REVIEW *New Phytol.* (2000), **147**, 201–222

Research review

Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis

DONALD R. ZAK[1]*, KURT S. PREGITZER[2], JOHN S. KING[2] AND WILLIAM E. HOLMES[1]

[1]*School of Natural Resources & Environment, University of Michigan, 430 E. University Avenue, Ann Arbor, Michigan 48109–1115, USA*
[2]*School of Forestry and Wood Products, Michigan Technological University, 1400 Townsend Drive, Houghton, Michigan 49931, USA*

Received 4 November 1999; accepted 14 February 2000

SUMMARY

There is considerable uncertainty about how rates of soil carbon (C) and nitrogen (N) cycling will change as CO_2 accumulates in the Earth's atmosphere. We summarized data from 47 published reports on soil C and N cycling under elevated CO_2 in an attempt to generalize whether rates will increase, decrease, or not change. Our synthesis centres on changes in soil respiration, microbial respiration, microbial biomass, gross N mineralization, microbial immobilization and net N mineralization, because these pools and processes represent important control points for the below-ground flow of C and N. To determine whether differences in C allocation between plant life forms influence soil C and N cycling in a predictable manner, we summarized responses beneath graminoid, herbaceous and woody plants grown under ambient and elevated atmospheric CO_2. The below-ground pools and processes that we summarized are characterized by a high degree of variability (coefficient of variation 80–800%), making generalizations within and between plant life forms difficult. With few exceptions, rates of soil and microbial respiration were more rapid under elevated CO_2, indicating that (1) greater plant growth under elevated CO_2 enhanced the amount of C entering the soil, and (2) additional substrate was being metabolized by soil microorganisms. However, microbial biomass, gross N mineralization, microbial immobilization and net N mineralization are characterized by large increases and declines under elevated CO_2, contributing to a high degree of variability within and between plant life forms. From this analysis we conclude that there are insufficient data to predict how microbial activity and rates of soil C and N cycling will change as the atmospheric CO_2 concentration continues to rise. We argue that current gaps in our understanding of fine-root biology limit our ability to predict the response of soil microorganisms to rising atmospheric CO_2, and that understanding differences in fine-root longevity and biochemistry between plant species are necessary for developing a predictive model of soil C and N cycling under elevated CO_2.

Key words: elevated atmospheric CO_2, soil microorganisms, fine-root longevity, fine-root biochemistry, soil respiration, microbial activity, microbial N transformations.

INTRODUCTION

The physiological activities of plants and soil microorganisms control the flow of carbon (C) and nitrogen (N) in terrestrial ecosystems, a relationship that is likely to be altered by rising atmospheric CO_2. Throughout many regions of the Earth, plant growth is limited by the microbial release of NH_4^+ during the decomposition of organic matter (Fig. 1). Although soil temperature and water potential influence this process, the amount of inorganic N available for plant uptake is controlled by the biochemical constituents of litter, their use during microbial biosynthesis, and the N required to synthesize proteins, nucleic acids and other N-containing compounds within microbial cells (Smith & Paul, 1990; Paul & Clark, 1996). Plant-derived substrates entering soil that stimulate microbial growth (i.e. simple carbohydrates and organic acids) create a biosynthetic demand for N, thus increasing microbial immobilization and potentially lessening the amount available for plant uptake, whereas substrates providing relatively small amounts of

*Author for correspondence (fax +1 734 936 2195; e-mail drzak@umich.edu).

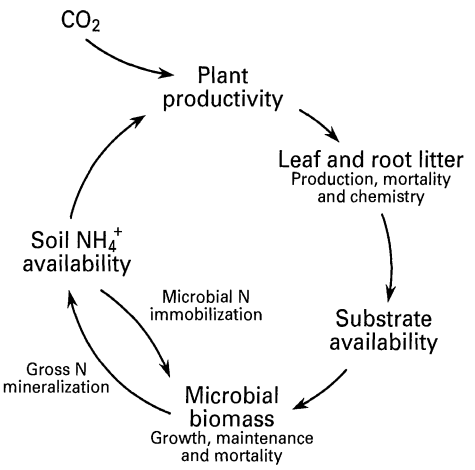

Fig. 1. A conceptual model illustrating the links between plant and microbial activity in terrestrial ecosystems, and the potential for this relationship to be altered by elevated [CO_2]. Plant production is often limited by quantities of N made available during the decomposition of fresh litter and organic matter in soil. At the same time, the growth and maintenance of soil microorganisms is controlled by the amount and type of organic compounds entering soil via plant litter production. Compounds in plant litter that fuel microbial growth also fuel a biosynthetic demand for N to build new N-containing compounds in microbial cells. If elevated CO_2 alters the amount of energy available for microbial growth in soil, it also has the potential to alter the microbial demand for N (i.e. microbial immobilization) and decrease the amount of inorganic N available for plant uptake.

energy for microbial metabolism (i.e. lignin and tannins) lessen the biosynthetic demand for N and decrease the rate of microbial immobilization. Rising atmospheric CO_2 has the potential to influence this relationship, because it can increase above- and below-ground plant growth (Poorter, 1993; Curtis & Wang, 1998), alter the production and chemical constituents of plant litter (Cotrufo *et al.*, 1994; Cotrufo & Ineson, 1995; King *et al.*, 1997) and influence the types of organic substrate available for microbial metabolism in soil. Therefore, changes in litter production under elevated CO_2 could alter the microbial demand for N and the flow of N between soil microorganisms and plant roots, a subject that has received growing attention over the past several years (Berntson & Bazzaz, 1997, 1998; Hungate *et al.*, 1997a,b, 1999; Zak *et al.*, 2000a).

Recently, a number of studies have focused on understanding whether elevated CO_2 alters soil C and N cycling; however, no clear pattern has emerged that allows us to generalize the response of soil microorganisms across different plants and ecosystems exposed to elevated CO_2. For example, rates of soil N cycling have been observed to increase (Zak *et al.*, 1993; Hungate *et al.*, 1997a,b), decrease (Diaz *et al.*, 1993; Berntson *et al.*, 1997, 1998) and remain constant (Zak *et al.*, 2000a) under elevated atmospheric CO_2, even in the same experiment (Hungate *et al.*, 1996). Understanding the factors that produce these divergent responses is important, because soil N availability controls the extent to which elevated CO_2 increases plant growth (McGuire *et al.*, 1995; Johnson *et al.*, 1997; Curtis & Wang, 1998; Zak *et al.*, 2000b), which in turn influences the amount of C that ecosystems sequester from the atmosphere. Consequently, it will be very difficult to predict long-term changes in ecosystem C storage as atmospheric CO_2 increases without discerning the mechanism(s) leading to the varied response of soil N cycling.

We contend that several gaps in our understanding of fine-root biology limit our ability to predict the response of soil microorganisms to rising atmospheric CO_2, and that differences in fine-root longevity between plant species can give rise to divergent responses of soil C and N cycling. For example, fine-root and mycorrhizal mortality constitute a substantial proportion of the organic substrates entering soil, often equivalent to or greater than inputs from above-ground plant tissues (Coleman, 1976; Fogel & Hunt, 1983; Vogt *et al.*, 1986). Nevertheless, we have a limited understanding of how fine-root production and longevity vary in plant taxa exposed to elevated CO_2 (Berntson & Bazzaz, 1996; Zak & Pregitzer, 1998) and whether differences in root longevity and mycorrhizal infection influence the types of organic substrate that are available for microbial metabolism. In this review we summarize changes in microbial processes under elevated atmospheric CO_2 that influence the flow of C and N in soil. We establish that a wide range of responses are exhibited by microbial communities beneath graminoid, herbaceous and woody plants exposed to elevated CO_2. We hypothesize that differences in fine-root production, mortality and biochemistry among plant taxa might influence microbial metabolism such that soil N availability could increase, decrease or not change under elevated atmospheric CO_2. Finally, we draw on evidence in the literature to support our idea that fine-root longevity and biochemistry could influence microbial activity in a manner that leads to very different patterns of soil C and N cycling.

ELEVATED [CO_2], FINE ROOTS AND SOIL MICROORGANISMS: A REVIEW

We reviewed 47 published studies that have evaluated the response of soil C and N cycling to elevated CO_2. These studies include a wide number of graminoid (14 spp.), herbaceous (8 spp.) and woody (18 spp.) plant species, as well as intact annual grasslands, tallgrass prairie and alpine pastures. From these reports we gathered information on soil respiration, microbial respiration, microbial biomass, gross N mineralization, microbial N immobilization and net N mineralization. Our analysis focused on these pools and fluxes, because they represent important control points for the below-

ground flow of C and N. We grouped information by plant life form (i.e. graminoid, herbaceous and woody) to discern whether differences in below-ground growth under elevated CO_2 predictably influence soil C and N dynamics. These results have been reported in a wide variety of formats (i.e. tables and figures) and units (i.e. by area and by mass). Our approach was to express values for each pool and flux in common terms, calculate the relative change between ambient and elevated-CO_2 treatments, describe experimental conditions and evaluate the range of responses. Results presented in bar and line graphs were digitized; the scale of each graph and the height of each data point were measured digitally to estimate values for that particular pool or process. Values reported in tables were taken directly from the publication and, when appropriate, converted to a common measure.

Soil and microbial respiration

The flux of CO_2 from soil represents the integrated response of plant roots and soil microorganisms to elevated atmospheric CO_2, and it can presage a change in the below-ground flow of C in soil. This process has been measured under a variety of experimental settings, which span a range of soil conditions (Table 1). With the exception of one observation (Ineson *et al.*, 1998), all studies report more rapid rates of soil respiration under elevated [CO_2]; however, very few of these increases are statistically significant (Table 1). In experiments with grasses and intact grasslands, soil respiration varied from a 10% decline beneath *Lolium perenne* to a 162% increase beneath *Bromus hordeaceus*. The mean response of grasses and grassland systems was a 51% increase; however, variability was relatively high (coefficient of variation (c.v.) = 100%). Observations of soil respiration beneath herbaceous plants are limited, but all studies report greater rates under elevated CO_2 (Table 1). In all cases, soil respiration beneath woody plants was more rapid under elevated atmospheric CO_2 ($42 \pm 24.1\%$; mean response $\pm$ SD); however, these increases also were variable and ranged from 5% to 93%.

Variation in the relative increase in soil respiration under elevated CO_2 likely results from a number of factors, some of which are difficult to quantify. Plants clearly differ in the degree to which elevated CO_2 stimulates above-ground and below-ground growth (Poorter, 1993; Rogers *et al.*, 1994; Curtis & Wang, 1998). Variability in growth response to elevated CO_2 undoubtedly translates to variability in soil respiration, because plant roots contribute substantially to the flux of CO_2 from soil. Soil microorganisms also contribute to soil respiration, and it is conceivable that elevated CO_2 differentially alters the types of plant-derived compounds entering soil that can be used for microbial metabolism. However, our understanding of this relationship is limited, making it difficult to draw any inference regarding its importance to soil respiration. The length of exposure to CO_2 could be another potential source of variability, especially if plant roots have not fully colonized soil during an experiment. Nevertheless, the fact that elevated CO_2 increased soil respiration in several intact grasslands where roots fully exploited soil before exposure to CO_2 suggests that such an effect is likely to be minimal (Hungate *et al.*, 1997b). Because we report the relative responses of soil respiration to elevated CO_2, it is also unlikely that differences in measurement technique (i.e. static vs dynamic chamber approaches) substantially contributed to the variability that we document in Table 1. Nevertheless, CO_2 fluxes from the soil of elevated CO_2 treatments might be overestimated if dynamic chamber measurements are made at the ambient concentration (i.e. greater diffusion gradient; D. Tingey, pers. comm.). However, there is insufficient information for an assessment of how such an effect has influenced rates reported in the literature.

Regardless of this high degree of variability, our analysis suggests that soil respiration will increase as atmospheric CO_2 continues to rise. Although there is considerable uncertainty regarding the relative contribution of roots, mycorrhizae and microbes to the flux of CO_2 from soil, more rapid rates of soil respiration under elevated CO_2 signal a greater below-ground flux of C in plants. Such a change has the potential to alter substrate availability for microbial metabolism, depending on how plants allocate the additional photosynthate acquired under elevated CO_2 to the production and maintenance of fine roots and mycorrhizae. Several pieces of evidence suggest that more rapid rates of soil respiration under elevated CO_2 largely result from greater root production, but the contribution of soil microorganisms to this greater flux is less clear.

