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Source: *Annual Review of Ecology, Evolution, and Systematics*, Vol. 37 (2006), pp. 611-636

Published by: Annual Reviews

Stable URL: <http://www.jstor.org/stable/30033845>

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Carbon-Nitrogen Interactions in Terrestrial Ecosystems in Response to Rising Atmospheric Carbon Dioxide

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Annu. Rev. Ecol. Evol. Syst. 2006. 37:611–36

First published online as a Review in Advance on August 16, 2006

The *Annual Review of Ecology, Evolution, and Systematics* is online at <http://ecolsys.annualreviews.org>

This article's doi:
10.1146/annurev.ecolsys.37.091305.110039

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1543-592X/06/1201-0611\$20.00

Key Words

biogeochemistry, global change, nitrogen, photosynthesis, stoichiometry

Abstract

Interactions involving carbon (C) and nitrogen (N) likely modulate terrestrial ecosystem responses to elevated atmospheric carbon dioxide (CO₂) levels at scales from the leaf to the globe and from the second to the century. In particular, response to elevated CO₂ may generally be smaller at low relative to high soil N supply and, in turn, elevated CO₂ may influence soil N processes that regulate N availability to plants. Such responses could constrain the capacity of terrestrial ecosystems to acquire and store C under rising elevated CO₂ levels. This review highlights the theory and empirical evidence behind these potential interactions. We address effects on photosynthesis, primary production, biogeochemistry, trophic interactions, and interactions with other resources and environmental factors, focusing as much as possible on evidence from long-term field experiments.

1. INTRODUCTION

Carbon (C) and nitrogen (N) are critical to many aspects of plant, herbivore, and microbial metabolism. Given rising levels of atmospheric CO₂ (hereafter *e*CO₂), the coupled cycling of C and N is also critical to ecosystem function today and in the future. Interactions involving C and N that might influence the global C cycle are of great importance to atmosphere-biosphere interactions, and thus to human society, because changes in *e*CO₂ impact global climate.

This importance is highlighted by several kinds of studies suggesting that C-N interactions may substantially constrain the CO₂ fertilization effect at local and global scales (Hungate et al. 2003, Luo et al. 2004, Oren et al. 2001, Reich et al. 2006). The mechanisms involve responses to resource supply rates, modifications of resource supply rates, or both (**Figure 1**). First, if plants experience multiple resource limitations, interactions of CO₂ and N supply could limit the CO₂ fertilization effect on biomass and C accumulation (Oren et al. 2001, Reich et al. 2006, Schneider et al. 2004). Such interactions occur at ecophysiological to ecosystem scales, and involve plant-microbial, plant-consumer, and plant-plant interactions, or all of these. If responses to *e*CO₂ are generally larger when N supply is high rather than low, as shown by contrasting response curves in a simple multiple limitation framework (**Figure 1**), then N supply rate would routinely influence *e*CO₂ response.

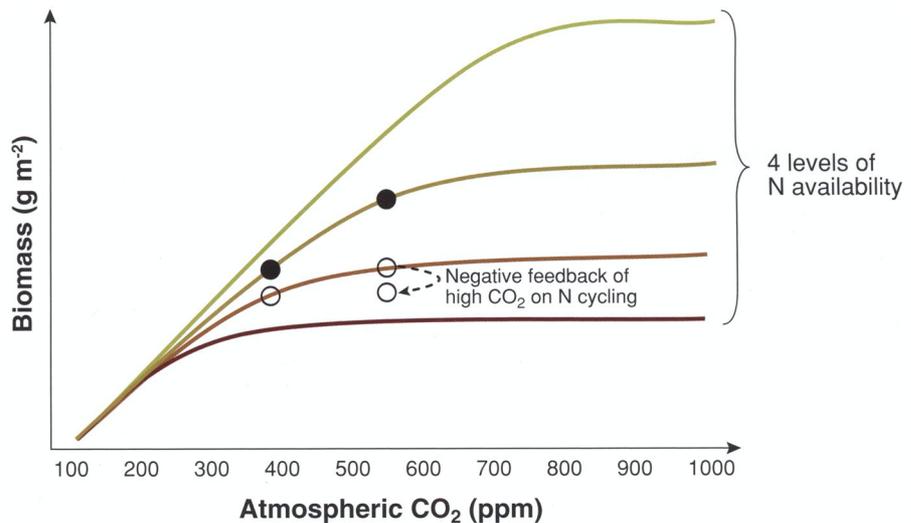


Figure 1

Simplified theoretical model of the way multiple resource limitation theory can lead to significant CO₂-N interactions (i.e., greater-than-additive) and of the way in which elevated CO₂-induced progressive N limitation of soil net N supply is superimposed on such multiple limitation responses. In this example, biomass increase with a 50% increase in CO₂ concentration is greater at higher than lower N supply rates (compare filled circles to open circles on the middle two N supply lines). Moreover, elevated CO₂ can diminish N supply rates, further suppressing biomass at elevated CO₂ (compare the two open circles connected with the dotted line).

Second, rising $e\text{CO}_2$ may result in feedbacks that lead to a suppression of plant N availability that limits the CO_2 fertilization effect, called progressive nitrogen limitation (PNL) (Luo et al. 2004). PNL could occur even if responses to $e\text{CO}_2$ were not influenced by N-supply level (i.e., effects of CO_2 and N availability were strictly additive) and would reduce CO_2 stimulation of biomass by reducing N supply, especially in N-limited conditions (note the downward shift in N supply owing to $e\text{CO}_2$; **Figure 1**). Finally, multiple resource limitations and PNL can work in tandem over time, exacerbating the extent to which lack of N availability may influence ecosystem responses to $e\text{CO}_2$.

The main C-N interactions proposed to influence responses to $e\text{CO}_2$ include the following: down-regulation of leaf N concentration and, hence, of net photosynthetic capacity; altered rates of herbivory, disease, or fungal mutualism due to changes in plant chemistry and stoichiometry; alterations of biogeochemical cycling; compositional change in plant or soil microbial communities; and increased N fixation rates or abundances of fixers, or both. Other drivers of environmental change (e.g., temperature or water) may also potentially influence C-N interactions under $e\text{CO}_2$.

Summarizing and synthesizing information about C-N interactions under $e\text{CO}_2$ is the central goal of this review. Unfortunately, empirical evidence of long-term C-N interactions under $e\text{CO}_2$ is still rare for either managed or unmanaged ecosystems. In a strange twist of fate, in contrast to the thousands of publications on laboratory $e\text{CO}_2$ experiments, we are severely limited, and will remain so over the next 25 years, in our ability to generalize about CO_2 -N interactions over meaningful ecosystem time frames, by the low number of realistic long-term experiments [such as those conducted without chambers using free-air CO_2 enrichment (FACE)]. This limitation is especially true for experiments using communities that are realistic approximations of natural or managed mixed species communities, as well as those examining CO_2 responses across a range of nutrient supply conditions (see **Table 1**). Thus, herein we focus on C-N interactions in a set of long-term studies (at least three years in length) of $e\text{CO}_2$ in near-natural conditions, emphasizing especially those that manipulate both CO_2 and N.

2. ABIOTIC AND BIOTIC CARBON-NITROGEN INTERACTIONS UNDER RISING ATMOSPHERIC CARBON DIOXIDE

The stoichiometry of tissue C:N ratios and the relative supply of carbon vis-à-vis nitrogen influence an enormous number of biotic and abiotic processes and relationships in terrestrial ecosystems. In this section we review some of these, organized by hierarchical and trophic scale.

