosynthesis (Li et al. 1999), plant biomass growth (Dijkstra et al. 2002), and litter mass (Johnson et al. 2003). However, total ecosystem N content slightly decreased, while more N accumulated in the litter layer, at elevated levels of CO₂ (Johnson et al. 2003). In addition, CO₂-stimulated plant N uptake declined over time, and soil N fixation initially increased and then decreased after a few years of the CO₂ experiment in the Florida scrub oak ecosystem (Hungate et al. 2004), indicating the possibility of PNL.

Overall, three experiments in the Texas grassland, Swiss calcareous grassland, and Florida woodland showed what is expected from a response influenced by PNL. The nonresponsive results from the CO₂ experiments in Alaska tussock tundra and Nevada desert do not contradict the PNL hypothesis. To refute this hypothesis, we would need results that show continual increase in C sequestration and NPP over the long term. The Duke Forest–replicated FACE experiment and the Kansas OTC experiment did show continual increases in C sequestration over durations of 4 to 8 years, suggesting that short-term N supply mechanisms can prevent N limitation from happening for a substantial period of time. No long-term data are available to suggest whether or not mechanisms of N supply would adapt under elevated CO₂ to sustain C sequestration in the Duke FACE project or even to reverse the trends of PNL occurring at the Texas grassland and Florida woodland.

Parameters for progressive nitrogen limitation

Because up-to-date experimental results are diverse and not conclusive, examining the PNL hypothesis remains a great challenge for the scientific community. To detect PNL, it is critical to select and measure appropriate parameters. Taking a system view that considers changes in whole-ecosystem N availability, the following three parameters are fundamental indicators of whether or not PNL may occur.

Whole-ecosystem nitrogen stocks. The ecosystem N stock is a critical parameter to indicate whether PNL will occur. Elevated CO₂ stimulates C influx and accumulation in the plant and soil pools. If the whole-ecosystem N stocks increase in proportion to the C increase, this will delay the onset of PNL or make PNL unlikely to occur. In this case, the additional C accumulation is balanced by the N increase. If the N stocks for the whole ecosystem do not change, or if they decrease, while C influx and accumulation increase in the initial phase of elevated-CO₂ experiments, PNL is unavoidable.

Total ecosystem N stocks are the sum of plant and soil N and thus are relatively easy to measure. However, total ecosystem N pools are large, and annual changes in N content resulting from elevated CO₂ are relatively small. It is very difficult to detect statistical significance in small increments in N content, especially in short-term CO₂ experiments. Thus, it is essential to have a time series of measurements of the ecosystem N content (ideally, once a year for several years) to detect small, incremental changes.

Nitrogen inputs into ecosystems. Nitrogen input through fixation is thought to be one of the primary processes that limit productivity in many natural ecosystems. Since N fixation requires an abundant supply of carbohydrates to meet its high energy demand, elevated CO₂ that stimulates C influx into the rhizosphere and soil could potentially stimulate N fixation. If elevated CO₂ indeed stimulates N fixation enough to balance additional C accumulation, PNL may be avoided. Alternatively, a decrease in N fixation at elevated CO₂ would accelerate PNL. Nitrogen fixation varies greatly over time and among ecosystems, largely because of constraints by multiple biotic and abiotic factors (Vitousek et al. 2002). Thus, quantification of N fixation requires high temporal and spatial resolution. To balance the N budget, it is also critical to monitor atmospheric N deposition. Carbon dioxide treatments could affect retention of deposited N, leading to levels of N accumulation in the elevated-CO₂ plots different from those in the control plots.

Nitrogen losses. If increases in biomass and soil C accumulation at elevated levels of CO₂ are accompanied by reduction of N losses through either gaseous or aqueous pathways (or both), N stocks in the ecosystem are likely to increase. In that situation, PNL may be avoided or at least partially alleviated. If elevated CO₂ stimulates N loss (Arnone and Bohlen 1998, Ineson et al. 1998), then PNL is likely to occur. N is lost from an ecosystem through many processes and in many chemical forms. Measuring all the forms and pathways of N loss is not a trivial task, but thorough quantification of those N-loss pathways is a key to understanding PNL.