Greater rates of soil respiration under elevated CO_2 could, in part, result from greater root biomass, increases in the specific respiration rate of roots (μmol CO_2 g^{-1} s^{-1}) or some combination of both. In a review, Rogers *et al.* (1994) reported that elevated CO_2 increased root biomass in the vast majority (87%) of studies that they summarized, a finding consistent with more rapid rates of soil respiration under elevated CO_2 (Table 1). Recently, Pregitzer *et al.* (2000) found a significant, positive relationship between soil respiration and the biomass of *Populus tremuloides* roots growing under ambient and elevated CO_2. In this experiment, elevated CO_2 did not influence the specific respiration rate of fine roots (i.e. less than 1 mm) (D. E. Rothstein *et al.*, unpublished), suggesting that a greater root biomass under elevated CO_2 contributed to more rapid rates of soil respiration. This differs from the observations of Edwards & Norby (1999), who found significant

Table 1. *Soil respiration (root plus microbial respiration) under ambient and elevated CO_2 (see Table notes for details)*

Plant species	Respiration (μg C m^{-2} d^{-1}) Ambient [CO_2]	Elevated [CO_2]	Relative change[a] (%)	CO_2 exposure[b] (μmol mol^{-1})	Soil conditions	Reference
I. Graminoid						
Avena fatua	5103	6592	29	710; SC	Serpentine	Luo *et al.* (1996)
Calycadenia multiglandulosa	1116	1276	14	710; SC	Serpentine	Luo *et al.* (1996)
Bromus hordeaceus	852	2232	162	710; SC	Serpentine	Luo *et al.* (1996)
Hemizonia congesta	1272	2868	125	710; SC	Serpentine	Luo *et al.* (1996)
Loluim multiflorum	2233	5210	133	710; SC	Serpentine	Luo *et al.* (1996)
Lolium perenne	13680	12276	−10	600; FA	Native soil	Ineson *et al.* (1998)
Scirpus olneyi[c]	6946	8087	16	705; SC	Native soil	Ball & Drake (1998)
Sorghum bicolor	3892	5208	34	720; LC		Prior *et al.* (1997a)
Spartina patens[c]	6532	7776	19	705; SC	Native soil	Ball & Drake (1998)
Spartina patens/Scirpus olneyi	5132	5650	10	705; SC	Native soil	Ball & Drake (1998)
Triticum aestivum[d]	1090	1707	56*	700; GC		Lekkerkerk *et al.* (1990)
Annual grassland, California	885	1205	36	710; SC	Sandstone	Hungate *et al.* (1997b)
Annual grassland, California	2105	2934	46	710; SC	Serpentine	Luo *et al.* (1996)
Annual grassland, California	2537	3683	45	710; SC	Sandstone	Luo *et al.* (1996)
Mean response			51			
SD			51.6			
II. Herbaceous						
Glycine max	307	371	21*	720; LC	34 kg N ha^{-1}	Prior *et al.* (1997b)
Plantago erecta	1169	2126	80	710; SC	Serpentine	Luo *et al.* (1996)
Vulpia michrostachys	3295	4891	48	710; SC	Serpentine	Luo *et al.* (1996)
Forb-dominated grassland, California	2015	2934	46	710; SC	Serpentine	Luo *et al.* (1996)
Mean response			49			
SD			24.2			
II. Woody						
Acer rubrum	756	964	27*	700; LC	Native soil	Edwards and Norby (1999)
Acer rubrum	756	871	15*	700; LC	Native soil + 3.3°C warming	Edwards and Norby (1999)
Acer saccharum	752	788	5*	700; LC	Native soil	Edwards and Norby (1999)
Acer saccharum	752	949	26*	700; LC	Native soil + 3.3°C warming	Edwards and Norby (1999)
Castanea sativa[c]	2422	3697	53*	700; GH		Rouhier *et al.* (1996)
Liriodendron tulipifera	6843	8512	24	500; LC	Native soil	Norby *et al.* (1992)
Liriodendron tulipifera	6843	8325	22	750; LC	Native soil	Norby *et al.* (1992)

Pinus ponderosa	1400	2200	57	525; LC	0 kg N ha^{-1}	Johnson *et al.* (1994)
Pinus ponderosa	1400	2700	93	700; LC	0 kg N ha^{-1}	Johnson *et al.* (1994)
Pinus ponderosa	3400	6000	76	700; LC	100 kg N ha^{-1}	Johnson *et al.* (1994)
Pinus ponderosa	1800	2600	44	525; LC	200 kg N ha^{-1}	Johnson *et al.* (1994)
Pinus ponderosa	1800	2400	33	700; LC	200 kg N ha^{-1}	Johnson *et al.* (1994)
Pinus ponderosa	1044	1844	76	+175; LC	Averaged over N treatments	Vose *et al.* (1995)
Pinus ponderosa	1044	1522	45	+350; LC	Averaged over N treatments	Vose *et al.* (1995)
Pinus ponderosa	5393	8951	66*	525; LC	Averaged over N treatments	Vose *et al.* (1997)
Pinus ponderosa	5393	7444	38*	700; LC	Averaged over N treatments	Vose *et al.* (1997)
Pinus taeda	4431	5478	24	+200; FA	Native soil	Andrews (2000)
Populus tremuloides	1883	2391	27	715; SC	Low-N soil	Mikan *et al.* (2000)
Populus tremuloides	3110	4782	54*	715; SC	High-N soil	Mikan *et al.* (2000)
Populus tremuloides	911	1131	24	707; LC	Low-N soil	Pregitzer *et al.* (2000)
Populus tremuloides	1959	2350	20*	707; LC	High-N soil	Pregitzer *et al.* (2000)
Quercus alba	4977	6071	22	500; LC	Native soil	Norby (1996)
Quercus alba	4977	6818	37	750; LC	Native soil	Norby (1996)
Model tropical forest	1970	3732	89*	610; GC	Sand, vermiculite + surface Litter	Körner & Arnone (1992)
Mean response			42			
SD			24.1			

[a]For example, the percentage change in soil respiration = 100 (elevated − ambient)/ambient.
[b]FA, FACE exposure; LC, large open-top chambers; SC, small open-top chambers; GC, growth chamber; GH, glasshouse.
[c]Values reported are the mode of soil respiration, not the mean.
[d]Soil respiration reported in mg C per soil column; the dimensions of the soil column were not given.
[e]Soil respiration reported in mg C per pot; the dimensions of the pots were not given.
Values have been summarized for soil beneath grasses, herbaceous plants and woody plants growing under ambient and elevated CO_2. Where soil respiration was reported in μmol CO_2 m^{-2} s^{-1}, we converted it to mg C m^{-2} d^{-1}. The increase or decrease in soil respiration at elevated CO_2 was calculated relative to the flux at ambient CO_2. An asterisk indicates a significant increase or decrease.

Table 2. *Response of microbial respiration to elevated atmospheric CO_2 (see Table notes for details)*

Plant species	Respiration (μg C g^{-1} d^{-1}) Ambient CO_2	Elevated CO_2	Relative Change[a] (%)	CO_2 exposure[b] (μmol mol^{-1})	Soil conditions	Reference
I. Graminoid						
Lolium perenne/Trifolium repens	5.4	5.8	7	700; GC		Newton *et al.* (1995)
Lolium perenne/Trifolium repens	45.7	51.7	13	700; GC		Ross *et al.* (1995)
Triticum aestivum	7.7	9.3	15	550; FA	214 kg N ha^{-1}; 24 kg P ha^{-1}	Prior *et al.* (1997b)
Tallgrass prairie, Kansas[c]	1.6	1.8	11	700; LC	0 kg N ha^{-1}	Rice *et al.* (1994)
Tallgrass prairie, Kansas[c]	1.4	2.2	60*	700; LC	45 kg N ha^{-1}	Rice *et al.* (1994)
Annual grassland, California	2.5	3.3	30	710; SC	Serpentine	Hungate *et al.* (1997b)
Annual grassland, California	3.5	7.1	103	710; SC	Serpentine + 200 kg N,P,K ha^{-1}	Hungate *et al.* (1997b)
Mean response			34			
SD			35.4			
II. Herbaceous						
Carex curvula	2.1	2.3	10	680; SC	0 kg N ha^{-1}	Niklaus & Körner (1996)
Carex curvula	2.4	3.9	62*	680; SC	45 kg N ha^{-1}	Niklaus & Körner (1996)
Gossypium hirsutum	8.7	11.6	33	550; FA	Non-irrigated	Wood *et al.* (1994)
Gossypium hirsutum	9.3	12.4	33	550; FA	Irrigated	Wood *et al.* (1994)
Forb-dominated grassland, California	2.5	3.3	30	710; SC	Serpentine	Hungate *et al.* (1997b)
Mean response			34			
SD			18.5			
III. Woody						
Ficus spp.[d]	0.20	0.24	19	610; GH	170 g N ha^{-1}	Insam *et al.* (1999)
Pinus ponderosa	2.43	2.33	−4	525; LC	0 kg N ha^{-1}	Johnson *et al.* (1994)
Pinus ponderosa	2.43	2.83	16	700; LC	0 kg N ha^{-1}	Johnson *et al.* (1994)
Pinus ponderosa	2.55	2.85	12	700; LC	100 kg N ha^{-1}	Johnson *et al.* (1994)
Pinus ponderosa	2.30	3.02	72	525; LC	200 kg N ha^{-1}	Johnson *et al.* (1994)
Pinus ponderosa	2.30	2.32	1	700; LC	200 kg N ha^{-1}	Johnson *et al.* (1994)
Populus grandidentata	0.77	0.99	28*	693; SC	Rhizosphere	Zak *et al.* (1993)
Populus grandidentata	0.58	0.67	15	693; SC	Bulk soil	Zak *et al.* (1993)
Mean response			20			
SD			23.3			

[a]For example, the percentage change in microbial respiration = 100 (elevated − ambient)/ambient.
[b]FA, FACE exposure; LC, large open-top chambers; SC, small open-top chambers; GC, growth chamber; GH, glasshouse.
[c]Tallgrass prairie dominated by *Andropogon gerardii* and *Sorghastum nutans*.
[d]Model tropical ecosystem containing *Elettaria cardamonum*, *Ficus benjamin*, *F. pumila*, *Heliconia humulis*, *Ctenanthe lubbersiana*, *Cecropia peltata*, *Epipremnum pinnatum*.
Values have been summarized from laboratory studies in which microbial respiration was measured in soil collected beneath grasses, herbaceous plants and woody plants. For studies in which microbial respiration was measured as CO_2-C accumulation, we divided the total amount of CO_2-C produced by the total days of incubation (i.e. μg C g^{-1} d^{-1}). The increase or decrease in microbial respiration at elevated CO_2 was calculated relative to the amount at ambient CO_2. An asterisk indicates a significant increase or decrease.

increases in the specific respiration rate of *Acer rubrum* and *Acer saccharum* roots (1–2 mm diameter) growing under elevated CO_2. However, root biomass increased to a much greater extent than did specific root respiration, leading Edwards & Norby to conclude that increases in root biomass contributed primarily to significantly greater rates of soil respiration under elevated CO_2. Other authors have concluded that increases in root biomass under elevated CO_2 contributed to a greater flux of CO_2 from soil (Nakayama *et al.*, 1994; Vose *et al.*, 1995); however, it is likely that a portion of this increase was due to greater microbial activity.

At present it is difficult to discern the contribution of microorganisms to the flux of CO_2 from soil. Nevertheless, insight can be gained from laboratory studies that measure microbial respiration in root-free soil under conditions conductive to microbial activity (i.e. field capacity and 25–30°C) (Table 2). Such an approach is useful for understanding changes in substrate availability under elevated CO_2 that influence microbial respiration, and thus the flux of CO_2 from soil. With one exception, all studies have reported greater rates of microbial respiration under elevated CO_2; however, the degree to which microbial respiration was stimulated under elevated CO_2 is highly variable (Table 2). For example, microbial respiration increased from 7% to 103% beneath grasses and intact grasslands, it varied from 10% to 30% beneath herbaceous plants and forb-dominated pasture, and it ranged from a 4% decline to a 72% increase beneath woody plants (Table 2). It is noteworthy that microbial respiration beneath single species, ponderosa pine (*Pinus ponderosa*), encompassed the entire range of response for woody plants. Variability in the response of microbial respiration to elevated CO_2 was similar to that of soil respiration; c.v. values ranged from 54% (herbaceous plants) to 100% (grasses and woody plants).