2.1. Carbon-Nitrogen Interactions at the Point of Carbon Capture: The Leaf

How might C-N interactions down-regulate photosynthetic capacity under $e\text{CO}_2$ and therefore limit the sustainability of potential productivity response? Growth at

Table 1 Long-term (three or more field seasons) studies of elevated CO₂ under contrasting replicated N treatments. Studies listed by longevity of treatments

Study & System	Location	Ecosystem	Treatment period	References
Swiss FACE	Eschikon, Switzerland	Managed grassland	1993–present	Zanetti et al. 1996, Schneider et al. 2004
Jasper Ridge Mini FACE	California, USA	Grassland (annual)	1997–present	Shaw et al. 2002, Dukes et al. 2005
BioCON FACE	Minnesota, USA	Grasslands	1998–present	Reich et al. 2001, 2004, 2006
California pine OTC	California, USA	Pine juveniles	1991–1997	Johnson et al. 1997, Haile-Mariam et al (2000)
Swiss model ecosystem OTC	Birmensdorf, Switzerland	Juvenile trees	1995–1998	Spinnler et al. (2002)
Japan FACE	Shizukuizhi, Japan	Rice	1998–2000	Okada et al. 2001, Kim et al. 2003
Swiss OTC	Central Alps, Switzerland	Alpine grassland	1991–1994	Schäppi & Körner 1996
Iceland OTC	Gunnarsholt, Iceland	Poplar juveniles	1994–1996	Sigurdsson et al. 2001

$e\text{CO}_2$ can lead to acclimation (i.e., down-regulation) of carboxylation capacity (V_{cmax}) driven by reduced ribulose biphosphate carboxylase (Rubisco) amount and activity. This can occur by either of two ways, or by both. Down-regulation can occur by a general decrease in leaf N (especially on a mass basis) owing to enhanced C uptake under $e\text{CO}_2$. Additionally, down-regulation of Rubisco amount and activity could occur owing to decreased expression of specific photosynthetic genes as a result of increased sucrose cycling in mesophyll cells that occurs when net C uptake exceeds the capacity for carbohydrate export and utilization (Ainsworth & Long 2005). Thus, if $e\text{CO}_2$ decreases soil N availability (see Section 2.4) and/or increases C pools in biomass, this should lead to lower leaf N (mass or area basis), leading to down-regulation of net photosynthetic capacity (Ellsworth et al. 2004, Nowak et al. 2004).

In a meta-analysis of FACE studies ($n = 3$ to 11), $e\text{CO}_2$ decreased mass-based leaf N by 13% (range of 95% confidence interval, 10% to 17%), and area-based N, V_{cmax} and Rubisco, by 5% (2% to 7%), 13% (11% to 16%), and 19% (6% to 31%), respectively (Ainsworth & Long 2005). For 104 studies [predominantly open-top chambers (OTC)], the $e\text{CO}_2$ -induced reduction in mass-based N was 11% on average (Luo et al. 2006). The repeated experimental observations of down-regulation of N, Rubisco, and V_{cmax} strongly suggest that $e\text{CO}_2$ is likely having similar effects globally, although species responses vary substantially.

Across all FACE studies, $e\text{CO}_2$ increased light-saturated photosynthetic capacity by 31% (Ainsworth & Long 2005), comparable to earlier meta-analyses based largely on chamber studies (Curtis & Wang 1998, Wand et al. 1999). Does the long-term $e\text{CO}_2$ -enhancement of net photosynthesis vary with N supply? Surprisingly, our only data come from two FACE studies, the managed pasture Swiss FACE (*Lolium perenne*) and the tallgrass prairie BioCON study in Minnesota (Table 1, Figure 2). Leaf-level

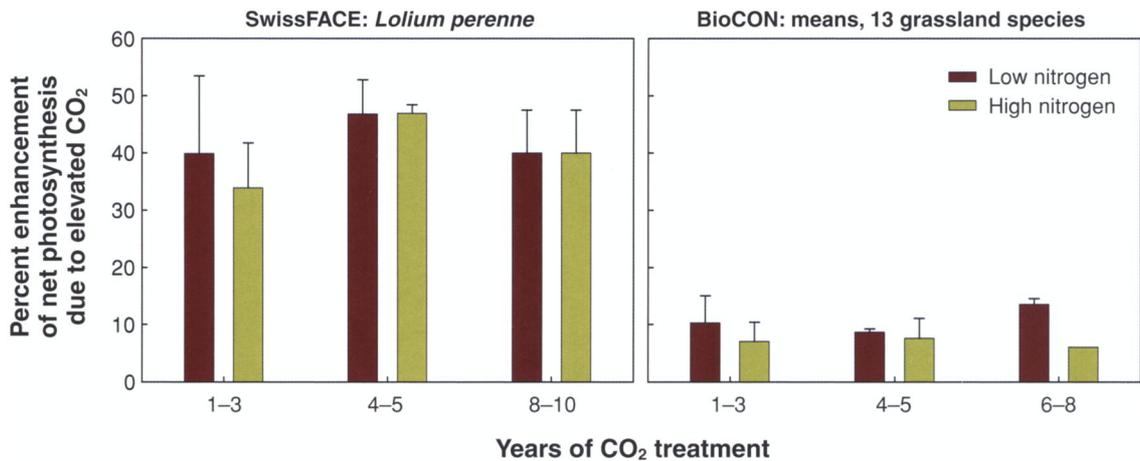


Figure 2

Effects of elevated CO₂ and N supply on light-saturated net photosynthesis in two long-term FACE studies, the managed pasture study in Eschikon, Switzerland (Swiss FACE) and the temperate grassland study in Cedar Creek, Minnesota (BioCON). For BioCON, data are the means of 11–13 species each year in monocultures. Data from Lee et al. (2001), Ainsworth & Long (2005), and T.D. Lee & P.B. Reich (unpublished data).

photosynthesis was increased by $e\text{CO}_2$ in both studies, but much more so for *Lolium* in the managed pasture than for the dozen grassland species in Minnesota. However, despite the different magnitude of photosynthetic enhancement, in neither study did this vary across contrasting N treatments at any point in time (**Figure 2**). Given that in both studies, biomass responses to $e\text{CO}_2$ differ at contrasting N supply rates (Schneider et al. 2004, Reich et al. 2006), the results suggest that plants are more prone to C-N interactions at the system scale than at the leaf scale, and these may be manifest in the size, organization or turnover rates of canopies and root systems.

2.2. Productivity and Biomass Accumulation

Both multiple resource limitation theory and PNL provide mechanisms by which C-N interactions can influence biomass and productivity responses to $e\text{CO}_2$. Direct evidence of such interactions can only be obtained from experiments with contrasting soil N supply rates, which we focus on herein (**Table 1**, plus additional shorter-term, multiyear studies).

In OTC studies with CO₂ and N manipulations, poplar seedlings and saplings (≈ 1 –5 years old) had greater aboveground growth stimulation by $e\text{CO}_2$ at high rather than at low N supply after two years in Michigan (Zak et al. 2000) and Italy (Liberloo et al. 2005) and three years in Iceland (Sigurdson et al. 2001), as did young North Carolina pine plantation trees (≈ 15 years old) after two years of CO₂ and N manipulations in a FACE system (Oren et al. 2001). In contrast, juvenile (1–7 years old) ponderosa pine in California and spruce-beech in Switzerland had similar responses

to $e\text{CO}_2$ at high and low N supply over six and four years in OTC, respectively (Haile-Mariam et al. 2000, Johnson et al. 1997, Spinnler et al. 2002). Results of these six studies suggest young trees may often, but not always, be more responsive to $e\text{CO}_2$ under enriched rather than ambient N conditions. There have been no such experiments with mature forests though, nor any long-term (more than three years) CO_2 and N studies with woody plants grown in natural settings. Thus, whether mature forests will be responsive to $e\text{CO}_2$ only at high N supply remains an open question, as does the general response of young forests over 5-, 10-, or 20-year time frames.