These three indicators—whole-ecosystem nitrogen stocks, nitrogen inputs into ecosystems, and nitrogen losses—are major parameters for the definition and detection of PNL. However, whole-ecosystem N stocks are not sensitive indicators, because small, incremental changes in N stocks occur

against huge and variable backgrounds. Biological N fixation (particularly through free-living bacteria) and gaseous and aqueous losses are difficult to quantify, because of high variability in both time and space. The following parameters are much more sensitive and can be used as partial indicators of PNL.

Whole-plant nitrogen amount. If the amount of whole-plant N increases in proportion to the increases in plant biomass at elevated levels of CO₂, then N sequestration in plant biomass—one of the major mechanisms underlying PNL—is occurring. Meanwhile, an increase of plant N indicates that the soil N supply has also increased, either through short-term adjustments in N supply or through long-term ecosystem N availability. Nevertheless, an initial increase in plant N at elevated CO₂, followed by a decline in N over time, could indicate PNL. In comparison with soil N pools, the plant N pool is very small. Thus, the change in plant N amount is much easier to detect and therefore a much more sensitive indicator for PNL.

Canopy nitrogen amount. Canopy N amount, or the sum of N in leaves within a canopy, directly regulates ecosystem photosynthetic C fixation. It is relatively easy to measure, and data are widely available. However, this index alone may not indicate whether PNL is occurring, because reallocation can increase the mass of N in the canopy when whole-plant and whole-ecosystem N amounts do not change. Despite this caveat, canopy N is generally a sensitive parameter and can be used to indicate whether soil N availability is limiting C production.

Annual nitrogen uptake. Like the whole-plant N amount, annual uptake of N is likely to indicate PNL if it shows an initial increase in response to elevated CO₂, followed by a decrease over time. If the annual N uptake does not change with elevated CO₂, N-use efficiency may increase and temporarily prevent PNL from happening.

Nitrogen mineralization. Microbial decomposition of litter and SOM releases mineral N. The microbial N release can be measured in the laboratory or field in terms of gross or net N transformations. If microbial N release increases, PNL is not occurring because of increased N supply to meet demand at elevated CO₂. Declining N mineralization is likely to indicate incipient PNL.

In addition to the above parameters, ecologists have used several other indicators of C-N interactions, including leaf N concentration and photosynthetic capacity. Leaf N concentration is strongly correlated with leaf photosynthetic capacity. Both leaf N concentration and photosynthetic capacity are sensitive indicators but by themselves do not indicate whether or not PNL will occur. Particularly when evaluating long-term ecosystem responses to rising atmospheric CO₂, it is essential to use ecosystem-level parameters as indicators of PNL.

Future research needs

Long-term field CO₂ experiments are extremely valuable for studying ecosystem C-N interactions. The C-N interactions in response to rising atmospheric CO₂ involve numerous processes. These processes operate at different time scales because of heterogeneity in the residence times of C and N pools in terrestrial ecosystems. Studying C-N interactions under elevated CO₂ is further complicated by CO₂-induced changes in soil water availability (Hungate et al. 2002, Mosier et al. 2002) and species composition. To examine the PNL hypothesis, it is imperative to make comprehensive measurements of various N processes over time in long-term CO₂ experiments.

The complexity of C and N interactions at different time scales also necessitates joint efforts between modelers and experimentalists. Models will be particularly useful in examining processes that are not easily measurable or that operate at time scales beyond the duration of CO₂ experiments. Combined experimental and modeling studies are powerful tools for understanding complex C-N interactions, evaluating whether or not PNL would eventually occur in a given situation, and, if it would, identifying the time scale at which it would occur.

The question of how to extrapolate results from CO₂ experiments to ecosystems in the real world remains challenging. Field experiments with elevated CO₂ usually result in step increases in C influx. A modeling study by Luo and Reynolds (1999) suggests that these step-change experiments create CO₂-induced extra N demand that is five- to tenfold higher than that in the real-world ecosystems where atmospheric CO₂ concentration is gradually increasing. In a step-change experiment with increasing CO₂, high N demand is likely to induce greater N stress and thereby stimulate stronger adjustments in short-term N redistribution and supply processes than in a gradual CO₂ increase in the real world. Unless the long-term N-supply processes were considerably stimulated, PNL would be unavoidable either in real-world ecosystems with a gradual CO₂ increase or in field experiments with step changes in CO₂. Ultimately, a mechanistic understanding of N-supply processes and their responses to various degrees of N demand in natural ecosystems is essential to improving the ability of scientists and managers to predict ecosystem responses to rising atmospheric CO₂.