Because substrate availability drives microbial metabolism in soil, it is likely that much of the variability in microbial respiration resulted from differences in plant growth response to elevated CO_2 and subsequent increases in litter production. Differences in the length of incubation contribute further to the variability in Table 2. Microbial respiration follows first-order kinetics (Zak *et al.*, 1993, 1999), and as a consequence the rate of microbial respiration declines as the length of incubation increases. Notwithstanding the high degree of variability, the relatively consistent increase in microbial respiration across studies suggests that greater plant growth under elevated CO_2 provides more organic substrates for microbial metabolism in soil. This seems to be true regardless of differences in the growth and C allocation patterns of grasses, herbs and woody plants. Nevertheless, the high degree of variability that we document suggests that we cannot currently predict the extent to which microbial metabolism will change under elevated CO_2.

Soil microbial biomass

The biomass of soil bacteria, actinomycetes and fungi is small on an ecosystem basis (*c.* 1–3% of C and N; Wardle, 1992), and it is unlikely that a change in microbial biomass alone would substantially alter soil N availability or C storage. However, the flow of substrates through microbial biomass is a key factor influencing soil N availability and C storage, so understanding the turnover of microbial biomass (biomass/assimilation rate) is central to predicting a change in soil C and N cycling under elevated CO_2. At present, few studies have simultaneously measured a change in microbial biomass and the assimilation of C or N (Hungate *et al.*, 1996, 1997a; Berntson & Bazzaz, 1997, 1998; Mikan *et al.*, 2000; Zak *et al.*, 2000a), making it difficult to predict how elevated CO_2 will influence the flow of C and N through microbial biomass. Nevertheless, a relatively large number of studies have measured microbial biomass (C or N) under a wide array of experiment settings in which plants are grown under ambient and elevated CO_2 (Table 3).

Although soil and microbial respiration generally increased under elevated CO_2 (Tables 1 and 2), the response of microbial biomass encompasses both large increases and large declines (Table 3). This is well illustrated by graminoid species growing under elevated CO_2, beneath which microbial biomass exhibited a 280% decline (*Vulpia michrostachys*) and a 100% increase (*Avena fatua*). Given that these species occur together in annual grasslands, the wide range of responses beneath them supports the idea that species-specific responses to elevated CO_2 can elicit very different changes in soil microbial communities. This might be true of grasses and intact grasslands in which microbial biomass increased, decreased or did not respond to elevated CO_2. Beneath herbaceous species, the response of microbial biomass encompassed a smaller range, spanning a 1% decline beneath *Carex curvula* to a 64% increase beneath *Plantago erecta*; nevertheless, variability was substantial (c.v. = 100%). The relative change in microbial biomass beneath woody plants ranged from a 52% decline to a 121% increase, somewhat narrower than the response beneath grasses.

The high degree of variability within plant life-form groups, in combination with responses that range from large declines to large increases, clearly indicates that shifts in substrate availability under elevated CO_2 can elicit a variety of changes in microbial biomass. However, it is not clear what factors contribute to this wide range of responses,

Table 3. *Response of microbial biomass (C) to elevated atmospheric CO_2 (see Table notes for details)*

Plant species	Biomass (μg C g^{-1}) Ambient CO_2	Elevated CO_2	Relative Change[a] (%)	CO_2 exposure[b] (μmol mol^{-1})	Soil conditions	Reference
I. Graminoid						
Agrostis capillaris	–	–	90*	700; GC		Diaz *et al.* (1993)
Avena fatua	200	333	66	710; SC		Hungate *et al.* (1996)
Avena fatua	1213	3017	148	710; SC	200 kg N,P,K ha^{-1}	Hungate *et al.* (1996)
Bromus hordeaceus	200	400	100	710; SC		Hungate *et al.* (1996)
Bromus hordeaceus	909	1212	33	710; SC	200 kg N,P,K ha^{-1}	Hungate *et al.* (1996)
Lolium multiflorum	400	187	−47*	710; SC		Hungate *et al.* (1996)
Lolium multiflorum	3636	3030	17	710; SC	200 kg N,P,K ha^{-1}	Hungate *et al.* (1996)
Lolium perenne	926	1019	9	600; FA	140 kg N ha^{-1}	Schortemeyer *et al.* (1996)
Lolium perenne	888	992	12	600; FA	560 kg N ha^{-1}	Schortemeyer *et al.* (1996)
Lolium perenne	167	177	6*	692; GC	110 kg N ha^{-1} + P,K,Mg	Schenk *et al.* (1995)
Lolium perenne/Trifolium repens	957	916	−4	700; GC		Ross *et al.* (1995)
Lolium perenne/Trifolium repens	1480	1480	0	700; GC		Newton *et al.* (1995)
Vulpia michrostachys	757	151	−280*	710; SC		Hungate *et al.* (1996)
Vulpia michrostachys	606	1212	100	710; SC	200 kg N,P,K ha^{-1}	Hungate *et al.* (1996)
Tallgrass prairie, Kansas[c]	1487	1555	4	700; LC	0 kg N ha^{-1}	Rice *et al.* (1994)
Tallgrass prairie, Kansas[c]	1354	1674	23	700; LC	45 kg N ha^{-1}	Rice *et al.* (1994)
Model grassland	195	195	0	550; GC	0–10 cm; nutrient poor	Kampichler *et al.* (1998)
Annual grassland, California[d]	324	457	41	710; SC	sandstone	Hungate *et al.* (1997a)
Annual grassland, California[d]	143	143	0	710; SC	sandstone + 200 kg N,P,K ha^{-1}	Hungate (1999)
Mean response			17			
SD			86.1			
II. Herbaceous						
Carex curvula	69	72	4	680; SC	0 kg N ha^{-1}	Niklaus & Körner (1996)
Carex curvula	71	70	−1	680; SC	45 kg N ha^{-1}	Niklaus & Körner (1996)
Lasthenia californica	333	545	63	710; SC		Hungate *et al.* (1996)
Lasthenia californica	2424	2424	0	710; SC	200 kg N,P,K ha^{-1}	Hungate *et al.* (1996)
Plantago erecta	424	697	64	710; SC		Hungate *et al.* (1996)
Plantago erecta	1212	1212	0	710; SC	200 kg N,P,K ha^{-1}	Hungate *et al.* (1996)
Rumex obtusifolius	–	–	40*	700; GC		Diaz *et al.* (1993)
Trifolium repens	704	820	16	600; FA	140 kg N ha^{-1}	Schortemeyer *et al.* (1996)
Trifolium repens	768	964	25	600; FA	560 kg N ha^{-1}	Schortemeyer *et al.* (1996)
Trifolium repens	201	225	12*	692; GC	110 kg N ha^{-1} + P,K,Mg	Schenk *et al.* (1995)
Forb-dominated grassland, California	238	323	36	710; SC	Serpentine	Hungate *et al.* (1997a)

Mean response			29			
SD			29.2			
III. Woody						
Artemisia tridentata	9	12	33	700; GC		Klironomos *et al.* (1996)
Artemisia tridentata	9	19	111*	700; GC	Hoaglands solution	Klironomos *et al.* (1996)
Betula alleghaniensis and B. papyrifera	3213	2817	−12	700; GH	Forest floor	Berntson & Bazzaz (1998)
Betula alleghaniensi	559	608	9	700; GH	Forest floor	Berntson & Bazzaz (1997)
Ficus spp.[e]	46	58	27	610; GH	170 g N ha^{-1}	Insam *et al.* (1999)
Populus × *euramericana*	1281	1088	−15	690; SC	Low-N soil; rhizosphere	Lussenhop *et al.* (1998)
Populus × *euramericana*	3879	2542	−52	690; SC	High-N soil; rhizosphere	Lussenhop *et al.* (1998)
Populus grandidentata	117	259	121*	693; SC	Rhizosphere	Zak *et al.* (1993)
Populus grandidentata	54	77	42*	693; SC	Bulk soil	Zak *et al.* (1993)
Populus tremuloides	7	9	28	715; SC	Low-N soil	Mikan *et al.* (2000)
Populus tremuloides	28	30	7	715; SC	High-N soil	Mikan *et al.* (2000)
Populus tremuloides	56	54	−3	707; LC	Low-N soil	Zak *et al* (2000b)
Populus tremuloides	246	252	2	707; LC	High-N soil	Zak *et al.* (2000b)
Pinus taeda	565	569	1	+200; FA	Native soil	Allen *et al.* (2000)
Quercus myrtifilia, Q. chapmanni, Q. geminata	176	152	−13	715; LC	Native soil	Hungate *et al.* (1999)
Mean response			19			
SD			45.7			

[a]For example, the percentage change in microbial biomass = 100(elevated − ambient)/ambient.
[b]FA, FACE exposure; LC, large open-top chambers; SC, small open-top chambers; GC, growth chamber; GH, glasshouse.
[c]Tallgrass prairie dominated by *Andropogon gerardii* and *Sorghastum nutans*.
[d]Plant composition: *Avena barbata, bromus hordeaceus, Nassela pulchra, Lotus wrangelianus, Hemizonia congesta*.
[e]Model tropical ecosystem containing *Elettaria cardamonum, Ficus benjamina, F. pumila, Heliconia humulis, Ctenanthe lubbersiana, Cecropia peltata, Epipremnum pinnatum*.
Microbial biomass C reported on an areal basis (mg C m^{-2}) was converted to a mass basis (μg g^{-1}) using bulk density (Db) and depth of soil sampling. When Db was not available for a particular study, we used a value of 1 Mg m^{-3}. We also converted reports of microbial biomass N to biomass C using a C:N of 10. Most studies measured microbial C or N using fumigation–extraction procedures, except for Klironomos *et al.* (1996), who estimated microbial biomass by direct observation. We converted their biomass estimates to C, assuming microbial biomass to be 47% C. The increase or decrease in microbial biomass at elevated CO_2 was calculated relative to the amount at ambient CO_2. An asterisk indicates a significant increase or decrease.

even beneath plants that occur together in nature. Unless we identify these factors we shall not be able to predict changes in the turnover of C and N through microbial biomass, and hence the cycling and storage of these elements in soil.

It is well established that plant productivity and microbial biomass are positively related across a wide range of soils (Schimel, 1986; Burke, 1989). Given that elevated CO_2 stimulates plant productivity with few exceptions (Poorter, 1993; Curtis & Wang, 1998), one would expect microbial biomass to increase under elevated CO_2 in a manner consistent with the growth response of plants. Although microbial biomass declined, increased and did not change under elevated CO_2, most (67%) studies that we summarized observed greater amounts of microbial biomass, suggesting that greater substrate inputs under elevated CO_2 led to a larger pool of microbial biomass. However, variability in plant growth response and substrate availability are insufficient to explain why microbial biomass declined in 19% of the studies and did not change in the remainder (14%).

Enhanced levels of bacterial and fungal grazing by soil animals might be partly responsible for declines in microbial biomass under elevated CO_2. Plant production fuels the flow of energy through soil food webs, and several studies have documented that greater below-ground plant growth under elevated CO_2 can stimulate the transfer of C from organisms occupying low trophic levels (i.e. bacteria and fungi) to those higher in the soil food web such as protozoa, nematodes and collembola (Runion *et al.*, 1994; Yeates *et al.*, 1997; Jones *et al.*, 1998; Lussenhop *et al.*, 1998; Hungate *et al.*, 2000). In some studies, bacterial or fungal biomass declined by a small margin under elevated CO_2, the result of enhanced predation by larger populations of protozoa, nematodes or collembola. For example, Lussenhop *et al.* (1998) observed a non-significant decline in rhizosphere microbial biomass (−15% to −52%) under elevated CO_2, whereas the number of protozoa increased by 150–550%. It is possible that enhanced bacterial grazing by protozoa and greater rates of fungal consumption by collembola could increase the turnover and decrease the biomass of soil microorganisms. However, in some instances, greater bacterial or fungal predation have increased the turnover of microbial biomass with no substantial change in its size (Klironomos *et al.*, 1996; Jones *et al.*, 1998; Kampichler *et al.*, 1998), suggesting that predation could also account for no change in microbial biomass under elevated CO_2. These observations indicate that trophic interactions figure prominently in whether microbial biomass declines or does not change under elevated CO_2 and whether greater turnover of microbial cells increases the flow of C and N in the plant–soil system. Such a response has the potential to influence the composition and function of soil food webs by altering bacterial and fungal assemblages (Schortenmeyer *et al.*, 1996; Jones *et al.*, 1998) and modifying their use of plant-derived substrates (Rillig *et al.*, 1997; Hungate *et al.*, 2000). Clearly, there is much to be learned about the composition and function of soil food webs and how they might be altered by changes in substrate availability under elevated CO_2. Developing our understanding of these dynamics seems central to predicting rates of soil C and N cycling under elevated CO_2.