In managed agricultural and unmanaged herbaceous ecosystems with both FACE and N manipulations, there are aboveground biomass data for wheat (2 years), rice (3 years), annual grassland (5 years), perennial grassland (7 years), and ryegrass (10 years) (**Table 1**). In wheat the $e\text{CO}_2$ effect was no different at high N than at low N (Ainsworth & Long 2005). In rice there was greater $e\text{CO}_2$ enhancement of tiller number and biomass at higher rather than lower N fertilization rates (15 versus 9 versus 4 g N m⁻² years⁻¹) during early- and mid-season stages (such as panicle initiation and anthesis), which disappeared, however, by the end of the growing season (Kim et al. 2003). In the ryegrass study in Switzerland, there was a consistent ($\approx 26\%$) enhancement over 10 years of harvestable aboveground biomass by $e\text{CO}_2$ at high N fertilization (56 g N m⁻² years⁻¹), and no enhancement with lower (14 g N m⁻² years⁻¹) fertilization rates (Schneider et al. 2004; **Figure 3**). The two natural system FACE studies (Jasper Ridge, California and BioCON, Minnesota) had divergent responses. In California annual grasslands there was no effect by five years of $e\text{CO}_2$ on aboveground biomass regardless of N supply (Dukes et al. 2005). In Minnesota perennial grassland N addition (4 g N m⁻² years⁻¹) caused a slightly smaller $e\text{CO}_2$ stimulation of total biomass in years 1–3 and then a significantly larger $e\text{CO}_2$ stimulation of biomass after year 3 of the study (Reich et al. 2006; **Figure 3**). Additionally, a three-year OTC study in Swiss alpine grasslands reported no statistically significant interactions of CO_2 with nutrients treatments (Schäppi & Körner 1996); however, after three years total biomass was 13% greater under $e\text{CO}_2$ in fertilized plots and 5% lower under $e\text{CO}_2$ in unfertilized plots, consistent with the patterns of the Minnesota grassland and Swiss-managed pasture results.

Summarizing these 12 agricultural, grassland, and woody plant studies that manipulated both CO_2 and N for at least two years, greater biomass accumulation under $e\text{CO}_2$ at high rather than low N availability was observed in two thirds of the cases (with neutral findings in the others). Thus, the evidence suggests that a general N limitation to the $e\text{CO}_2$ effect is common, although not ubiquitous.

Measurements of leaf area index (LAI), soil CO_2 flux, and root biomass have been made in too few of the studies, and in insufficient intensity within studies, to come to any meaningful general conclusion regarding the interactions of CO_2 with N on these properties and processes, despite their general stimulation by $e\text{CO}_2$ (Ainsworth & Long 2005, Luo et al. 2006, Nowak et al. 2004). However, in the Japanese FACE study, $e\text{CO}_2$ influenced the seasonal trajectory of LAI in a manner consistent with a progressive within-season N limitation. LAI was enhanced early by $e\text{CO}_2$ but declining relative N availability eventually eliminated the LAI enhancement (Kim et al. 2003). Kim et al. (2003) state that management of rice under $e\text{CO}_2$ must

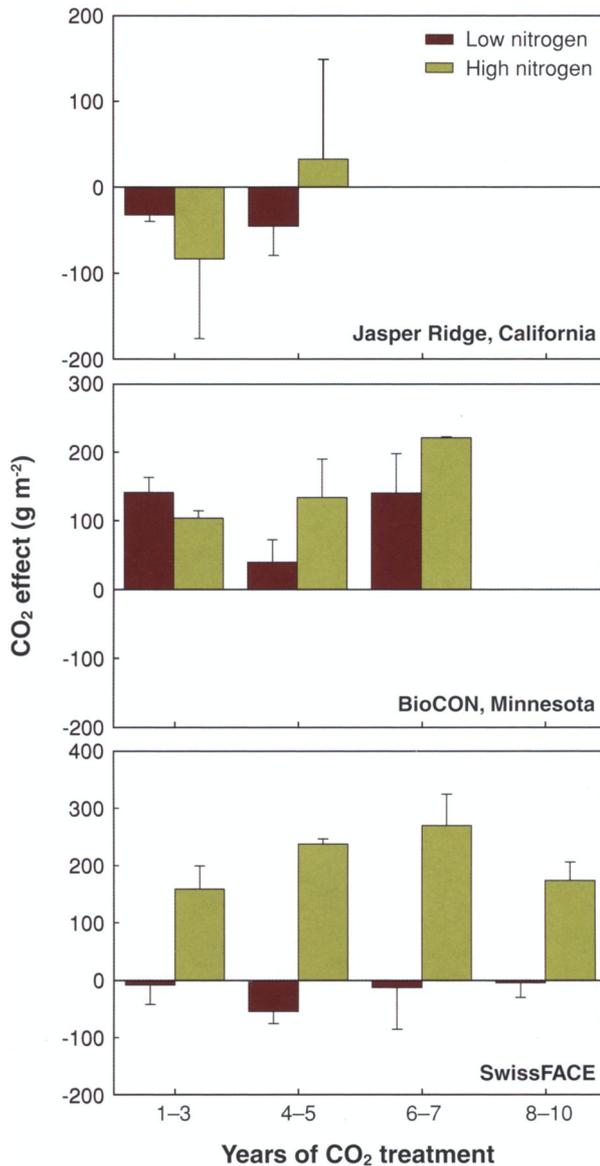


Figure 3

Effects of elevated CO₂ and N supply on biomass accumulation in three long-term studies, the annual grassland study at Jasper Ridge, California; managed pasture study in Eschikon, Switzerland (Swiss FACE) and the temperate grassland study in Cedar Creek, Minnesota (BioCON). The effect of CO₂ on biomass is defined as the biomass at elevated CO₂ minus biomass at ambient CO₂, estimated separately at contrasting low and high N supply. For the BioCON study, the data are for total biomass (above and belowground, 0–20 cm). For the other two studies, only aboveground biomass data are available. Data from Dukes et al. (2005), Reich et al. (2006), Schneider et al. (2004), and P.B. Reich (unpublished data).

therefore include a ramping up of N fertilization sufficient to maintain enhanced LAI and stable tissue N concentrations in order for the $e\text{CO}_2$ supply to be converted into increased C uptake.

2.3. Net Ecosystem Production and Carbon Sequestration

Net ecosystem production (NEP), the sum of net primary production (NPP) minus total heterotrophic respiration, is one explicit measure of ecosystem C uptake. Does response of NEP to $e\text{CO}_2$ depend on N supply? Given that plant biomass production is one key component of NEP, the larger $e\text{CO}_2$ enhancement of plant biomass with added N (see above) indicates the potential for added N to stimulate the response of NEP to $e\text{CO}_2$. Whether this potential is realized depends on how $e\text{CO}_2$ affects soil heterotrophic respiration from standing and soil surface detritus, and heterotrophic respiration from microbial breakdown of soil organic carbon stocks, and whether this response is sensitive to added N (van Groenigen et al. 2006). In the Swiss FACE pasture, mean soil C pools were higher with $e\text{CO}_2$, and the difference was slightly larger with added N; none of the changes were statistically significant, however, even after 10 years of experimental treatment (Xie et al. 2005). In the cold perennial grassland in Minnesota (BioCON), soil C also did not change significantly with $e\text{CO}_2$, but any tendency was toward a loss of C, with the loss being greater when N was also added (Dijkstra et al. 2005). Overall, changes in NEP due to $e\text{CO}_2$ in these (and other) experiments are apparently small on an annual basis and appear to exhibit little sensitivity to N additions. However, our ability to detect such effects is limited by the magnitude of the changes in soil C and the scarcity of ongoing experiments—only long-term experimentation in more systems than currently being studied will adequately characterize the sign and magnitude of CO_2 -N effects on C storage.

Assuming that we are years, if not decades, from having adequate direct tests of long-term C sequestration under contrasting CO_2 -N regimes, data syntheses are an alternative tool. A synthesis that divided up data (total $n = 80$) from a broad array of indoor, open-top chamber, and FACE experiments into contrasting N fertilization levels suggests that soil C is insensitive to $e\text{CO}_2$ in the absence of N supplements and that exogenous N is needed for $e\text{CO}_2$ to increase soil carbon (van Groenigen et al. 2006). A different synthesis (Luo et al. 2006) that directly contrasted only the limited number ($n = 6$) of studies that included contrasting N treatments (i.e., with versus without N additions) also found that soil C only increased in response to $e\text{CO}_2$ when high N was added. However, based on the same data set (but not shown in Luo et al. 2006), the larger sample ($n = 28$) of studies without N addition did show a significant increase (of 6.5%) in soil C in response to $e\text{CO}_2$. Thus, these recent meta-analyses (Luo et al. 2006, van Groenigen et al. 2006) suggest that evidence is still equivocal regarding whether $e\text{CO}_2$ increases soil C under ambient soil conditions (i.e., without added N). However, both analyses indicate that the effects of $e\text{CO}_2$ on soil C depends on N supply; in both cases, responses of soil C to $e\text{CO}_2$ were larger with added N than without.