Scientists also need to search for general principles and patterns underlying the diverse responses observed in CO₂ experiments. Without generalizable principles, it is difficult to incorporate experimental results into model predictions. At present, two contrasting types of models have been developed in the literature. One type of model links nutrient cycling to plant production and C sequestration (VEMAP 1995, Rastetter et al. 1997) and generally predicts long-term photosynthetic down-regulation and reduced stimulation in plant production with elevated CO₂. The other type of model does not incorporate N regulation of C processes at all and usually predicts large amounts of C sequestration. Without considering N constraints, for example, several dynamic

global vegetation models (DGVMs) have predicted that 350 to 890 Pg C will be sequestered by the terrestrial biosphere during this century in response to rising atmospheric CO₂ (IPCC 2001). The N required to balance this predicted C storage exceeds the potential supply from a combination of mechanisms, leaving a substantial discrepancy between N demand and potential supply (Hungate et al. 2003). This discrepancy highlights three issues that need to be resolved. First, N feedbacks need to be incorporated into DGVMs if these models are to be used to predict future C stocks. Second, scientific bodies such as the Intergovernmental Panel on Climate Change must carefully select properly constrained models for projecting the policy ramifications of PNL and its consequences for global C management. Finally, researchers must find ways to rigorously test the PNL hypothesis in order to accurately inform models that predict future global C stocks. Until we understand this issue fully, our ability to contribute to one of the most important discussions of our era will be very limited.

Acknowledgments

We thank Weixing Zhu for his critical comments. This work was conducted as a part of the Nitrogen Working Group supported by the National Center for Ecological Analysis and Synthesis, a center funded by the National Science Foundation (DEB-94-21535), the University of California at Santa Barbara, and the State of California. The preparation of the manuscript was supported by grants from the Terrestrial Carbon Program at the Office of Science (Biological and Environmental Research, or BER), US Department of Energy (DE-FG03-99ER62800); from the National Institute of Global Environmental Change, South Central Regional Center; and from the National Science Foundation (DEB 0092642). Research at the Duke Forest FACE facility was supported by the Office of Science (BER) program, US Department of Energy. This work contributes to the Global Change and Terrestrial Ecosystems (GCTE) core project of the International Geosphere-Biosphere Programme. GCTE Focus 1 was supported by the NASA Earth Science Enterprise.

References cited

Arnone JA III, Bohlen PJ. 1998. Stimulated N₂O flux from intact grassland monoliths after two growing seasons under elevated atmospheric CO₂. *Oecologia* 8: 331–335.

Baisden WT, Amundson R, Brenner DL, Cook AC, Kendall C, Harden JW. 2002. A multiisotope C and N modeling analysis of soil organic matter turnover and transport as a function of soil depth in a California annual grassland soil chronosequence. *Global Biogeochemical Cycles* 16: 1135–1160.

Billings SA, Schaeffer SM, Evans RD. 2002. Trace N gas losses and N mineralization in Mojave desert soils exposed to elevated CO₂. *Journal of Soil Biology and Biochemistry* 34: 1777–1784.

Billings SA, Zitzer SF, Weatherly H, Schaeffer SM, Charlet T, Arnone JA, Evans RD. 2003. Effects of elevated carbon dioxide on green leaf tissue and leaf litter quality in an intact Mojave Desert ecosystem. *Global Change Biology* 9: 729–735.

Cheng WG, Inubushi K, Yagi K, Sakai H, Kobayashi K. 2001. Effects of elevated carbon dioxide concentration on biological nitrogen fixation,

nitrogen mineralization and carbon decomposition in submerged rice soil. *Biology and Fertility of Soils* 34: 7–13.