It is also likely that microbial biomass might not respond to elevated CO_2 under experimental conditions in which plant roots have not fully colonized soil. In this situation, the influence of root-associated C inputs on microbial physiology is likely to be small compared with the influence of the relatively large pool of native soil organic matter already present in soil. In a recent experiment, we observed that elevated atmospheric CO_2 substantially increased net fine-root production only when soil N was in abundant supply (Pregitzer *et al.*, 2000). Although the input of organic substrates from fine-root production clearly increased under elevated CO_2, we observed no change in the biomass of soil microorganisms during a 3-yr experiment with *Populus tremuloides* (Zak *et al.*, 2000a). In this soil, fine root biomass was 1/100 of the labile pool of organic matter and 1/1000 of the total organic matter content. It is possible that the increase in root-derived substrates entering soil from enhanced fine-root production under elevated CO_2 was insufficient to alter the influence of native organic matter on microbial physiology.

Most studies reporting changes in microbial biomass and activity under elevated CO_2 are characterized by relatively large below-ground inputs of plant C and relatively small pools of soil organic matter. In California annual grasslands, in which plant C inputs under elevated CO_2 can increase or decrease microbial biomass, soil organic matter is 13–15-fold that in plant roots and below-ground detritus (ambient CO_2, calculated from Hungate *et al.* (1997a,c)). Plant roots in these grassland soils are equivalent to the biomass of soil microorganisms, in contrast to our experiment in which microbial biomass was 50–90-fold that in plant roots (calculated from Pregitzer *et al.* (2000) and Zak *et al.* (2000b)). Similarly, microbial biomass and activity were not altered by elevated CO_2 in a calcareous grassland in which microbial biomass was 0.05% of the soil organic matter content (Niklaus, 1998). In this ecosystem, the annual increase in below-ground plant growth under elevated CO_2 ranged from 260 to 360 g C m^{-2}, which is 1–2% of the C already stored in mineral soil (12 kg C m^{-2}) (Körner *et al.*, 1997; Niklaus, 1998). Because only a fraction of this extra C enters soil annually, increased below-ground litter under elevated CO_2 represented

only a small fraction of the organic substrates already present. This supports the idea that microbial biomass will not change in situations where increases in root production under elevated CO_2 are small compared with amount of organic matter already present in soil. Consequently, with short-term experiments, it will be difficult to determine the influence of greater above-ground or below-ground plant growth on microbial communities in soil that contain relatively large quantities of organic matter. This is probably true in alpine pastures, tallgrass prairies and forests, in which large quantities of organic matter accumulate in surface soil horizons.

At present we do not have the ability to predict changes in soil microbial biomass under elevated CO_2, nor do we have a sufficient understanding of the factors controlling the turnover of substrates through microbial biomass. The highly variable response of microbial biomass beneath graminoid, herbaceous and woody plants growing under elevated CO_2 clearly indicates that differences in plant life form have little bearing on the response of microbial communities. Rather, it seems that developing an understanding of trophic interactions in soil, changes in the composition and function of soil food webs, and the relative importance of additional substrate produced under elevated CO_2 might help to explain the divergent responses that we document. We cannot predict changes in soil C and N cycling without understanding how a greater substrate availability under elevated CO_2 will influence microbial biomass and the flow of C and N through it.

Soil nitrogen dynamics

There is considerable debate regarding the response of soil N dynamics to elevated CO_2, because observations indicate that rates of soil N cycling can increase (Zak *et al.*, 1993; Hungate *et al.*, 1997a), decline (Berntson & Bazzaz, 1997, 1998) or not change (Zak *et al.*, 2000b), even within the same ecosystem (Hungate *et al.*, 1996). Understanding changes in rates of gross N mineralization and microbial immobilization lie at the heart of predicting whether elevated CO_2 will alter the amount of N available for plant uptake (i.e. net N mineralization) (Fig. 1). These processes occur simultaneously in soil, albeit at different rates depending on the organic compounds present and the amount of N required during microbial biosynthesis. For example, an abundant supply of carbohydrates will fuel microbial growth and protein synthesis, and any amino acids produced during the degradation of 'free' proteins in soil solution will be directly assimilated by microbial cells for protein synthesis. Microbes will assimilate NH_4^+ and NO_3^- from soil solution to satisfy any need for additional N during protein biosynthesis. However, when carbohydrate supply is insufficient to meet the maintenance requirements of microbial cells, mortality occurs and the constituents of the dead cells begin to serve as substrates for surviving microorganisms. Under energy-limited conditions (i.e. low carbohydrate supply), amino acids produced during the degradation of 'free' proteins are deaminated, and the resulting organic acids are used to generate cellular energy. Ammonium is subsequently released into soil solution, where it can be assimilated by plant roots, participate in cation exchange reactions, or enter into other microbially mediated processes. Thus, the amount and type of plant-derived substrates produced under elevated CO_2 control the amount of energy available for microbial growth and maintenance, and, in turn, control whether inorganic N will be assimilated from, or released into, soil solution.

In Table 4 we have compiled studies in which gross rates of N mineralization have been estimated beneath plants grown under ambient and elevated CO_2. Most measurements have been made with isotope dilution techniques in short-term (2–5 d) laboratory incubations (Hart *et al.*, 1994). However, several experiments have labelled entire plant–soil systems with ^{15}N to estimate these processes (Berntson & Bazazz, 1997, 1998; Mikan *et al.*, 2000). Nevertheless, only a small number of studies have measured gross N mineralization, making it difficult to draw inferences about how this process might change under elevated CO_2 (Table 4). Of the handful of studies described in the literature, there are no reports of statistically significant changes gross N mineralization under elevated CO_2.

The variability in the response of gross N mineralization to elevated CO_2 is striking within and among the three plant life forms. In annual grasslands, elevated CO_2 increased rates of gross mineralization by 5%, but this response also was extremely variable (c.v. = 400%). Only one observation of gross mineralization has been made beneath herbaceous plants (33% increase), whereas the largest number of observations have been made beneath woody plants (Table 4). Elevated CO_2 produced a 49% decline beneath *Betula alleghaniensis* and a 17% increase beneath *Populus tremuloides*, two early-successional trees with similar life history traits. Beneath woody plants, the mean response was a 13% decline and the coefficient of variation was 200%.

Although observations are limited, the broad range of response in gross N mineralization suggests that organic substrates entering soil under elevated CO_2 substantially differ in the way in which they influence microbial growth and the demand for N during biosynthesis. Lower rates of gross N mineralization under elevated CO_2 imply that substrate inputs under elevated CO_2 stimulated growth and bio-

Table 4. *Gross nitrogen mineralization under ambient and elevated CO_2 (see Table notes for details)*

Plant species	Gross N mineralization ($\mu g\ Ng^{-1}\ d^{-1}$) Ambient CO_2	Elevated CO_2	Relative Change[a] (%)	CO_2 exposure[b] ($\mu mol\ mol^{-1}$)	Soil conditions	Reference
I. Graminoid						
Annual grassland, California	1.51	1.82	20	710; SC	Sandstone	Hungate *et al.* (1997a)
Annual grassland, California[c]	1.23	1.11	−19	710; SC	Sandstone	Hungate *et al.* (1997c)
Annual grassland, California[c]	4.81	5.43	13	710; SC	Sandstone; 200 kg N,P,K ha^{-1}	Hungate (1997c)
Mean response			5			
SD			(20.8)			
II. Herbaceous						
Annual grassland, California	0.99	1.31	33	710; SC	Serpentine	Hungate *et al.* (1997a)
Mean response			?			
SD						
III. Woody						
Betula alleghaniensis and B. papyrifera	2.21	1.29	−41	700; GH	Forest floor	Berntson & Bazzaz (1998)
Betula alleghaniensis	159.50	81.71	−49	700; GH	Forest floor	Berntson & Bazzaz (1997)
Populus tremuloides[d]	0.07	0.07	0	715; SC	Low-N soil	Mikan *et al.* (2000)
Populus tremuloides[e]	0.18	0.21	17	715; SC	High-N soil	Mikan *et al.* (2000)
Populus tremuloides	0.24	0.22	−8	707; LC	Low-N soil	Zak *et al.* (2000b)
Populus tremuloides	0.47	0.48	1	707; LC	High-N soil	Zak *et al.* (2000b)
Quercus myrtifilia, Q. chapmanni, Q. geminata				715; LC	Native soil	Hungate *et al.* (1999)
Mean response			−13			
SD			25.9			

[a]For example, the percentage change in gross N mineralization = 100(elevated − ambient)/ambient.
[b]FA, FACE exposure; LC, large open-top chambers; SC, small open-top chambers; GC, growth chamber; GH, glasshouse.
[c]Plant composition: *Avena barbata, Bromus hordeaceus, Nassela pulchra, Lotus wrangelianus, Hemizonia congesta.*
[d]Isotope dilution conducted in the field by labelling soil within small chambers.
Values have been summarized from laboratory studies using isotope dilution techniques to estimate gross rates of soil N transormation beneath grasses, herbaceous plants and woody plants. Rates reported on an areal basis ($mg\ N\ m^{-2}\ d^{-1}$) were converted to a mass basis ($\mu g\ Ng^{-1}\ d^{-1}$) using soil bulk density (Db) and depth of soil sampling. When Db was not available for a particular study, we used a value of 1 $Mg\ m^{-3}$. The increase or decrease in gross N mineralization at elevated CO_2 was calculated relative to the flux at ambient CO_2. An asterisk indicates a significant increase or decrease.

synthesis, whereas higher rates of gross N mineralization imply the input of low-energy-yielding substrates that lessen the biosynthetic need for N and increase the catabolism of amino acids and amino sugars for energy. Nevertheless, it is difficult to interpret the impact of gross N mineralization on microbial N dynamics without understanding the response of microbial immobilization.

Reports of microbial immobilization are more abundant in the literature (Table 5), but only a handful of investigators have measured gross N mineralization and microbial immobilization in concert (Berntson & Bazazz, 1997, 1998; Hungate *et al.*, 1997a,c; Mikan *et al.*, 2000; Zak *et al.*, 2000a). Beneath grasses and intact grasslands, microbial immobilization exhibited large declines (-64%) and large increases (+501%); with few exceptions, these responses are not statistically significant (Table 5). It is noteworthy that this range of responses includes species that occur together within the annual grasslands of California (Table 5), further suggesting that species-specific differences in substrate production under elevated CO_2 might lie at the heart of understanding divergent responses by soil microbial communities. It is also noteworthy that N fertilization did not modify the response of microbial immobilization to elevated CO_2 in a predictable manner; N fertilization both increased and decreased the response to CO_2, depending on the plant species present. Such a response probably reflects species-specific changes in below-ground allocation and tissue chemistry in response to greater N availability. The overall mean response of microbial immobilization was a 77% increase beneath grasses and intact grasslands, but there was considerable variability (c.v. = 220%).

Fewer studies have documented changes in microbial immobilization beneath herbaceous species; for those that have done so, variability is very high. For example, the mean response beneath herbaceous species was a 186% increase, but responses ranged from a 67% decline (*Plantago erecta*) to an 876% increase (*Lasthenia californica*). Similar to the response of grasses, about half of the studies reported increases in microbial immobilization. Moreover, N fertilization also did not influence the response of microbial immobilization to elevated CO_2 in a predictable manner beneath herbaceous plants: it led to higher immobilization beneath *Lasthenia californica* and lower immobilization beneath *Plantago erecta* (Table 5). As with grasses, these species are clearly responding in very different ways to greater C and N availability. Variability in microbial immobilization beneath woody plants was similar to that of grasses and forbs, displaying large increases as well as large declines under elevated CO_2 (Table 5). Microbial immobilization declined beneath *Betula* spp., exhibited little change or increased beneath *Populus tremuloides*, and increased beneath *Quercus* spp. growing under elevated CO_2. The mean response was a 55% increase beneath woody plants (c.v. = 209%).

Rates of gross N mineralization and microbial immobilization both displayed large increases and declines under elevated CO_2. We suggest that a change in the types of organic substrate entering under elevated CO_2 is the agent responsible for these observations. Greater rates of microbial immobilization under elevated CO_2 indicate a more abundant supply of substrates that stimulate microbial biosynthesis, thus increasing the amount of N needed for the synthesis of amino acids, proteins, nucleic acids and other N-containing compounds. In contrast, lower rates of microbial immobilization suggest the input of substrates that yield little energy for microbial metabolism, and thus do not stimulate an additional need for N. Of the studies in which gross N mineralization and microbial immobilization have been measured in concert (Berntson & Bazazz, 1997, 1998; Hungate *et al.*, 1997a,c; Mikan *et al.*, 2000; Zak *et al.*, 2000a), all possible permutations of increases and decreases in gross N mineralization and microbial immobilization are present, further suggesting that substrate inputs under elevated CO_2 stimulate microbial biosynthesis to very different degrees beneath different plant species.