These findings underscore the importance of considering N supply and availability when projecting $e\text{CO}_2$ -induced changes in soil C sequestration to the global scale

(Hungate et al. 2003). The following sections discuss processes that are involved in such C-N interactions.

2.4. Carbon Dioxide Effects on Soil Organic Matter Turnover and N Cycling

Nitrogen mineralization largely controls N availability to plants, so effects of $e\text{CO}_2$ on N mineralization have the potential to feed back to N-limited plant growth, and thus C gain in ecosystems. The effects of $e\text{CO}_2$ on organic matter mineralization are also important because, although $e\text{CO}_2$ usually increases photosynthesis, changes in respiration are equally important for total system C balance. Elevated CO_2 has been postulated to decrease litter quality, increase C input to soil, and increase soil water content, and these in turn are expected to alter organic matter mineralization, plant N uptake, and whole-system carbon balance. In this section, we summarize findings from field experiments examining these effects.

Despite early speculation (Strain & Bazzaz 1983) based on the common observation that $e\text{CO}_2$ reduces the N concentration of live plant leaves (see above), exhaustive tests suggest little influence of $e\text{CO}_2$ on the C:N ratio of litter or on the rate of plant litter decomposition (Norby et al. 2001), nor any dependence thereof on soil N supply (Henry et al. 2005; M.A. de Graaff, K.J. van Groenigen, J. Six, B. Hungate, C. van Kessel, in review). Because of large interspecific differences in decomposition rates between plant species (Dijkstra et al. 2006), $e\text{CO}_2$ is likely to have larger effects on litter decomposition by altering the composition of the plant assemblage (Dukes et al. 2005), though this indirect mechanism is likely to be very system specific (Henry et al. 2005).

In contrast to litter C:N ratios, $e\text{CO}_2$ has been shown to influence soil organic matter decomposition through several mechanisms. Plant growth affects mineralization of soil organic matter via the priming effect (Kuzakov 2002). In the context of $e\text{CO}_2$ and soil N availability, there are three hypotheses describing mechanisms modulating the priming effect: competition, microbial activation, and preferential substrate use (Cheng 1999, Kuzakov 2002). The microbial activation hypothesis (Kuzakov 2002) predicts that $e\text{CO}_2$ increases the input of C-rich organic matter from the growth and death of roots to which soil microorganisms respond initially by immobilizing available nutrients, later by mining older soil organic matter. The preferential substrate use hypothesis holds that soil microorganisms prefer the higher-quality substrates from plant roots, such that when $e\text{CO}_2$ enhances C input to soil, decomposition of older soil organic matter declines; this hypothesis assumes that soil nutrients do not limit decomposition. Where nutrients do limit decomposition, the competition hypothesis is relevant, whose predictions vary depending on the presumed winner in plant-microbe competition for soil nutrients: When plants triumph, greater plant uptake of nutrients reduces decomposition (Hu et al. 2001), whereas when microorganisms emerge victorious, the reduced nutrient availability caused by immobilization restricts plant growth responses to $e\text{CO}_2$ (Gill et al. 2002). These hypotheses are broad, and collectively explain a wide range of experimental results, but we still lack a general reconciliation of when each is dominant.

Does greater plant growth in $e\text{CO}_2$ affect decomposition of soil organic matter, and does this depend on soil nutrient status? Experiments have assessed this directly by measuring gross and net rates of soil N transformations. There is little evidence that $e\text{CO}_2$ alters gross N mineralization: No overall significant effect was found via meta-analysis (M.A. de Graaff, K.J. van Groenigen, J. Six, B. Hungate, C. van Kessel, in review), nor do the field experiments assessed here show any strong response or any dependence on soil N supply (Table 2). In a synthesis including pot and greenhouse studies, $e\text{CO}_2$ enhanced gross immobilization (M.A. de Graaff, K.J. van Groenigen, J. Six, B. Hungate, C. van Kessel, in review), yet this generalization does not reflect the field experiments considered here, where $e\text{CO}_2$ effects on gross consumption of inorganic N are small to nonexistent (Table 2). In summary, effects of $e\text{CO}_2$ on soil gross N mineralization and immobilization appear to be small compared to background variation, and there is no clear pattern revealing a dependence on soil N supply. Nevertheless, it is difficult to state with certainty that such effects are

Table 2 Summary of gross N transformation measurements conducted in elevated CO_2 field experiments*

Ecosystem	Years	Gross NH_4^+ mineralization ($\text{g N g}^{-1} \text{d}^{-1}$)		Gross NH_4^+ consumption ($\text{g N g}^{-1} \text{d}^{-1}$)		Seasonal gross NH_4^+ flux $\text{g N m}^{-2} \text{years}^{-1}$
		Ambient	Elevated	Ambient	Elevated	
Tallgrass prairie ¹	7.5	2.5 ± 0.4	2.3 ± 0.4	3.1 ± 0.8	2.7 ± 0.6	72
Lolium pasture ²	7.2	6.2 ± 0.9	7.7 ± 1.3	8.0 ± 0.8	10.2 ± 1.2	217
Trifolium pasture ²	7.2	6.9 ± 0.5	5.6 ± 1.1	8.8 ± 0.6	7.4 ± 0.7	194
Loblolly pine ³	3.5	1.6 ± 0.5	1.6 ± 0.5	2.8 ± 0.4	2.9 ± 0.4	60
Sweetgum ⁴	1.5	0.7 ± 0.2	0.6 ± 0.1	0.7 ± 0.3	0.9 ± 0.3	20
Aspen ⁵	2.5	0.9 ± 0.3	1.3 ± 0.3	1.7 ± 0.8	2.3 ± 0.8	42
Scrub-oak ⁶	1.0	2.9 ± 0.4	2.0 ± 0.2	2.9 ± 0.5	2.4 ± 0.6	68
Perennial grassland ⁷	5.0	2.0 ± 0.1	2.1 ± 0.1			55
Perennial grassland (+N)	5.0	2.2 ± 0.2	2.3 ± 0.2			61

*Gross NH_4^+ mineralization and consumption were calculated using the ^{15}N isotope dilution technique over 24- to 48-h periods. As a first approximation of seasonal flux rates, values were expressed on a real basis for a 180-day period using the average rates of both fluxes (mineralization and immobilization) for both CO_2 treatments, and assuming a bulk density of 1 g cm^{-3} . The largest change in plant uptake reported to date is for the tallgrass prairie site, where eight years of CO_2 enrichment caused an increment in plant N content of 9.8 g N m^{-2} , or $1.2 \text{ g N m}^{-2} \text{ years}^{-1}$ (Jastrow et al. 2000 for belowground, and calculated for aboveground from Owensby et al. 1999 and personal communication). After four years of CO_2 enrichment in the loblolly pine plantation, total plant N increased by 1.91 g N m^{-2} , or $0.4 \text{ g N m}^{-2} \text{ years}^{-1}$ (Finzi & Schlesinger 2003). If driven by increases in gross NH_4^+ transformations, such changes could easily go undetected.

¹Average over depths and times (Williams et al. 2001).

²Average over depths and times for the low N treatment ($14 \text{ g N m}^{-2} \text{ years}^{-1}$) (Richter et al. 2003).

³Finzi & Schlesinger (2003).

⁴Zak et al. (2003).

⁵Average of high and low O_3 treatments (Zak et al. 2003).

⁶Average over times (Hungate et al. 1999).