Dijkstra P, Hymus G, Colavito D, Vieglais D, Cundari C, Johnson D, Hungate BA, Hinkle CR, Drake BG. 2002. Elevated atmospheric CO₂ stimulates shoot growth in a Florida scrub-oak ecosystem. *Global Change Biology* 8: 90–103.

Field CB. 1999. Diverse controls on carbon storage under elevated CO₂: Toward a synthesis. Pages 373–391 in Luo Y, Mooney HA, eds. *Carbon Dioxide and Environmental Stress*. San Diego: Academic Press.

Finzi AC, Schlesinger WH. 2003. Soil-N cycling in a pine forest exposed to 5 years of elevated carbon dioxide. *Ecosystems* 6: 444–456.

Finzi AC, DeLucia EH, Hamilton JG, Richter DD, Schlesinger WH. 2002. The nitrogen budget of a pine forest under free-air CO₂ enrichment. *Oecologia* 132: 567–578.

Gill RA, Polley HW, Johnson HB, Anderson LJ, Maherali H, Jackson RB. 2002. Nonlinear grassland responses to past and future atmospheric CO₂ ecosystems in sequestering anthropogenic CO₂. *Nature* 417: 279–282.

Hamilton JG, DeLucia EH, George K, Naidu SL, Finzi AC, Schlesinger WH. 2002. Forest carbon balance under elevated CO₂. *Oecologia* 131: 250–260.

Holland EA, Dentener FJ, Braswell BH, Sulzman JM. 1999. Contemporary and pre-industrial global reactive nitrogen budgets. *Biogeochemistry* 46: 7–43.

Hu S, Chapin FS III, Firestone MK, Field CB, Chiariello NR. 2001. Nitrogen limitation of microbial decomposition in a grassland under elevated CO₂. *Nature* 409: 188–191.

Hungate BA. 1999. Ecosystem responses to rising atmospheric CO₂: Feedbacks through the nitrogen cycle. Pages 265–285 in Luo Y, Mooney HA, eds. *Carbon Dioxide and Environmental Stress*. San Diego: Academic Press.

Hungate BA, Jackson RB, Field CB, Chapin FS III. 1996. Detecting changes in soil carbon in CO₂ enrichment experiments. *Plant and Soil* 187: 135–145.

Hungate BA, Reichstein M, Dijkstra P, Johnson D, Hymus G, Tenhunen JD, Hinkle CR, Drake BG. 2002. Evapotranspiration and soil water content in a scrub-oak woodland under carbon dioxide enrichment. *Global Change Biology* 8: 289–298.

Hungate BA, Dukes JS, Shaw MR, Luo Y, Field CB. 2003. Nitrogen and climate change. *Science* 302: 1512–1513.

Hungate BA, Stiling PD, Dijkstra P, Johnson DW, Ketterer ME, Hymus GJ, Hinkle CR, Drake BG. 2004. CO₂ elicits long-term decline in nitrogen fixation. *Science* 304: 1291.

Ineson P, Taylor K, Harrison AF, Poskitt J, Benham DG, Tipping E, Woof C. 1998. Effects of climate change on nitrogen dynamics in upland soils. *Global Change Biology* 4: 143–152.

[IPCC] Intergovernmental Panel on Climate Change. 2001. *Climate Change 2001: The Scientific Basis*. New York: Cambridge University Press.

Jastrow JD, Miller RM, Owensby CE. 2000. Long-term effects of elevated atmospheric CO₂ on below-ground biomass and transformations to soil organic matter in grassland. *Plant and Soil* 224: 85–97.

Johnson DW, Ball JT, Walker RF. 1997. Effects of CO₂ and nitrogen fertilization on vegetation and soil nutrient content in juvenile ponderosa pine. *Plant and Soil* 190: 29–40.

Johnson DW, Hungate BA, Dijkstra P, Hymus G, Drake B. 2001. Effects of elevated carbon dioxide on soils in a Florida scrub oak ecosystem. *Journal of Environmental Quality* 30: 501–507.

Johnson DW, Hungate BA, Dijkstra P, Hymus G, Hinkle CR, Stiling P, Drake BG. 2003. The effects of elevated CO₂ on nutrient distribution in a fire-adapted scrub oak forest. *Ecological Applications* 13: 1388–1399.