Although the documented responses of gross N mineralization and microbial immobilization are few and variable, a larger number of studies have measured net N mineralization beneath graminoid, herbaceous and woody plants. Net N mineralization is the difference between gross N mineralization and microbial immobilization, and it is the balance of these processes that supplies plants with most of the N that they assimilate from soil solution. This process has been measured by incubating root-free soil in the field or laboratory (Hart *et al.*, 1994). Net N mineralization also has been estimated as the net increment of N that plants take up over the course of a particular experiment; it assumes no losses of N to leaching or denitrification.

Under elevated CO_2, rates of net N mineralization have increased and decreased beneath grasses and intact grasslands, ranging from a 10% decline to a 51% increase. In these ecosystems, four studies reported more rapid rates of net N mineralization and two reported declines, although none of these was statistically significant. The mean response beneath graminoid species was a 23% increase (c.v. = 112%). Beneath herbaceous species, most (four of five) studies documented an increase in net N mineralization, but most of these increases were small (4–9%) and none of the responses was significant. On average, net N mineralization increased beneath herbaceous plants growing under elevated CO_2 (11%) (Table 6); however, variability was high (c.v. = 218%). Net N mineralization beneath woody plants growing under elevated CO_2

Table 5. *Gross nitrogen immobilization under ambient and elevated CO_2 (see Table notes for details)*

Plant species	Gross N immobilization ($\mu g\ Ng^{-1}\ d^{-1}$) Ambient CO_2	Elevated CO_2	Relative Change[a] (%)	CO_2 exposure[b] ($\mu mol\ mol^{-1}$)	Soil conditions	Reference
I. Graminoid						
Avena fatua	0.42	2.50	501*	710; SC		Hungate *et al.* (1996)
Avena fatua	2.71	4.16	54	710; SC	200 kg N,P,K ha^{-1}	Hungate *et al.* (1996)
Bromus hordeaceus	1.67	1.88	13	710; SC		Hungate *et al.* (1996)
Bromus hordeaceus	0.83	2.71	225	710; SC	200 kg N,P,K ha^{-1}	Hungate *et al.* (1996)
Lolium multiflorum	0.83	0.63	−25	710; SC		Hungate *et al.* (1996)
Lolium multiflorum	1.66	6.25	276	710; SC	200 kg N,P,K ha^{-1}	Hungate *et al.* (1996)
Vulpia michrostachys	1.46	0.52	−64	710; SC		Hungate *et al.* (1996)
Vulpia michrostachys	1.75	0.83	−52	710; SC	200 kg N,P,K ha^{-1}	Hungate *et al.* (1996)
Annual grassland, California	2.08	1.94	−7	710; SC	Sandstone	Hungate *et al.* (1997a)
Annual grassland, California	1.98	1.75	−11	710; SC	Serpentine	Hungate *et al.* (1997a)
Annual grassland, California[c]	0.86	0.83	−3	710; SC	Sandstone	Hungate *et al.* (1997c)
Annual grassland, California[c]	1.48	3.83	16*	710; SC	Sandstone; 200 kg N,P,K ha^{-1}	Hungate *et al.* (1997c, 1999)
Mean response			77			
SD			169.9			
II. Herbaceous						
Lasthenia californica	1.25	3.33	167*	710; SC		Hungate *et al.* (1996)
Lasthenia californica	0.62	6.04	867	710; SC	200 kg N,P,K ha^{-1}	Hungate *et al.* (1996)
Plantago erecta	2.50	0.83	67*	710; SC		Hungate *et al.* (1996)
Plantago erecta	2.91	2.16	−26	710; SC	200 kg N,P,K ha^{-1}	Hungate *et al.* (1996)
Forb-dominated grassland, California	1.98	1.75	−11	710; SC	Serpentine	Hungate *et al.* (1997a)
Mean response			186			
SD			391.1			
III. Woody						
Betula alleghaniensis and *B. papyrifera*	2.05	1.00	−50	700; GH	Forest floor	Berntson & Bazzaz (1998)
Betula alleghaniensis	8.49	4.90	−42	700; GH	Forest Floor	Berntson & Bazzaz (1997)
Populus tremuloides[d]	0.13	0.42	223	715; SC	Low-N soil	Mikan *et al.* (2000)
Populus tremuloides	0.32	0.99	209	715; SC	High-N soil	Mikan *et al.* (2000)
Populus tremuloides	0.45	0.42	−6	707; LC	High-N soil	Zak *et al.* (2000b)
Quercus myrtifilia, Q. chapmanni, Q. geminata	0.10	0.14	47	715; LC	Native soil	Hungate *et al.* (1999)
Mean response			55			
SD			114.6			

[a]For example, the percentage change in gross N immobilization = 100(elevated−ambient)/ambient.
[b]FA, FACE exposure; LC, large open-top chambers, SC, small open-top chambers; GC, growth chamber; GH, glasshouse.
[c]Plant composition: *Avena barbata, Bromus hordeaceus, Nassela pulchra, Lotus wrangelianus, Hemizonia congesta*; microbial immobilization estimated as the difference between NH_4^+ consumption and gross nitrification.
[d]Isotope dilution conducted in the field by labelling soil within small chambers.
Values have been summarized from laboratory studies using isotope dilution techniques to estimate gross rates of soil N transformations beneath grasses, herbaceous plants and woody plants. Rates reported on an areal basis ($mg\ C\ m^{-2}\ d^{-1}$) were converted to a mass basis ($\mu g\ N\ g^{-1}\ d^{-1}$) using soil bulk denmsity (Db) and depth of soil sampling. When Db was not available for a particular study, we used a value of 1 $Mg\ m^{-3}$. The increase or decrease in gross N mineralization at elevated CO_2 was calculated relative to the flux at ambient CO_2. An asterisk indicates a significant increase or decrease.

Table 6. *Net nitrogen mineralization under ambient and elevated CO_2 (see Table notes for details)*

Plant species	Net N mineralization ($\mu g\ N\ g^{-1}\ d^{-1}$) Ambient CO_2	Elevated CO_2	Relative Change[a] (%)	CO_2 exposure[b] ($\mu mol\ mol^{-1}$)	Soil conditions	Reference
I. Graminoid						
Lolium perenne/Trifolium repens	2.39	2.30	−4	700; GC		Newton *et al.* (1995)
Triticum aestivum[c]	0.95	1.14	20	700; GC	0 kg N ha^{-1}	Billes *et al.* (1993)
Triticum aestivum[c]	1.24	1.69	36	700; GC	50 kg ha^{-1}	Billes *et al.* (1993)
Triticum aestivum	0.41	0.37	−10	550; FA	214 kg N ha^{-1}; 24 kg P ha^{-1}	Prior *et al.* (1997b)
Model grassland	5.65	5.21	−8	550; GC	0–10 cm; nutrient poor	Kampichler *et al.* (1998)
Annual grassland, California[c]	0.04	0.06	47	710; SC	Sandstone	Hungate *et al.* (1997a)
Annual grassland, California[c]	0.04	0.06	51	710; SC	Serpentine	Hungate *et al.* (1997a)
Mean response			19			
SD			26.4			
II. Herbaceous						
Carex curvula[d]	12.8	11.0	−14	680; SC	Native soil	Arnone (1997)
Gossypium hirsutum	0.88	0.93	6	550; FA	Non-irrigated	Wood *et al.* (1994)
Gossypium hirsutum	0.96	1.05	9	550; FA	Irrigated	Wood *et al.* (1994)
Lolium perenne/Trifolium repens	3.71	3.86	4	700; GC		Ross *et al.* (1995)
Forb-dominated grassland, California[c]	0.04	0.06	51	710; SC	Serpentine	Hungate *et al.* (1997a)
Mean response			11			
SD			24.0			
III. Woody						
Betula alleghaniensis and *B. papyrifera*	7.30	3.30	−54	700; GH	Forest floor	Berntson & Bazzaz (1998)
Betula alleghaniensis	0.15	0.35	127	700; GH	Forest floor	Berntson & Bazzaz (1997)
Pinus taeda	0.10	0.11	16	+200; FA	Native soil	Allen *et al.* (2000)
Populus tremuloides[c]	0.22	0.22	0	715; SC	Low-N soil	Mikan *et al.* (2000)
Populus tremuloides[c]	0.32	0.50	57*	715; SC	High-N soil	Mikan *et al.* (2000)
Populus tremuloides	0.06	0.06	5	707; LC	Low-N soil	Zak *et al.* (2000b)
Populus tremuloides	0.32	0.31	−2	707; LC	High-N soil	Zak *et al.* (2000b)
Populus grandidentata	0.47	2.60	553*	693; SC	Bulk soil	Zak *et al.* (1993)
Mean response			8			
SD			195.2			

[a]For example, the percentage change in net N mineralization = 100(elevated − ambient)/ambient.
[b]FA, FACE exposure; LC, large open-top chambers, SC, small open-top chambers; GC, growth chamber; GH, glasshouse.
[c]Net N mineralization estimated as the accumulation of N in plant biomass during the experiment; it assumes no losses of N to leaching and denitrification.
[d]Net N mineralization estimated with ion-exchange resin bags; units are $\mu mol\ N\ mol^{-1}$ of ion exchange capacity. Data are the means of 1993 and 1994 growing seasons.
Values have been summarized from laboratory and field studies in which net N mineralization was in soil collected beneath grasses, herbaceous plants and woody plants. We converted areal estimates of N mineralization ($g\ N\ m^{-2}\ d^{-1}$) to a mass basis ($\mu g\ N\ g^{-1}\ d^{-1}$) using Db and the depth of soil sampling; we assumed 1.0 $Mg\ m^{-3}$ where published Db values were not available. The increase or decrease in net N mineralization at elevated CO_2 was calculated relative to the flux at ambient CO_2. An asterisk indicates a significant increase or decrease.

Table 7. *A summary of the below-ground response of plant roots and soil microorganisms to elevated CO_2 (see Table notes for details)*

Pool or process	Mean response (%)	Coefficient of variation (%)	Significant responses/ total observations	Percentage of observations displaying:		
				Increase	Decrease	No change
Soil respiration	+45	80	12/41	96	4	0
Microbial respiration	+28	96	3/20	95	5	0
Microbial biomass	+19	326	8/45	62	18	20
Gross N mineralization	−3	800	0/10	40	40	20
Microbial immobilization	+93	231	3/24	50	42	8
Net N mineralization	+44	285	2/19	68	11	21

Changes in soil respiration, microbial respiration, microbial biomass, gross N mineralization, microbial immobilization and net N mineralization are presented, because they are key pools and fluxes controlling the cycling of C and N. Responses have been averaged across graminoid, herbaceous and woody plants grown under ambient and elevated CO_2 in soil. Positive values (+) represent an increase and negative values (−) represent a decrease under elevated CO_2. We assume that the values ranging from +3 to −3% constituted no response.

was also variable (c.v. = 222%), displaying increases, decreases and no change (Table 6). Responses beneath woody plants encompassed a 553% increase and a 54% decrease, the largest range of response among the three plant life forms. Of the 19 observations in Table 6, only two changes in net N mineralization under elevated CO_2 are significant.

Similarly to gross N mineralization and microbial immobilization, there are no clear patterns of response across or within graminoid, herbaceous and woody plant species. In some arid ecosystems, greater plant water-use efficiency under elevated CO_2 can increase soil matric potential and enhance rates of soil N cycling (Hungate *et al.*, 1997a), but this plant-mediated change in the soil environment is likely to be of limited importance in ecosystems in which water is not limiting. Notwithstanding this effect, the consistent high degree of variability in microbial N transformations makes it impossible to generalize about how greater plant growth under elevated CO_2 will alter the cycling of N in soil and change the amounts of N available for plant uptake. This is particularly troublesome because the links between plant litter production, substrate chemistry, microbial biosynthesis and the uptake or release of N by microbial communities ultimately control the extent to which elevated CO_2 increases the productivity of terrestrial ecosystems.