⁷Average across diversity treatments (West et al. 2006).

unimportant, because very small changes in gross N transformations (empirically undetectable using isotope dilution) would be more than adequate to explain changes in plant N acquisition caused by $e\text{CO}_2$. Scaling gross N cycling rates measured in the field as a first approximation of annual gross fluxes, in cases where $e\text{CO}_2$ has been shown to increase plant N uptake, gross N mineralization need increase (or immobilization decrease) by only 2% or less to explain the differences observed (Table 2). Thus, even if only a small portion of gross N turnover is available to plants, still only small changes in gross N mineralization would be needed to explain the differences observed.

Net N mineralization is typically on the same scale as plant N uptake, and thus less challenged by a low signal-to-noise ratio compared to gross N transformation measurements. $e\text{CO}_2$ often has no detectable effect on net N mineralization or on proxies of soil N availability, such as resin bags or resin sticks (Finzi & Schlesinger 2003, Matamala & Drake 1999, D.W. Johnson et al. 2003). In contrast, in the absence of N supplements $e\text{CO}_2$ has been found to reduce net N mineralization under field conditions in a cold perennial grassland (Reich et al. 2001, 2006) and in a warm perennial grassland (Gill et al. 2002). Also in the absence of N supplements, $e\text{CO}_2$ has been found to reduce N availability as estimated by extractable inorganic nitrogen (Hu et al. 2001, Hungate et al. 1999, Matamala & Drake 1999), in some cases despite having no effect on net N mineralization. In total, these findings indicate that under typical ambient soil conditions, $e\text{CO}_2$ has neutral or negative effects on net N mineralization rates.

Do these effects of $e\text{CO}_2$ on net N mineralization and plant available N, in turn, depend on soil N supply? Added N gradually (by years 5–6) reversed the depressing effect of $e\text{CO}_2$ on net N mineralization in the cold perennial grassland (Figure 4) and resulted in a substantially greater stimulation of total plant N pools under $e\text{CO}_2$ at high rather than low N, likely contributing to the greater plant biomass response to $e\text{CO}_2$ with added N in that ecosystem (Figure 3) that began only after several years (Reich et al. 2006). Similarly, in the Swiss FACE study, $e\text{CO}_2$ consistently reduced total aboveground N pools in ryegrass over 10 years at low N fertilization ($14 \text{ g N m}^{-2} \text{ years}^{-1}$), with no temporal trend, and for the first 5 years at high N fertilization ($56 \text{ g N m}^{-2} \text{ years}^{-1}$), but with a gradual switch from suppression to stimulation at high N over 10 years (Schneider et al. 2004). Thus, these two long-term grassland studies with radically different N cycles (ambient versus $4 \text{ g N m}^{-2} \text{ years}^{-1}$ in BioCON; 14 versus $56 \text{ g N m}^{-2} \text{ years}^{-1}$ in Swiss FACE) have similar results (Figure 4). In both studies there was an interactive effect of N supply on the $e\text{CO}_2$ effect on total plant available N pools that had a significant temporal trajectory, with stimulation of net N mineralization and of total plant available N by $e\text{CO}_2$ developing over time in N-rich conditions, first showing up in the fifth year in BioCON and the sixth year in Swiss FACE. It is unfortunate that, to our knowledge, only three grassland studies (Table 1) provide long-term direct evidence of the degree to which $e\text{CO}_2$ effects on the N cycle are dependent upon (the collective set of factors that regulate) the overall level of plant available N.

What can we conclude about the possible importance of $e\text{CO}_2$ -induced changes in organic matter mineralization? Although a far cry from a mechanistic understanding

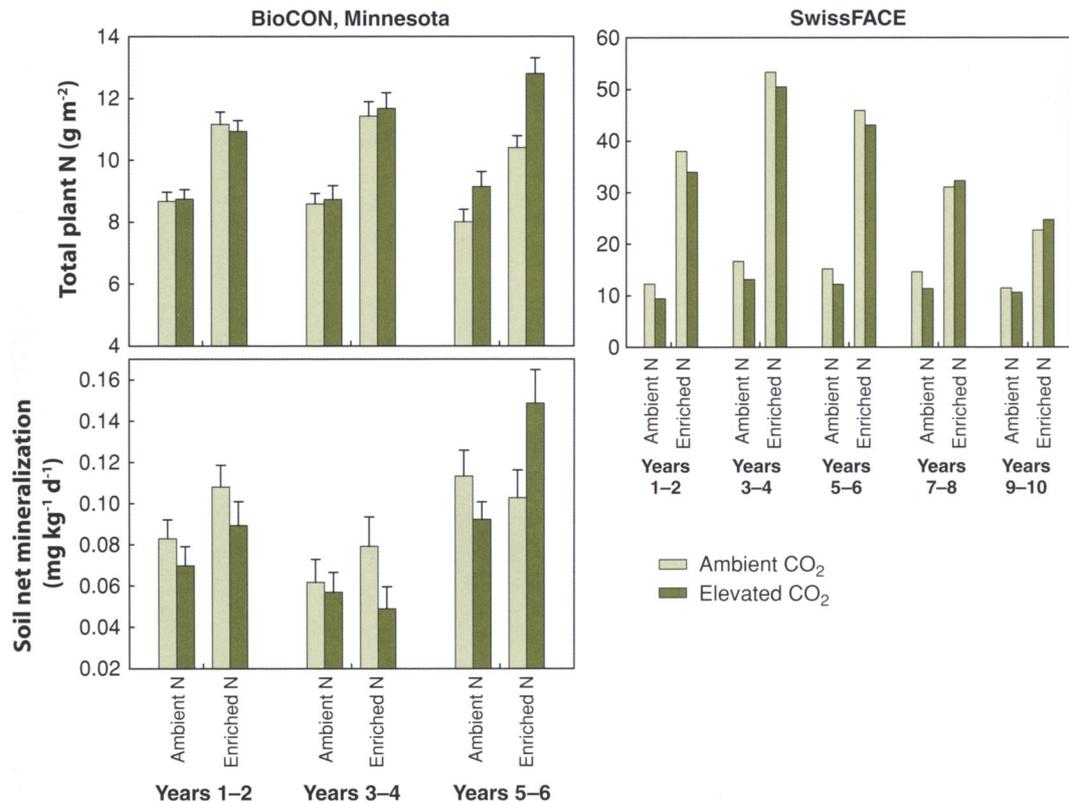


Figure 4

Mid-summer soil net N mineralization rates and plant N pools at elevated and ambient CO₂, at ambient and enriched soil N for a cold temperate grassland in Minnesota (BioCON) and a managed pasture in Switzerland (Swiss FACE). Using annual data in BioCON, there was significant interaction between CO₂ and N, because elevated CO₂ suppressed net N mineralization at both ambient and enriched N supply in the first four years of the study, but in year 5–6 CO₂ stimulated net N mineralization at enriched N supply. A similar interaction over time was noted for plant N pools in both studies. Data from Reich et al. (2006) and Schneider et al. (2004).

of the importance of the priming effect, two conclusions emerge from the finding that *e*CO₂ increases soil organic C consistently only when N is also added. First, even if N addition augments the priming effect with *e*CO₂, any loss of soil C caused by priming is insufficient to offset the increased C input to soil caused by *e*CO₂-enhanced plant production with added N. Thus, the priming effect, if it occurs, does not appear to be large enough to dominate soil C balance response to *e*CO₂ with added N. Second, a *e*CO₂-induced enhancement of the priming effect may partly explain why *e*CO₂ often does not increase soil organic carbon in the absence of N additions, even when plant production increases, for example, in the Swiss FACE experiment (Xie et al. 2005). Quantifying the importance of the priming effect under field conditions, and

thereby assessing its importance compared to other, simpler mechanisms (e.g., the simple fact that plant responses to $e\text{CO}_2$ are larger with added nutrients) remains an important challenge to global change research.

2.5. Nitrogen Fixation: Interactions with Other Nutrients

Symbiotic biological N_2 fixation often increases with $e\text{CO}_2$ (Wilson 1933, Soussana & Hartwig 1996). Fixation of atmospheric N_2 requires reduced C, which higher rates of photosynthesis in response to $e\text{CO}_2$ can supply. Bacterial symbionts use this C surplus to fix N_2 , providing needed N to the plants (Hartwig 1998).