King JS, Pregitzer KS, Zak DR, Sober J, Isebrands JG, Dickson RE, Hendrey GR, Karnosky DF. 2001. Fine-root biomass and fluxes of soil carbon in young stands of paper birch and trembling aspen as affected by elevated atmospheric CO₂ and tropospheric O₃. *Oecologia* 128: 237–250.

Leadley PW, Niklaus PA, Stocker R, Korner C. 1999. A field study of the effects of elevated CO₂ on plant biomass and community structure in a calcareous grassland. *Oecologia* 118: 39–49.

Levi MP, Cowling EB. 1969. Role of nitrogen in wood deterioration, VII: Physiological adaptation of wood-destroying and other fungi to substrates deficient in nitrogen. *Phytopathology* 59: 460–468.

- Li J-H, Dijkstra P, Hinkle CR, Wheeler RM, Drake BG. 1999. Photosynthetic acclimation to elevated atmospheric CO₂ concentration in the Florida scrub-oak species *Quercus germinata* and *Quercus myrtifolia* growing in their native environment. *Tree Physiology* 19: 229–234.
- Lichter J. 1998. Primary succession and forest development on coastal Lake Michigan sand dunes. *Ecological Monographs* 68: 487–510.
- Luo Y, Reynolds JF. 1999. Validity of extrapolating field CO₂ experiments to predict carbon sequestration in natural ecosystems. *Ecology* 80: 1568–1583.
- Luo Y, Field CB, Mooney HA. 1994. Predicting responses of photosynthesis and root fraction to elevated CO₂: Interaction among carbon, nitrogen and growth. *Plant, Cell and Environment* 17: 1195–1204.
- Luo Y, Medlyn B, Hui D, Ellsworth D, Reynolds JF, Katul G. 2001. Gross primary productivity in the Duke Forest: Modeling synthesis of the free-air CO₂ enrichment experiment and eddy-covariance measurements. *Ecological Applications* 11: 239–252.
- Matamala R, Schlesinger WH. 2000. Effects of elevated atmospheric CO₂ on fine root production and activity in an intact temperate forest ecosystem. *Global Change Biology* 6: 967–980.
- McMurtrie RE, Comins HN. 1996. The temporal response of forest ecosystems to doubled atmospheric CO₂ concentration. *Global Change Biology* 2: 49–57.
- Mosier AR, Morgan JA, King JY, LeCain D, Milchunas DG. 2002. Soil-atmosphere exchange of CH₄, CO₂, NO_x, and N₂O in the Colorado shortgrass steppe under elevated CO₂. *Plant and Soil* 240: 201–211.
- Niklaus PA, Leadley PW, Stocklin J, Körner C. 1998. Nutrient relations in calcareous grassland under elevated CO₂. *Oecologia* 116: 67–75.
- Niklaus PA, Alpehi D, Ebersberger D, Kampichler C, Kandeler E, Tschirko D. 2003. Six years of in situ CO₂ enrichment evoke changes in soil structure and soil biota of nutrient-poor grassland. *Global Change Biology* 9: 585–600.
- Norby RJ, Cotrufo MF, Ineson P, O'Neill EG, Canadell JG. 2001. Elevated CO₂, litter chemistry, and decomposition: A synthesis. *Oecologia* 127: 153–165.
- Norby RJ, et al. 2002. Net primary productivity of a CO₂-enriched deciduous forest and the implications for carbon storage. *Ecological Applications* 12: 1261–1266.
- Oren R, et al. 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO₂-enriched atmosphere. *Nature* 411: 469–472.
- Owensby CE, Ham JM, Knapp AK, Auen LM. 1999. Biomass production and species composition change in a tallgrass prairie ecosystem after long-term exposure to elevated atmospheric CO₂. *Global Change Biology* 5: 497–506.
- Perakis SS, Hedin LO. 2002. Nitrogen loss from unpolluted South American forests mainly via dissolved organic compounds. *Nature* 415: 416–419.