Summary and synthesis

The response of soil microorganisms to changes in plant production under elevated CO_2 is highly variable, making broad generalizations difficult. Variability within a plant life form for most variables was equivalent to variability between plant life forms, indicating that these groupings have a limited usefulness for understanding how soil C and N cycling will change under elevated CO_2 (Table 7). The average number of studies per variable was eight when grouped by plant life form, but increased to 25 when averaged across plant life forms. The relatively limited number of observations within a life form group might, in part, contribute to the present uncertainty in microbial responses to elevated CO_2. Alternatively, there might be very different patterns of plant C allocation within a life-form type that obscure our ability to make generalizations about the response of soil microbial communities to elevated CO_2. It would be useful to group microbial response by plant phylogeny; however, the limited number of studies currently in the literature precludes such an analysis.

In Table 7 we have summarized the overall responses of soil microbial communities to elevated CO_2. We calculated the mean response averaged across plant life-form group and c.v. The clearest and least variable responses were those of soil respiration and microbial respiration. These processes had the lowest overall coefficient of variation, and the most common response across studies was greater rates of soil and microbial respiration (Table 7). Taken together, these results suggest that greater amounts of C are being allocated below ground under elevated CO_2, and that soil microorganisms are metabolizing the additional substrate. This is consistent with ^{14}C labelling experiments investigating the transfer of C between plant roots and soil microorganisms, in which the amount of ^{14}C substrate entering soil and its use by the microbial community increased under elevated CO_2 (Lekkerkerk *et al.*, 1990; Rouhier *et al.*, 1996; Paterson *et al.*, 1999; Mikan *et al.*, 2000).

Although it is clear that greater plant growth under elevated CO_2 increases substrate availability and microbial metabolism, it is not clear how increases in substrate availability influence microbial biomass, gross N mineralization, microbial immobil-

ization, and hence the rate at which N becomes available to plants (i.e. net N mineralization). These pools and processes all displayed large increases as well as large declines under elevated CO_2. Given the values in Table 7, we can be least confident about a change in gross N mineralization (c.v. = 800%), followed by microbial biomass, net N mineralization and microbial immobilization. It is noteworthy that an equivalent number of studies have documented higher and lower rates of gross N mineralization and microbial immobilization under elevated CO_2. This observation, in combination with the fact that microbial biomass can increase or decrease, strongly suggests that elevated CO_2 can differentially alter the turnover of C and N through microbial biomass. Arguments about whether soil N cycling will increase (Zak *et al.*, 1993) or decrease (Diaz *et al.*, 1993) under elevated CO_2 are unproductive because both responses occur in nature; this is clearly documented in Table 7. Our challenge is to understand why these divergent responses occur. Without doing so, we are unable to predict whether rates of soil C and N cycling will increase, decline or not change as atmospheric CO_2 continues to rise over the next several decades.

A soil environment more conducive to microbial activity, resulting from increases in plant water-use efficiency under elevated CO_2, seems to be an important factor controlling microbial responses to CO_2 in arid ecosystems (Hungate *et al.*, 1997a). Higher soil matric potentials beneath annual grasslands exposed to elevated CO_2 are known to stimulate microbial activity and soil N cycling. However, it is likely that this mechanism cannot account for the high degree of variability across studies, especially those in mesic environments. Large declines and increases in microbial immobilization and microbial biomass imply that elevated CO_2 induced changes in plant-derived substrates. This change seems to have increased and decreased the amount of energy available for microbial metabolism, which, in turn, drives the biosynthetic demand for N within microbial cells. We contend that understanding how elevated CO_2 alters fine-root production, longevity and biochemistry among plant taxa should serve as a starting point for resolving the uncertainty surrounding the response of soil microbial communities to elevated CO_2.

ELEVATED [CO_2], FINE ROOTS AND THE RESPONSE OF SOIL MICROORGANISMS: A HYPOTHESIS

Most studies at elevated CO_2 have been conducted over time steps ranging from weeks to years, suggesting that root-derived substrates have a proportionately greater influence on soil C availability than above-ground litter. For example, fine-root and mycorrhizal mortality occur throughout the growing season (Hendrick & Pregitzer, 1992; Fitter *et al.*, 1997) and these organic matter inputs enter mineral soil directly. In tree species, 40–50% of fine roots die and disappear within a single year (Hendrick & Pregitzer, 1992; Burke & Raynal, 1994; Fahey & Hughes, 1994), and this proportion can be even greater in grasslands (Fitter *et al.*, 1997). Moreover, elevated CO_2 can disproportionately increase net fine-root production over mortality (Pregitzer *et al.*, 1995; Day *et al.*, 1996; Pregitzer *et al.*, 2000), although such a response is not universal (Berntson & Bazazz, 1996; Fitter *et al.*, 1997). In contrast, above-ground litter production in most experiments occurs as a seasonal pulse deposited at the soil surface. Given the relatively short duration of most experiments with elevated CO_2 and the direct input of root-derived substrates into mineral soil, it likely that fine-root turnover and rhizodeposition exert a much greater influence on soil microorganisms than above-ground litter. Unfortunately, there are no studies of below-ground dynamics under elevated CO_2, or otherwise, in which fine-root demography and biochemistry are directly coupled to substrate availability and microbial activity (i.e. respiration, N immobilization, gross mineralization). Experiments in which these physiologically linked processes are measured in concert have the potential to explain why rates of soil N cycling increase, decrease or do not change under elevated CO_2.

A few studies have observed changes in root demography under elevated CO_2, and there seems to be a commonality among them. Elevated CO_2 increases fine-root production in most species, but this response can be dampened by low soil fertility (Pregitzer *et al.*, 1995; Berntson & Bazzaz, 1996; Day *et al.*, 1996; Tingey *et al.*, 1996; Fitter *et al.*, 1997; Kubiske *et al.*, 1998; Pregitzer *et al.*, 2000). Increases in fine-root production under elevated CO_2 undoubtedly give rise to a greater standing crop of fine roots, which has been documented in a large number of studies (Rogers *et al.*, 1994, 1997). Nevertheless, the influence of elevated CO_2 on fine-root mortality is more variable. Day *et al.* (1996) observed that elevated CO_2 decreased fine-root mortality in *Quercus* spp., whereas elevated [CO_2] increased fine-root mortality in *Populus* × *euramericana* (Pregitzer *et al.*, 1995). Fine-root mortality also increased in *Betula papyrifera* grown under elevated CO_2, but elevated CO_2 had no effect on the mortality of *Acer rubrum* fine roots (Berntson & Bazazz, 1996). In an experiment with *Pinus radiata*, elevated CO_2 increased fine-root mortality by 244% relative to mortality under ambient CO_2 (Thomas *et al.*, 1999). The fine roots of *Populus tremuloides* also exhibited greater mortality under elevated CO_2, but only when soil N was abundant (Kubiske *et al.*, 1998; Pregitzer *et al.*, 2000). In grasslands exposed to elevated CO_2, fine-

root mortality substantially increased in a species-rich plant community growing on peat, whereas it increased by a small margin in a species-poor plant community on limestone-derived soil (Fitter *et al.*, 1997). These studies demonstrate that elevated CO_2 can increase, decrease or not change fine-root mortality, depending on the species of plant and the level of soil fertility. Given the diversity of plant life forms and patterns of C allocation within and between plant taxa, it is conceivable that elevated atmospheric [CO_2] influences fine-root production and mortality in a species-specific manner. Consequently, experiments are needed to quantify fine-root demography across a range of plant life-history traits before we can draw any conclusion regarding the below-ground response of plants to elevated CO_2.

Species-specific patterns of fine-root longevity could influence the types of substrate available for microbial metabolism in soil. As the ontogeny of an individual fine root progresses, cortical browning occurs (Hendrick & Pregitzer, 1992) owing to the deposition of condensed tannins (Richards & Considine, 1981; McKenzie & Peterson, 1995a,b) and lignin (Van Fleet, 1957; Schreiber, 1996). Therefore, condensed tannin concentrations and lignin should increase as fine roots live longer, thereby causing a decline in the quality of substrates available for microbial growth. The relationship between longevity and the concentration of condensed tannins and lignin has been observed in the fine roots of trees and perennial herbs (McKenzie & Peterson, 1995a,b; Schreiber, 1996); however, we are unaware of similar studies for annuals or perennial grasses. In some plants, fine roots can disappear well before the process of browning occurs (Hendrick & Pregitzer, 1992) and condensed tannins and lignin have accumulated. There are two alternative explanations for this observation: (1) browning does not occur, or occurs to a limited extent, in some species, or (2) fine roots have been consumed or decomposed well before the browning process has occurred. The first has important implications for our ideas and suggests that the relationship between longevity and biochemistry might not be universal in plants. The second alternative is consistent with our idea: young, white roots with low concentrations of condensed tannins and lignin are more likely to be browsed by soil animals or decomposed by microorganisms than older, brown roots with high concentrations of these compounds. However, there is insufficient information to distinguish between these alternatives at present. Notwithstanding this limitation, it is clear that condensed tannins and lignin increase with fine-root longevity in woody and herbaceous species, an effect that has important implications for the types of substrate available for microbial metabolism in soil.

If elevated CO_2 increases the longevity of small-diameter roots (<0.5 mm), then one would expect the concentration of lignin and condensed tannins to increase too. This is consistent with greater concentrations of total phenolics and condensed tannin in the lateral roots of *Pinus taeda* grown under elevated CO_2 (Gebauer *et al.*, 1998); however, fine-root longevity was not measured in this study. Such a response should be the opposite for species in which elevated CO_2 lowers longevity: if fine-root life span declines, then the concentration of lignin and condensed tannins in root litter should also decline. Although greater concentrations of these compounds in fine roots would slow their decomposition, they would not stimulate microbial biosynthesis and N immobilization to a significant extent, at least in the short term. Nevertheless, a change in the percentage of lignin and condensed tannins implies a concomitant change in the proportion of other biochemical constituents in litter, which might have a more important influence on rates of microbial immobilization and soil N cycling.

Concentrations of non-structural carbohydrate (i.e. simple sugars and starch) and N in fine roots are likely to be important factors contributing to changes in microbial biosynthesis and N immobilization under elevated CO_2. Non-structural carbohydrates are energy-rich substrates for microbial growth, and greater inputs from fine roots should fuel a biosynthetic need for N. Moreover, there is a consistent, negative relationship between the concentration of non-structural carbohydrate and N in most plant tissues. Mooney *et al.* (1995) suggested that this relationship results from the fact that amino acid and starch syntheses compete for a common pools of photosynthate, and are therefore mutually exclusive biosynthetic processes. As a consequence, fine roots with higher concentrations of non-structural carbohydrate should stimulate microbial biosynthesis, but these tissues contain less N to build amino acids, proteins, nucleic acids and other N-containing compounds in microbial cells. Therefore, increases in non-structural carbohydrate concentrations have the potential for greatly stimulating a biosynthetic need for N, leading to higher rates of microbial immobilization. The extent to which microbial immobilization would be enhanced would depend on the degree to which non-structural carbohydrate and N concentrations are affected by elevated CO_2.

There are potential differences between plant species in the amount of photosynthate allocated to non-structural carbohydrates in fine roots (Eissenstat & Yanai, 1997), and there are clear seasonal patterns within species as well (Nguyen *et al.*, 1990). However, only a handful of studies have measured non-structural carbohydrates in fine roots grown under elevated CO_2, and fewer still have made concurrent measurements of non-structural carbohydrates, N and fine-root longevity. Elevated CO_2 did not alter the non-structural carbohydrate con-

centration of live fine roots in one study of *Pinus ponderosa* and *Pinus taeda* (King *et al.*, 1997), but it produced increases in others (BassiriRad *et al.*, 1996). In soil with low N, elevated CO_2 did not alter the non-structural carbohydrate concentration or longevity of fine roots of *Populus tremuloides* (Pregitzer *et al.*, 2000; Rothstein *et al.*, 2000). However, in soil with high N, plants exposed to elevated CO_2 produced fine roots with lower longevity and fewer non-structural carbohydrates, but this change in chemistry was not significant (Rothstein *et al.*, 2000). Crookshanks *et al.* (1998) also observed a non-significant increase in soluble sugar concentrations of *Fraxinus excelsior*, *Quercus petraea* and *Pinus sylvestris* fine roots. However, elevated CO_2 influenced fine-root starch concentration in a species-specific manner: it declined in *Quercus petraea*, increased in *Pinus sylvestris* and was not altered in *Fraxinus excelsior*.