Is this expected increase in N_2 fixation observed in ecosystems exposed to $e\text{CO}_2$? Sometimes. In experiments where phosphorus (P), potassium (K), and/or other non-N nutrients have been added, N_2 fixation often shows a positive response to $e\text{CO}_2$ (van Groenigen et al. 2006). For example, in a long-term experiment of pasture receiving annual supplements of P, K, and magnesium (Mg), $e\text{CO}_2$ increased N_2 fixation (Zanetti et al. 1996, Lüscher et al. 2000). But in the absence of such nutritional supplements, N_2 fixation is often unresponsive to $e\text{CO}_2$ (van Groenigen et al. 2006; see Section 3.3 below). In the cases where significant increases in N_2 fixation have been observed (e.g., Lee et al. 2003a), they may occur as a short-term response to $e\text{CO}_2$ (e.g., Dakora & Drake 2000, Hungate et al. 1999), a response that can subsequently decline (Hungate et al. 2004, van Groenigen et al. 2006) (**Figure 5**). In the cold temperate grassland experiment in Minnesota, evidence from 1998–2004 was mixed: two of four species showed some evidence of a decline over time in $e\text{CO}_2$ stimulation of N_2 fixation and the other two did not (J.B. West, T.D. Lee, S. Hobbie, and P.B. Reich, unpublished data). Unfortunately, little other evidence is available to further evaluate this question.

Nitrogen fixation often declines as soil N supply increases (e.g., Hartwig 1998, Lee et al. 2003b), because N uptake from soil is less costly than fueling N_2 -reduction, and thus N addition should depress the proportion of N derived from N_2 fixation, as shown experimentally by Lee et al. (2003b). N addition did reduce the response of N_2 fixation to exposure to $e\text{CO}_2$ in managed pasture (Zanetti et al. 1996, Lüscher et al. 2000) and in rice crops (Hoque et al. 2001). In each of these experiments, nonnitrogenous fertilizers were applied to both treatments, likely promoting the N_2 fixation response to $e\text{CO}_2$ with low N additions. In a more global analysis of all available observations, there was no effect of N addition on the response of N_2 fixation to $e\text{CO}_2$ (van Groenigen et al. 2006).

Elevated CO_2 can increase or decrease the relative abundance of N_2 -fixing plants, suggesting that some of the effects of $e\text{CO}_2$ on N_2 fixation may be manifest as changes in plant communities rather than changes in N_2 fixation on a per plant basis. For example, in the New Zealand FACE pasture experiment, legumes responded positively to $e\text{CO}_2$, constituting an increasing proportion of the total community biomass and productivity (Ross et al. 2004). Two leguminous understory trees increased biomass production with $e\text{CO}_2$ in a loblolly pine plantation (Mohan et al. 2006), but there have been no assessments of whether these species fix N under field conditions. Positive competitive responses of legumes to $e\text{CO}_2$ are by no means universal. Legumes

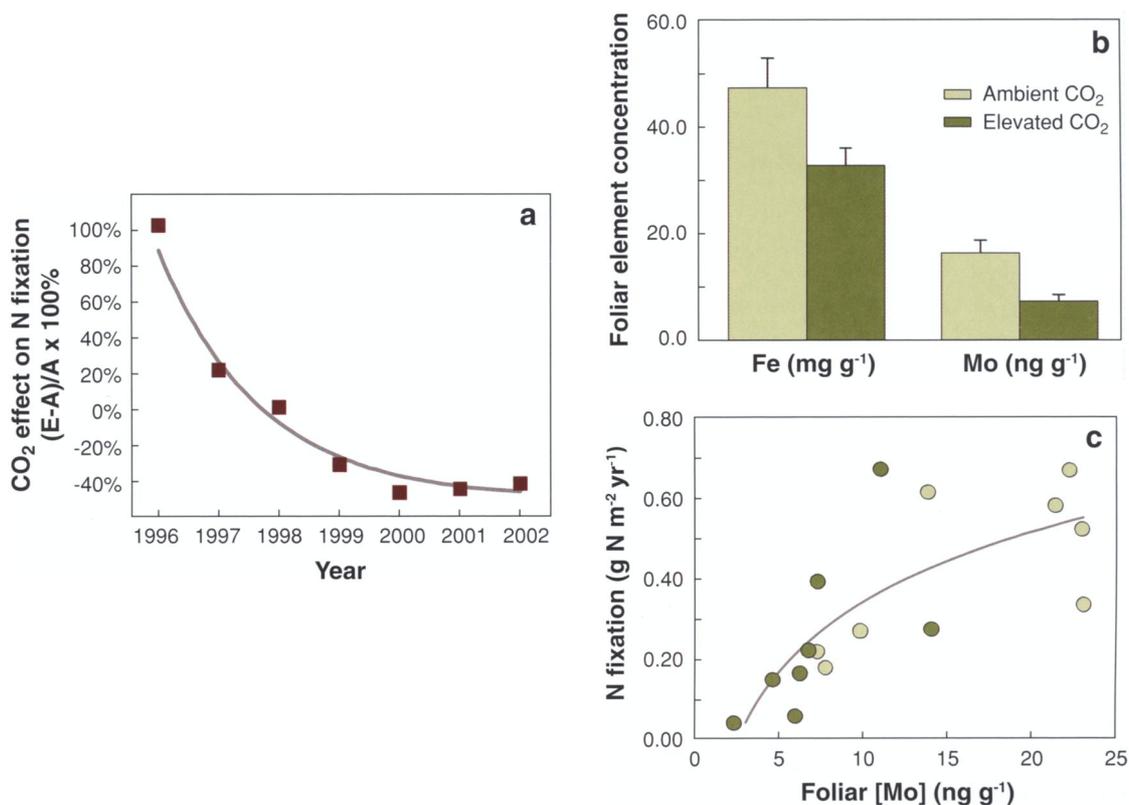


Figure 5

(a) The relative effect of elevated CO₂ on N fixation of *G. elliotii*, (b) the concentration of Fe and Mo, for ambient (light green) and elevated (dark green) treatments, and (c) correlation between foliar Mo concentration in *G. elliotii* and N fixation rate for plots exposed to ambient or elevated CO₂. From Hungate et al. 2004.

exhibited no significant abundance responses to eCO₂ in a calcareous grassland after six years of exposure to eCO₂ (Niklaus & Körner 2004), nor in an alpine grassland after four years (Arnone 1999). Similarly, over eight years in diverse assemblages in the Minnesota grassland, legumes made up a much larger fraction of plant cover in ambient than enriched N, but a similar fraction at eCO₂ as at ambient CO₂ at both N levels (P.B. Reich, unpublished data; **Figure 6**). In addition, eCO₂ can even depress productivity of N-fixing legumes as observed over time in the Florida scrub-oak ecosystem (Hungate et al. 2006).

