Research review

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

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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

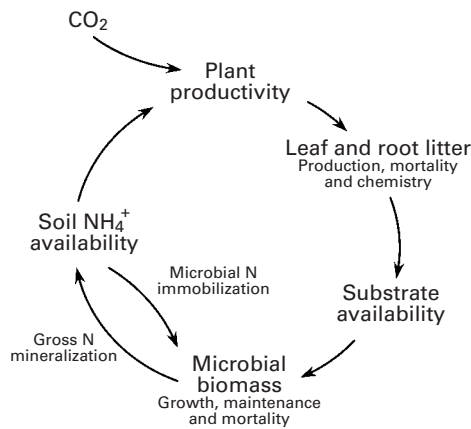*Author for correspondence (fax +1 734 936 2195; e-mail drzak@umich.edu).

**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 ± 24.1%; mean response ± 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 ($\mu$mol 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 ($\mu g$ C $m^{-2}$ $d^{-1}$) | | Relative change[a] (%) | $CO_2$ exposure[b] ($\mu mol$ $mol^{-1}$) | Soil conditions | Reference |
|---|---|---|---|---|---|---|
| | Ambient [$CO_2$] | Elevated [$CO_2$] | | | | |
| **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*[e] | 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 ($\mu$g C 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* | 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. $\mu$g 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 ($\mu$g C g$^{-1}$) | | Relative Change[a] (%) | $CO_2$ exposure[b] ($\mu$mol mol$^{-1}$) | Soil conditions | Reference |
|---|---|---|---|---|---|---|
| | Ambient $CO_2$ | Elevated $CO_2$ | | | | |
| **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* | 3 213 | 2 817 | −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* | 1 281 | 1 088 | −15 | 690; SC | Low-N soil; rhizosphere | Lussenhop *et al.* (1998) |
| *Populus × euramericana* | 3 879 | 2 542 | −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 $= 100(\text{elevated} - \text{ambient})/\text{ambient}$.

[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 & Bazzaz, 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 ($\mu g\ Ng^{-1}\ d^{-1}$) | | Relative Change[a] (%) | $CO_2$ exposure[b] ($\mu mol\ mol^{-1}$) | Soil conditions | Reference |
|---|---|---|---|---|---|---|
| | Ambient $CO_2$ | Elevated $CO_2$ | | | | |
| **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$^{-1}$) | | Relative Change[a] (%) | $CO_2$ exposure[b] ($\mu$mol mol$^{-1}$) | Soil conditions | Reference |
|---|---|---|---|---|---|---|
| | Ambient $CO_2$ | Elevated $CO_2$ | | | | |
| **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 [CO₂], 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 & Bazazz, 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 [CO₂] 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 micro-organisms 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.

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