Although elevated CO_2 can decrease the N concentration of fine roots (Jackson & Reynolds, 1996; Berntson & Bazzaz, 1997, 1998; Pregitzer *et al.*, 2000), we do not have sufficient information to understand the extent to which non-structural carbohydrates change in concert with fine-root N concentration or root longevity. Inherent differences between plant taxa in the degree to which photosynthate is allocated to the pool of non-structural carbohydrates in fine roots, and whether allocation to non-structural carbohydrates controls their life span, will be vital in understanding changes in the production of root-derived substrates under elevated CO_2. Given the importance carbohydrates and N for microbial metabolism in soil, a change in their concentration in fine roots has the potential to alter rates of microbial immobilization and modify soil N cycling. Experiments that couple measurements of fine-root longevity and biochemistry with gross rates of microbial N transformations holds promise for understanding the very different responses of soil N cycling under elevated CO_2.

In summary, the physiological activity of soil microorganisms is largely constrained by the input of organic substrates from plant production, and changes in the dynamics of fine roots and mycorrhizae under elevated CO_2 that modify substrate availability (i.e. amount and biochemistry) will probably alter the function and composition of the soil microbial community. However, we have a limited understanding of physiological links between the allocation of photosynthate to fine-root production and maintenance, the relationship between fine root maintenance and longevity, and the extent to which changes in the longevity of plant roots influence substrate availability and the need for N during microbial biosynthesis. We also do not understand how the composition and function of the microbial communities can change in response to altered substrate availability under elevated CO_2, nor do we understand how compositional shifts in microbial communities will alter the flow of C through soil food webs at an ecosystem level (Jones *et al.*, 1998). Clearly, there is much to be learned about fine-root demography in plants of contrasting life history traits and how fine-root longevity is modified by atmospheric CO_2 and other environmental factors. Molecular techniques for studying microbial community composition (Vestal & White, 1989; Lee & Fuhrman, 1991), assays for specific physiological functions (Sinsabaugh & Findlay, 1995; Sinsabaugh *et al.*, 1998), and the use of stable isotopes provide an opportunity to address whether changes in root longevity and biochemistry influence the function and composition of microbial communities. Experiments in which species-specific changes in fine-root longevity and biochemistry are directly coupled to gross rates of microbial N transformations have the potential to deepen our current understanding of plant–microbe interactions in soil. We argue that specific-specific responses of fine-root longevity and biochemistry to elevated CO_2 lie at the heart of understanding why soil N cycling increases in some studies and declines in others.

ACKNOWLEDGEMENTS

We thank Bruce Hungate, Jeff Andrews, Andy Allen for sharing their unpublished data with us, and Bruce Hungate for confirming N transformation rates calculated from his papers. We are grateful for the assistance of Matt Tomlinson, who spent numerous hours obtaining published reports from the library. This review was supported by grants from the US Department of Energy's Program for Ecosystem Research (PER Grant DE-FG02–93ER6166) and the US Environmental Protection Agency (EPA-C-R 824979-01).

REFERENCES

Allen AS, Andrews JA, Finzi AC, Matamala R, Richter DD, Schlesinger WH. 2000. Effects of free-air CO_2 enrichment (FACE) on below-ground processes in a loblolly pine (*Pinus taeda*) forest. *Ecological Applications* **10**: 437–448.

Andrews J. 1999. *Changes to belowground carbon dioxide dynamics under experimental CO_2 enrichment of a forest ecosystem.* PhD thesis, Duke University, Durham, NC, USA.

Arnone JA III. 1997. Indices of plant N availability in an alpine grassland under elevated atmospheric CO_2. *Plant and Soil* **190**: 61–66.

Ball AS, Drake BG. 1998. Stimulation of soil respiration by carbon dioxide enrichment of marsh vegetation. *Soil Biology and Biochemistry* **30**: 1203–1205.

BassiriRad H, Thomas RH, Reynolds JF, Strain BR. 1996. Differential responses of root uptake kinetics of NH_4^+ and NO_3^- to enriched atmospheric CO_2 concentrations in field-grown loblolly pine. *Plant, Cell & Environment* **19**: 367–371.

Berntson GM, Bazazz FA. 1996. The allometry of root production and loss in seedlings of *Acer rubrum* (Aceraceae) and *Betula papyrifera* (Betulaceae): implications for root dynamics in elevated CO_2. *American Journal of Botany* **83**: 608–616.

Berntson GM, Bazazz FA. 1997. Nitrogen cycling in microcosms of yellow birch exposed to elevated CO_2: simultaneous positive and negative below-ground feedbacks. *Global Change Biology* **3**: 247–258.

Berntson GM, Bazazz FA. 1998. Regenerating temperate forest mesocosms in elevated CO_2: belowground growth and nitrogen cycling. *Oecologia* **113**: 115–125.

Billes G, Rouhier H, Bottner P. 1993. Modifications of the carbon and nitrogen allocations in the plant (*Triticum aestivum*) soil system in response to increased atmospheric CO_2 concentration. *Plant and Soil* **157**: 215–225.

Burke IC. 1989. Control of nitrogen mineralization in a sagebrush steppe landscape. *Ecology* **70**: 1115–1126.

Burke MK, Raynal DJ. 1994. Fine-root phenology, production and turnover in a northern hardwood forest ecosystem. *Plant and Soil* **162**: 135–146.

Coleman DC. 1976. A review of root production processes and their influence on biota in terrestrial ecosystems. In: Anderson JM, MacFadeyen A, eds. *The role of terrestrial and aquatic organisms in decomposition processes*. Oxford, UK: Science Publications, 417–434.

Cotrufo MF, Ineson P. 1995. Effects of enhanced atmospheric CO_2 and nutrient supply on the quality and subsequent decomposition of fine roots of *Betula pendula* Roth. and *Picea sitchensis* (Bong.) Carr. *Plant and Soil* **170**: 267–277.

Cotrufo MF, Ineson P, Rowland AP. 1994. Decomposition of tree leaf litters grown under elevated CO_2: effect of litter quality. *Plant and Soil* **163**: 121–130.

Crookshanks M, Taylor G, Broadmeadow M. 1998. Elevated CO_2 and tree root growth: contrasting responses in *Fraxinus excelsior*, *Quercus petraea* and *Pinus sylvestris*. *New Phytologist* **138**: 241–250.

Curtis PS. 1996. A meta-analysis of leaf gas exchange and nitrogen in trees grown under elevated carbon dioxide. *Plant, Cell & Environment* **19**: 127–137.

Curtis PS, Wang XZ. 1998. A meta-analysis of elevated CO_2 effects on woody plant mass, form, and physiology. *Oecologia* **113**: 299–313.

Day FP, Weber EP, Ross Hinkle C, Drake B. 1996. Effects of elevated atmospheric CO_2 on fine root length and distribution in an oak–palmetto scrub ecosystem in central Florida. *Global Change Biology* **2**: 143–148.

Díaz S, Grime JP, Harris J, McPherson E. 1993. Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. *Nature* **364**: 616–617.

Edwards NT, Norby RJ. 1999. Below-ground respiratory responses of sugar maple and red maple seedlings to atmospheric CO_2 enrichment and elevated temperatures. *Plant and Soil* **206**: 85–97.

Eissenstat DM, Yanai RD. 1997. The ecology of root lifespan. *Advances in Ecological Research* **27**: 1–60.

Fahey TJ, Hughs JW. 1994. Fine-root dynamics in a northern hardwood forest ecosystem, Hubbard Brook Experimental Forest, NH. *Journal of Ecology* **82**: 533–548.

Fitter AH, Graves JD, Wolfenden J, Self GK, Brown TK, Bogie D, Mansfield TA. 1997. Root production and turnover and carbon budgets of two contrasting grasslands under ambient and elevated atmospheric carbon dioxide concentrations. *New Phytologist* **137**: 247–255.

Fogel R, Hunt G. 1983. Contribution of mycorrhizae and soil fungi to nutrient cycling in a Douglas-fir ecosystem. *Canadian Journal of Forest Research* **12**: 219–232.

Gebauer RLE, Strain BR, Reynolds JF. 1998. The effect of elevated CO_2 and N availability on tissue concentrations and whole plant pools of carbon-based secondary compounds in loblolly pine. *Oecologia* **113**: 29–36.

Hart SC, Stark JM, Davidson EA, Firestone MK. 1994. Nitrogen mineralization, immobilization, and nitrification. In: Weaver RW, Angle JS, Bottomley PS, eds. *Methods of soil analysis, part 2: microbiological and biochemical properties*. Segoe, WI, USA: Soil Science Society of America, 985–1018.

Hendrick RL, Pregitzer KS. 1992. The demography of fine roots in a northern hardwood forest. *Ecology* **73**: 1094–1104.

Hungate BA. 1999. Ecosystem responses to rising atmospheric CO_2: feedbacks through the nitrogen cycle. In: Lou Y, Mooney HA, eds. *Carbon dioxide and environmental stress*. San Diego, CA, USA: Academic Press, 265–285.

Hungate BA, Canadell J, Chapin FS III. 1996. Plant species mediate change in soil microbial N in response to elevated CO_2. *Ecology* **77**: 2505–2515.

Hungate BA, Chapin FS III, Zhong H, Holland EA, Field CB. 1997a. Stimulation of grassland nitrogen cycling under carbon dioxide enrichment. *Oecologia* **109**: 149–153.

Hungate BA, Dijkstra P, Johnson DW, Hinkle CR, Drake B. 1999. Elevated CO_2 increases nitrogen fixation and decreases soil nitrogen mineralization in Florida scrub oak. *Global Change Biology* **5**: 1–9.

Hungate BA, Holland EA, Jackson RB, Chapin FS III, Mooney HA, Field CB. 1997b. The fate of carbon in grasslands under carbon dioxide enrichment. *Nature* **388**: 576–579.

Hungate BA, Jaeger CH III, Gamara G, Chapin SF II, Field CB. 2000. Soil microbiota in two annul grasslands: responses to elevated atmospheric CO_2. *Oecologia* (In press.)

Hungate BA, Lund C, Pearson HL, Chapin FS III. 1997c. Elevated CO_2 and nutrient addition alter soil N cycling and N trace gas fluxes with early season wet-up in a California annual grassland. *Biogeochemistry* **37**: 89–109.

Ineson P, Coward PA, Hartwig UA. 1998. Soil gas fluxes of N_2O, CH_4 and CO_2 beneath *Lolium perenne* under elevated CO_2: the Swiss free air carbon dioxide enrichment experiment. *Plant and Soil* **198**: 89–95.

Insam H, Bååth E, Berreck M, Frostegård Å, Gerzabek MH, Kraft A, Schinner F, Schweiger P, Tschuggnall G. 1999. Responses of the soil microbiota to elevated CO_2 in an artificial tropical ecosystem. *Journal of Microbiological Methods* **36**: 45–54.

Jackson RB, Reynolds HL. 1996. Nitrogen and ammonium uptake for single- and mixed-species communities grown at elevated CO_2. *Oecologia* **105**: 74–80.

Johnson D, Geisinger D, Walker R, Newman J, Vose J, Elliot K, Ball T. 1994. Soil pCO_2 soil respiration, and root activity in CO_2-fumigated and nitrogen-fertilized ponderosa pine. *Plant and Soil* **165**: 129–138.

Johnson DW, Ball JT, Walker RF. 1997. Effects of CO_2 and nitrogen fertilization on vegetation and soil nutrient content in juvenile ponderosa pine. *Plant and Soil* **190**: 29–40.

Jones TH, Thompson LJ, Lawton JH, Bezemer TM, Bardgett RD, Blackburn TM, Bruce KD, Cannon PF, Hall GS, Hartley SE, Howson G, Hones CG, Kampichler C, Kandler E, Richie DA. 1998. Impacts of rising atmosphere carbon dioxide on model terrestrial ecosystems. *Science* **280**: 441–443.

Kampichler C, Kandeler E, Bardgett RD, Jones TH, Thompson J. 1998. Impact of elevated atmospheric CO_2 concentration on soil microbial biomass and activity in a complex, weedy field model ecosystem. *Global Change Biology* **4**: 335–346.

King, JS, Thomas RB Strain BR. 1997. Morphology and tissue quality of seedling root systems of *Pinus taeda* and *Pinus ponderosa* as affected by varying CO_2, temperature, and nitrogen. *Plant and Soil* **195**: 107–119.

Klironomos JN, Rillig MC, Allen MF. 1996. Below-ground microbial and microfaunal responses to *Artemisia tridentata* grown under elevated atmospheric CO_2. *Functional Ecology* **10**: 527–534.

Körner C, Arnone JA III. 1992. Responses to elevated carbon dioxide in artificial tropical ecosystems. *Science* **257**: 1672–1675.

Körner C, Diemer M, Schappi B, Niklaus P, Arnone J. 1997. The response of alpine grassland to four seasons of CO_2 enrichment: a synthesis. *Acta Oecologia* **18**: 165–175.