What about free-living and associate N fixation in soil? In salt marsh, associative N₂ fixation increased after four months of eCO₂ (Dakora & Drake 2000), but in longer-term field experiments, eCO₂ did not appear to alter N₂ fixation by free-living heterotrophs. During years three to six of experimental treatment, soil N fixers were unresponsive to eCO₂ in a loblolly pine plantation (K. Hofmackel &

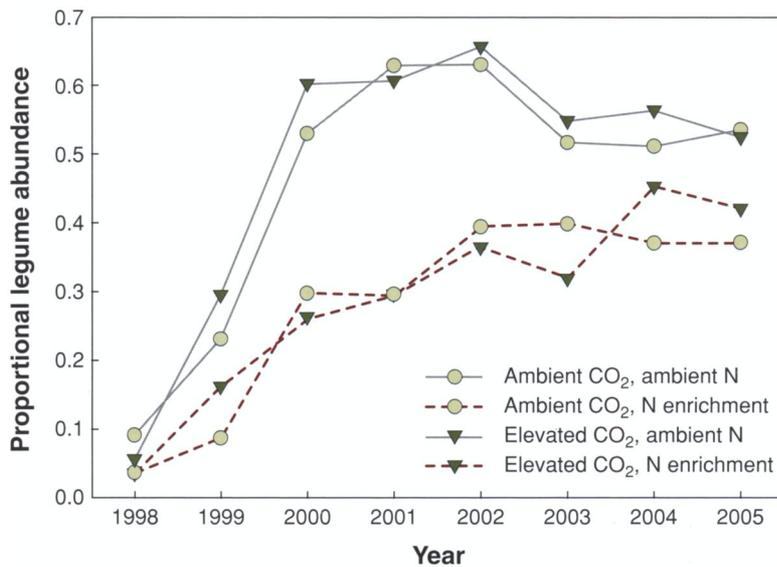


Figure 6

Legume abundance (total for four species) as a proportion of total abundance (based on aboveground biomass) in 16-species assemblages in the BioCON Minnesota FACE study over 8 years of treatment. From P.B. Reich, unpublished data.

W.H. Schlesinger, unpublished data). Bacterial N fixers were also unresponsive to $e\text{CO}_2$ in a desert ecosystem (Billings et al. 2003).

2.6. Carbon-Nitrogen Interactions: Other Trophic Dimensions

Elevated CO_2 usually increases plant growth rates, leaf C:N ratios, and secondary compounds, such as lignin and phenolics, and decreases N-based metabolites (Ainsworth & Long 2005, Matros et al. 2006). The increased C-based compounds and decreased N-based metabolites consequently impact trophic interactions with pathogens, herbivores, and mycorrhizal symbionts (Chakraborty & Datta 2003, N.C. Johnson et al. 2003, Matros et al. 2006, Mitchell et al. 2003).

2.6.1. Plant pathogens. The shift in the balance of C- and N-based secondary metabolites can enhance resistance to pathogen invasion under $e\text{CO}_2$. In inoculation experiments with *potato virus Y*, for example, the titer of viral coat-protein was markedly reduced in leaves under $e\text{CO}_2$ (Matros et al. 2006). Also, oats infected with the Barley Yellow Dwarf Virus showed a greater biomass response to $e\text{CO}_2$ than did uninfected oat plants (Malmström & Field 1997). $e\text{CO}_2$ also reduced disease incidence and severity of a red maple fungal pathogen due to reduced stomatal opening, reduced leaf N, and increased defensive chemistry (McElrone et al. 2005).

Increased plant resistance to pathogens and leaf C:N ratios under $e\text{CO}_2$ could, in theory, result in increased plant photosynthesis and production (e.g., Strengbom

& Reich 2006) and reduced decomposition. However, some studies have shown the opposite results, that $e\text{CO}_2$ promoted foliar diseases such as rusts, leaf spots, and blights owing to increased canopy size and density, decreased water stress, and increased canopy spore-trapping (Mitchell et al. 2003, Wand et al. 1999). In addition, some fungal pathogens produce more spores on host tissues under $e\text{CO}_2$ because of increased fecundity (Chakraborty & Datta 2003, Hibberd et al. 1996). The effects of $e\text{CO}_2$ and N on foliar fungal disease severity may also depend on the plant photosynthetic pathway (Mitchell et al. 2003). Given the diverse observed responses, it is difficult to draw general conclusions about the effects of $e\text{CO}_2$ on the interaction of plants and pathogens, let alone whether it systematically varies with N.

2.6.2. Herbivory. Plant-herbivore interactions are likely altered under $e\text{CO}_2$ because $e\text{CO}_2$ -induced changes in plant chemistry affect the quality of herbivore diets and can alter host plant preferences (Lindroth 1996). Decreased leaf N concentration and increased secondary compounds under $e\text{CO}_2$ reduce palatability and nutritional quality of foliage to some insect pests, especially at early larval stages (Agrell et al. 2000, Lindroth 1996). The detrimental effects may decrease herbivore growth rates, performance, and fecundity and increase insect developmental time and mortality (Lindroth 1996). However, some insect herbivores fed with CO_2 -enriched foliage increased their consumption rate to accumulate requisite amounts of N (i.e., compensatory consumption), especially at late larval stages (Agrell et al. 2000, Knepp et al. 2005). Nonetheless, overall herbivory may decrease under $e\text{CO}_2$ owing to decreased abundance of insects (Knepp et al. 2005).

However, N availability can exert considerable effects on these processes. For instance, survival rate and longevity of the silkworm (*Erisan*, a generalist herbivore) were lower when they were fed with birch, oak, and maple leaves under $e\text{CO}_2$ and infertile soil conditions than under more fertile conditions (Koiike et al. 2006). Responses to increased N deposition can counteract and mitigate the effects of $e\text{CO}_2$ on insect performance (Hättenschwiler & Schafellner 1999, Kerslake et al. 1998). However, the heterogeneity of changes in the chemistry (types of compounds) as well as the stoichiometry of plant tissues under $e\text{CO}_2$ makes it difficult to generalize.

2.6.3. Soil mutualists. Unlike pathogens and insect herbivores, mycorrhizal fungi [including arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi] form mutualistic association with most plant roots and link C transfers from plant to soil. Generally, stimulated supply of carbohydrate to roots under $e\text{CO}_2$ promotes the growth and colonization of mycorrhizae, resulting in increased uptake of limiting nutrients (i.e., N and P), which in turn facilitate plant growth and resistance to drought and pathogens (Allen et al. 2005, Olsrud et al. 2004, Treseder & Allen 2000). However, mycorrhizal responses to $e\text{CO}_2$ decline with added N (Treseder & Allen 2000). Additionally, mycorrhizae can be important mediators of plant community responses to $e\text{CO}_2$, which can be further adjusted by soil N availability (N.C. Johnson et al. 2003). Under a mycogenic view, increased fungal biomass could increase competition for nutrients between plants and mycorrhizae, shifting the fungal community toward less nutrient-limited species (Alberton et al. 2005).

3. ADDITIONAL MULTIPLE FACTOR INTERACTIONS

All of the processes and interactions discussed above are potentially sensitive to other environmental factors. Hence, the occurrence or strength of specific C-N interactions under $e\text{CO}_2$ may also depend on environmental factors such as soil pH, air or soil temperature, moisture availability, and/or other resources. In the following section, we briefly discuss a few potentially important multiple factor interactions, but note that empirical evidence about such interactions is generally quite scarce.

3.1. Interactive Effects of Temperature and Elevated Carbon Dioxide on Carbon-Nitrogen Interactions

Climate warming and $e\text{CO}_2$ could interactively alter plant and soil N cycling, which in turn could influence response to $e\text{CO}_2$. Experiments found altered $e\text{CO}_2$ fertilization response due to temperature only at temperatures substantially different than typical or optimal thermal environments (e.g., Dukes et al. 2005, Tjoelker et al. 1998). Such studies are rare, however, especially in the field, and typically can only detect dramatic interactions, given their limited replication (Norby & Luo 2004). Thus, we must use our understanding of interactions to deduce potential effects of temperature on coupled C-N cycling under $e\text{CO}_2$.

Temperature has both direct physiological (Tjoelker et al. 1999) and indirect biogeochemical (e.g., Shaw & Harte 2001, Wan et al. 2005) impacts on tissue-N concentrations. In cold regions, warming often increases leaf N concentrations because of enhanced soil N mineralization (Rustad et al. 2001, Shaw & Harte 2001), which in turn could enhance NPP responses to $e\text{CO}_2$ (e.g., **Figure 3**).

For instance, warming increased soil N availability and thereby enhanced relative responses of photosynthesis to $e\text{CO}_2$ (Kellomäki & Wang 1996) and a 3°C temperature increase alleviated the suppressive $e\text{CO}_2$ effects on N cycling (Loiseau & Soussana 2000). In a warmer region though, the initial stimulation of N mineralization by warming disappeared quickly (Wan et al. 2005), resulting in progressively larger decreases in green leaf N concentrations over time in warmed rather than in control plots (An et al. 2005). Whether interactive effects of $e\text{CO}_2$ and temperature on C and N dynamics are common or consistent requires further study, especially for long-term processes.