- Peterson AG, et al. 1999. The photosynthesis–leaf nitrogen relationship at ambient and elevated atmospheric carbon dioxide: A meta-analysis. *Global Change Biology* 5: 331–346.
- Pregitzer KS, Zak DR, Curtis PS, Kubiske ME, Teeri JA, Vogel CS. 1995. Atmospheric CO₂, soil nitrogen and turnover of fine roots. *New Phytologist* 129: 579–585.
- Rastetter EB, Ågren GI, Shaver GR. 1997. Responses of N-limited ecosystems to increased CO₂: A balanced-nutrition, coupled-element-cycles model. *Ecological Applications* 7: 444–460.
- Reich PB, et al. 2001. Plant diversity enhances ecosystem responses to elevated CO₂ and nitrogen deposition. *Nature* 411: 809–824.
- Richter DD, Markewitz D, Heine PR, Jin V, Raikes J, Tian K, Wells CG. 2000. Legacies of agriculture and forest regrowth in the nitrogen of old-field soils. *Forest Ecology and Management* 138: 233–248.
- Rygielgicz PT, Johnson MG, Ganio LM, Tingy DT, Storm MJ. 1997. Lifetime and temporal occurrence of ectomycorrhizae on ponderosa pine (*Pinus ponderosa* Laws.) seedlings grown under varied atmospheric CO₂ and nitrogen levels. *Plant and Soil* 189: 275–287.
- Schäfer KVR, Oren R, Lai CT, Katul GG. 2002. Hydrologic balance in an intact temperate forest ecosystem under ambient and elevated atmospheric CO₂ concentration. *Global Change Biology* 8: 895–911.
- Schlesinger WH, Lichter J. 2001. Limited carbon storage in soil and litter of experimental forest plots under increased atmospheric CO₂. *Nature* 411: 466–469.
- Smith SD, Huxman TE, Zitzer SF, Charlet TN, Housman DC, Coleman JS, Fenstermaker LK, Seemann JR, Nowak RS. 2000. Elevated CO₂ increases productivity and invasive species success in an arid ecosystem. *Nature* 408: 79–82.
- Staddon PL, Heinemeyer A, Fitter AH. 2002. Mycorrhizas and global environmental change: Research at different scales. *Plant and Soil* 244: 253–261.
- Sterner RW, Elser JJ. 2002. *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton (NJ): Princeton University Press.
- Tissue DT, Oechel WC. 1987. Response of *Eriophorum vaginatum* to elevated CO₂ and temperature in the Alaskan tussock tundra. *Ecology* 68: 401–410.
- Tissue DT, Magonigal JP, Thomas RB. 1997. Nitrogenase activity and N₂ fixation are stimulated by elevated CO₂ in a tropical N₂-fixing tree. *Oecologia* 109: 28–33.
- van Groenigen JW, van Kessel C. 2002. Salinity-induced patterns of natural abundance carbon-13 and nitrogen-15 in plant and soil. *Soil Science Society of America Journal* 66: 489–498.
- [VEMAP] Vegetation/Ecosystem Modeling and Analysis Project. 1995. Vegetation/ecosystem modeling and analysis project: Comparing biogeography and biogeochemistry models in a continental-scale study of terrestrial ecosystem responses to climate change and CO₂ doubling. *Global Biogeochemical Cycles* 9: 407–437.
- Vitousek PM, et al. 2002. Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* 57: 1–45.
- Williams MA, Rice CW, Owensby CE. 2000. Carbon dynamics and microbial activity in tallgrass prairie exposed to elevated CO₂ for 8 years. *Plant and Soil* 227: 127–137.
- . 2001. Nitrogen competition in a tallgrass prairie ecosystem exposed to elevated carbon dioxide. *Soil Science Society of America Journal* 65: 340–346.
- Zak DR, Pregitzer KS, Curtis PS, Teeri JA, Fogel R, Randlett DL. 1993. Elevated atmospheric CO₂ and feedback between carbon and nitrogen cycles. *Plant and Soil* 151: 105–117.
- Zak DR, Pregitzer KS, King JS, Holmes WE. 2000. Elevated atmospheric CO₂, fine roots and the response of soil microorganisms: A review and hypothesis. *New Phytologist* 147: 201–222.