Kubiske ME, Pregitzer KS, Zak DR, Mikan CJ. 1998. Growth and C allocation of *Populus tremuloides* genotypes in response to atmospheric CO_2 and soil N availability. *New Phytologist* **140**: 251–260.

Lee S, Fuhrman J. 1991. Spatial and temporal variation of natural bacterioplankton assemblages studied by total genomic DNA cross-hybridization. *Microbial Ecology* **26**: 189–200.

Lekkerkerk LJA, van De Geijn SC, van Veen J. 1990. Effects of elevated atmospheric CO_2 levels on the carbon economy of a soil planted with wheat. In: Bouwman AF, ed. *Soils and the greenhouse effect*. New York, USA: John Wiley & Sons, 423–429.

Luo Y, Jackson RB, Field CB, Mooney HA. 1996. Elevated CO_2 increases belowground respiration in California grasslands. *Oecologia* **108**: 130–137.

Lussenhop J, Treonis A, Curtis PS, Teeri JA, Vogel CS. 1998. Response of soil biota to elevated atmospheric CO_2 in poplar model systems. *Oecologia* **113**: 247–251.

McGuire AD, Melillo JM, Joyce LA. 1995. The role of nitrogen

in the response of forest net primary productivity to elevated atmospheric carbon dioxide. *Annual Review of Ecology and Systematics* **26**: 473–503.

McKenzie E, Peterson CA. 1995a. Root browning in *Pinus banksiana* Lamb. and *Eucalyptus pilularis* Sm. 1. Anatomy and permeability of the white and tannin zones. *Botanica Acta* **108**: 127–137.

McKenzie E, Peterson CA. 1995b. Root browning in *Pinus banksiana* Lamb. and *Eucalyptus pilularis* Sm. 2. Anatomy and permeability of the cork zone. *Botanica Acta* **108**: 138–143.

Mikan CJ, Zak DR, Pregitzer KS, Kubiske ME. 2000. Combined effects of atmospheric CO_2 and N availability on the belowground carbon and nitrogen dynamics of aspen mesocosms. *Oecologia*. (In press.)

Mooney HA, Fichtner K, Schulze E-D. 1995. Growth, photosynthesis, and storage of carbohydrates and nitrogen in *Plaseolus lunatus* in relation to resource availability. *Oecologia* **104**: 17–23.

Nakayama FS, Huluka G, Kimball BA, Lewin KF, Nagy J, Hendry GR. 1994. Soil carbon dioxide fluxes in natural and CO_2-enriched systems. *Agricultural and Forest Meteorology* **70**: 131–140.

Newton PCD, Clark H, Bell CC, Glasgow EM, Tate KR, Ross DJ, Yeates GW, Saggar S. 1995. Plant growth and soil processes in temperate grassland communities at elevated CO_2. *Journal of Biogeography* **22**: 235–240.

Nguyen PV, Dickman DI, Pregitzer KS, Hendrick R. 1990. Late-season changes in allocaiton of starch and sugar to shoots, coarse roots, and fine roots in 2 hybrid poplar clones. *Tree Physiology* **7**: 95–105.

Niklaus PA. 1998. Effects of elevated atmospheric CO_2 on soil microbiota in calcareous grassland. *Global Change Biology* **4**: 451–458.

Niklaus PA, Körner C. 1996. Responses of soil microbiota of a late successional alpine grassland to long term CO_2 enrichment. *Plant and Soil* **184**: 219–229.

Norby RJ. 1996. Oaks in a high-CO_2 world. *Annales des Sciences Forestières* **53**: 413–429.

Norby RJ, Gunderson CA, Wullschleger SD, O'Neill EG, MacCracken MK. 1992. Productivity and compensatory responses of yellow-poplar trees in elevated CO_2. *Nature* **357**: 322–324.

Paterson E, Hodge A, Thornton B, Millard P, Killham K. 1999. Carbon partitioning and rhizosphere C-flow in *Lolium perenne* as affected by CO_2 concentration, irradiance and belowground conditions. *Global Change Biology* **5**: 669–678.

Paul EA, Clark FE. 1996. *Soil microbiology and biochemistry, 2nd edn.* New York, USA: Academic Press.

Poorter H. 1993. Interspecific variation in the growth response of plants to an elevated ambient CO_2 concentration. *Vegetatio* **104/105**: 77–97.

Pregitzer KS, Zak DR, Curtis PS, Kubiske ME, Teeri JA, Vogel CS. 1995. Atmospheric CO_2, soil nitrogen and turnover of fine roots. *New Phytologist* **129**: 579–585.

Pregitzer KS, Zak DR, Maziasz J, DeForest J, Curtis PS, Lussenhop J. 2000. Fine root growth, mortality, and morphology in a factorial elevated atmospheric CO_2 × soil N availability experiment. *Ecological Applications* **10**: 18–33.

Prior SA, Rogers HH, Runion GB, Torbert HA, Reicosky DC. 1997a. Carbon dioxide-enriched agroecosystem: influence of tillage on short-term soil carbon dioxide efflux. *Journal of Environmental Quality* **26**: 244–252.

Prior SA, Torbert HA, Runion GB, Rogers HH, Wood CW, Kimball BA, LaMorte RL, Pinter PJ, Wall GW. 1997b. Free-air carbon dioxide enrichment of wheat: soil carbon and nitrogen dynamics. *Journal of Environmental Quality* **26**: 1161–1166.

Rice CW, Garcia FO, Hampton CO, Owensby CE. 1994. Soil microbial response in tallgrass prairie to elevated CO_2. *Plant and Soil* **165**: 67–74.

Richards D, Considine JA. 1981. Suberization and browning of grapevine roots. In: Brouwer R, ed. *Structure and function of plant roots*. London, UK: Dr W. Junk Publishing, 111–115.

Rillig MC, Scow KM, Klironomos JN, Allen MF. 1997. Microbial carbon-substrate utilization in the rhizosphere of *Gutierrezia sarothrae* grown in elevated atmospheric carbon dioxide. *Soil Biology and Biochemistry* **29**: 1387–1394.

Rogers HH, Runion GB, Krupa SV. 1994. Plant response to atmospheric CO_2 enrichment with emphasis on roots and rhizosphere. *Environmental Pollution* **83**: 115–189.

Rogers HH, Runion GB, Krupa SV, Prior SA. 1997. Plant responses to atmospheric carbon dioxide enrichment: implications in root–soil–microbe interactions. In: Allen LH Jr, Kirkham MB, Olszyk DM, Whitman CE, eds. *Advances in carbon dioxide effect research (ASA Special Publication no. 61)*. Segoe, WS, USA: Agronomy Society of America, 1–34.

Ross DJ, Tate KR, Newton PCD. 1995. Elevated CO_2 and temperature effects on soil carbon and nitrogen cycling in ryegrass/white clover turves of an Endoaquept soil. *Plant and Soil* **176**: 37–49.

Rothstein DE, Zak DR, Pregitzer KS, Curtis PS. 2000. The kinetics of nitrogen uptake by *Populus tremuloides* grown under experimental atmospheric CO_2 and soil N availability treatments. *Tree Physiology* **20**: 265–270.

Rouhier H, Billès G, Billès L, Bottner P. 1996. Carbon fluxes in the rhizosphere of sweet chestnut seedlings (*Castanea sativa*) grown under two atmospheric CO_2 concentrations: ^{14}C partitioning after pulse labeling. *Plant and Soil* **180**: 101–111.

Runion GB, Curl EA, Rogers HH, Backman PA, Rodrigues-Kabana R, Helms BE. 1994. Effects of free-air CO_2 enrichment on microbial populations in the rhizosphere and phyllosphere of cotton. *Agricultural and Forest Meterology* **70**: 117–130.

Schenk U, Manderscheid R, Hugen J, Weigel H-J. 1995. Effects of CO_2 enrichment and intraspecific competition in biomass partitioning, nitrogen content and microbial biomass carbon in soil of perennial ryegrass and white clover. *Journal of Experimental Botany* **46**: 987–993.

Schimel DS. 1986. Carbon and nitrogen turnover in adjacent grassland and cropland ecosystems. *Biogeochemistry* **23**: 345–357.

Schortemeyer M, Hartwig U, Hendry G, Sadowsky MJ. 1996. Microbial community changes in the rhizospheres of white clover and perennial ryegrass exposed to free air carbon dioxide enrichment (FACE). *Soil Biology and Biochemistry* **28**: 1717–1724.

Schreiber L. 1996. Chemical composition of Casparian strips isolated from *Clivia miniata* Reg. roots: evidence for lignin. *Planta* **199**: 596–601.

Sinsabaugh RL, Findlay S. 1995. Microbial production, enzyme activity and carbon turnover in surface sediments of the Hudson River estuary. *Microbial Ecology* **30**: 127–141.

Sinsabaugh RL, Klug MJ, Collins HP, Yeager P, Petersen SO. 1998. Characterizing soil microbial communities. In: Robertson GP, Bledsoe CS, Coleman DC, Sollins P, eds. *LTER soil methods standards*. Oxford, UK: Oxford University Press, 476–525.

Smith JL, Paul EA. 1990. The significance of soil microbial biomass estimations. In: Bollag J, Stotzky G, eds. *Soil biochemistry*. New York, USA: Marcel Dekker, Inc., 357–393.

Thomas SM, Whitehead D, Reid JB, Cook FJ, Adams JA, Leckie AC. 1999. Growth, loss and vertical distribution of *Pinus radiata* fine roots growing at ambient and elevated CO_2 concentration. *Global Change Biology* **5**: 107–121.

Tingey DT, Johnson MG, Phillips DL, Johnson DW, Ball JT. 1996. Effects of elevated CO_2 and nitrogen on the synchrony of shoot and root growth in ponderosa pine. *Tree Physiology* **16**: 905–914.

Van Fleet DS. 1957. Histochemical studies of phenolase and polyphenolase in the develoment of the endodermis in the genus *Smilax*. *Bulletin of the Torrey Botanical Club* **84**: 9–28.

Vestal JR, White DC. 1989. Lipid analysis in microbial ecology. *BioScience* **39**: 535–541.

Vogt KA, Grier CC, Vogt DJ. 1986. Production, turnover, and nutrient dynamics of above- and belowground detritus in world forests. *Advances in Ecological Research* **15**: 303–377.

Vose JM, Elliot KJ, Johnson DW, Tingey DT, Johnson MG. 1997. Soil respiration response to three years of elevated CO_2 and N fertilization in ponderosa pine (*Pinus ponderosa* Doug. ex Laws.). *Plant and Soil* **190**: 19–28.

Vose JM, Elliott KJ, Johnson DW, Walker RF, Johnson MG, Tingey DT. 1995. Effects of elevated CO_2 and N fertilization on soil respiration from ponderosa pine (*Pinus ponderosa*) in open-top chambers. *Canadian Journal of Forest Research* **25**: 1243–1251.

Wardle DA. 1992. A comparative assessment of the factors which

influence microbial biomass carbon and nitrogen levels in soil. *Biological Reveiws* **67**: 321–358.

Wood CW, Torbert HA, Rogers HH, Runion GB, Prior SA. 1994. Free-air CO_2 enrichment effects on soil carbon and nitrogen. *Agricultural and Forest Meteorology* **70**: 103–116.

Yeates GW, Tate KR, Newton PCD. 1997. Responses of the fauna of a grassland soil to doubling of atmospheric carbon dioxide concentration. *Biology and Fertility of Soil* **25**: 307–315.

Zak DR, Holmes WE, MacDonald NW, Pregitzer KS. 1999. Soil temperature, matric potential, and the kinetics of microbial respiration and net N mineralization in northern hardwood forests. *Soil Science Society of America Journal* **63**: 575–584.

Zak DR, Pregitzer KS. 1998. Integration of ecophysiological and biogeochemical approaches to ecosystem dynamics. In: Groffman PM, Pace ML, eds. *Successes, limitations, and frontiers in ecosystem science*. New York, USA: Springer-Verlag, 372–403.

Zak DR, Pregitzer KS, Curtis PS, Holmes WE. 2000a. Atmospheric CO_2 and the composition and function of soil microbial communities. *Ecological Applications* **10**: 47–59.

Zak DR, Pregitzer KS, Curtis PS, Teeri JA, Fogel R, Randlett DL. 1993. Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. *Plant and Soil* **151**: 105–117.

Zak DR, Pregitzer KS, Curtis PS, Vogel CS, Holmes WE, Lussenhop J. 2000b. Atmospheric CO_2, soil N availability, and the allocation of biomass and nitrogen in *Populus tremuloides*. *Ecological Applications* **10**: 34–46.