3.2. Influence of Water on Carbon-Nitrogen Interactions under Elevated CO_2

Intersite or interannual variation in water supply has been long hypothesized to be a potential regulator of $e\text{CO}_2$ effects, with positive responses expected in drier years or drier microenvironments. The main rationale for this hypothesis is that plants under $e\text{CO}_2$ have lower stomatal conductance (by far the most consistent $e\text{CO}_2$ effect out of dozens studied), which should ameliorate soil water deficits, all else being equal (Morgan et al. 2004).

Most studies do support the notion of modestly increased soil water under $e\text{CO}_2$. However, despite this, based on comparisons of responses in relatively dry versus wet

years, there is weak support for the hypothesized greater $e\text{CO}_2$ stimulation of productivity in dry versus wet years. Studies of aboveground biomass responses of grasslands and deserts supported this hypothesis in Kansas, Colorado, and Switzerland, but not in Texas, Minnesota, California, or New Zealand, and results from Nevada were opposite (Dukes et al. 2005, Hammerlynk et al. 2002, Morgan et al. 2004, Niklaus & Körner 2004, Owensby et al. 1999; also P.B. Reich, unpublished data). Unfortunately, it is difficult to draw a specific conclusion from these studies. Years that are relatively wet versus dry can vary in many other ways that could potentially confound or complicate the water availability effects, as can complex water-C-N interactions.

By reducing evapotranspiration, $e\text{CO}_2$ has been found to increase soil water content, and this has been invoked as a possible driver of increased plant N uptake and soil N turnover in some grassland ecosystems (Hungate et al. 1997, Rice et al. 1994). However, such effects will be modest or nonexistent if soil water enhancement under $e\text{CO}_2$ is modest or nonexistent, as can occur because of increased LAI (Ainsworth & Long 2005, Hungate et al. 2006).

We propose an alternative hypothesis. When water shortage is a predominant limitation compared with C or N, little positive response to $e\text{CO}_2$ or added N might occur, because the relative supply of each is high compared to water. According to such a hypothesis, we would expect a three-way interaction among C, N, and water availability: Response to high levels of all three would be greater than additive, but additionally $e\text{CO}_2$ might compensate for mild water limitation but be of little impact when water limitations were severe.

Direct evidence would come from experimental manipulations of water and CO_2 , and given widespread water limitations, one might expect reports of many such experiments. However, to our knowledge there is only one such experiment in the world, at Jasper Ridge, California, which involves a largely annual plant community in a strongly Mediterranean system. In the first five years of that study there was no evidence of a water- CO_2 interaction on productivity (Dukes et al. 2005), but there was also, surprisingly, no evidence of either a CO_2 effect or a water limitation on total NPP, and no water-N or water- CO_2 -N interactions. Dukes et al. (2005) hypothesized that a number of factors could be responsible for the lack of CO_2 or water effects as well as the lack of interactions, including phosphorus limitation.

In summary, elevated CO_2 does often slightly increase soil water, and higher soil water is usually associated with higher rates of net N mineralization, so moister conditions could ameliorate both the synergistic CO_2 -N interactions and PNL. This effect will likely occur only in systems where water and N colimit plant production, because the interaction requires a convergence of N-limited growth and water-limited soil activity. Also, this mechanism is self-limiting, because it will likely decline to the extent $e\text{CO}_2$ enhances growth and leaf area, minimizing changes in soil water content.

3.3. Multiple Element Interactions

The finding that positive responses of N fixation to $e\text{CO}_2$ depend on the availability of other nutrients illustrates the importance of element interactions beyond C and N. For example, the reduction in N_2 -fixation in the vine, *Galactia elliottii*, in the

scrub-oak ecosystem was accompanied by reduced foliar molybdenum (Mo) and iron (Fe), essential elements for N₂ fixation (**Figure 5**). After nine years of experimental treatment, eCO₂ tended to reduce extractable Mo concentrations in the soil (P = 0.069), implicating Mo limitation of N₂ fixation in this ecosystem (B. Duval & B.A. Hungate, unpublished data). Limitation of N fixation by Mo may not be a general phenomenon in terrestrial ecosystems, but the finding illustrates the importance of element interactions that may lurk lower in the periodic table than terrestrial biogeochemists are accustomed to looking.

Phosphorus limitation of the response of N₂ fixation to eCO₂ may be more common. In controlled-environment and short-term studies, low P availability often restricts the responses of N₂ fixers to eCO₂ (Edwards et al. 2006, Sa & Israel 1998). The small biomass and NPP responses to eCO₂ in the long-term calcareous grassland experiment in Switzerland were attributed to limitation of N₂ fixation by P (Niklaus & Körner 2004). In CO₂-P experiments using calcareous grassland mesocosms, growth of N fixers only responded to eCO₂ when P was also added (e.g., Niklaus & Körner 2004). Across all field experiments examined (van Groenigen et al. 2006), N fixation was far more responsive to eCO₂ in ecosystems receiving inputs of P (often in combination with K and Mg).

It is not known whether and to what extent interactions among the cycles of other elements influence ecosystem responses to eCO₂, in part because few experiments have documented responses or potential drivers. A survey of crop and short-term experiments showed that eCO₂ reduced plant concentrations of other elements, including P, K, Ca, S, Mg, Fe, Zn, Mn, and Cu (Loladze 2002). Consistent with findings from these short-term experiments, eCO₂ significantly reduced concentrations of many of these elements in long-term field studies (Hagedorn et al. 2001, D.W. Johnson et al. 2003; also S. Natali, unpublished data). Fe and Mo are involved in biological N transformations, so changes in their concentrations and availabilities with eCO₂ have implications for N cycling (e.g., Hungate et al. 2003).

Although eCO₂ caused no change in foliar concentrations of Zn in the Florida scrub-oak ecosystem, eCO₂ caused a 30% increase in Zn concentrations in the surface litter layer and a 17% reduction in Zn availability in the underlying mineral soil. Thus, eCO₂ can alter trace-element cycling through mechanisms other than changes in foliar chemistry. One such mechanism involves carbonic acid formation and base cation availability, both of which were found to increase in the loblolly pine experiment after three years of CO₂ enrichment (Andrews & Schlesinger 2001); such changes could exacerbate base cation losses in ecosystems subject to acid deposition.

In cases where eCO₂ does alter foliar element concentrations, reductions are by no means the universal response. Elevated CO₂ increased foliar concentrations of Mn by nearly 40% in the Florida scrub-oak site (D. Johnson et al. 2003), and increased concentrations of P and Zn in spruce-beech community exposed to eCO₂ for four years (Hagedorn et al. 2001) contrary to results from short-term controlled environment studies (Loladze 2002). Significant reductions and increases in element concentrations underscores the potential for global changes like rising CO₂ to alter the stoichiometries of plant-soil systems, which could in some cases shift or exacerbate nutrient limitations. For example, the 30% reduction in the N:P ratio of vegetation

in an acidic loam soil for spruce-beech suggests P limitation. Limitation by elements other than N could also contribute to negative or small responses to elevated CO₂ and other global changes in annual grasslands (Dukes et al. 2005, Shaw et al. 2002). These results show that eCO₂ has the potential to alter ecosystem stoichiometries. For the most part, however, the consequences of such changes are not well understood.

SUMMARY POINTS

1. Evidence from long-term field studies suggests that both progressive N limitation under eCO₂ and a significant interaction between CO₂ and plant available N supply that constrains NPP responses to eCO₂ are likely to be common, although not ubiquitous, in many natural and managed ecosystems. The combination of progressive N limitation and an interaction of CO₂ and plant available N supply will likely lead to smaller NPP enhancement under eCO₂ than widely anticipated.
2. Surprisingly, given the importance implications to Earth's C balance and future climate, there will likely be only a very small number (less than five) of long-term experiments that manipulate both CO₂ and N from which to extrapolate to the globe.

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