Plant and Soil **187:** 277–288, 1996.

Influence of rhizodeposition under elevated CO_2 on plant nutrition and soil organic matter

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Received 2 February 1996. Accepted in revised form 15 July 1996

Key words: carbon isotopes, carbon storage, elevated CO_2, phosphorus, rhizodeposition, root exudation, soil organic matter

Abstract

Atmospheric CO_2 concentrations can influence ecosystem carbon storage through net primary production (NPP), soil carbon storage, or both. In assessing the potential for carbon storage in terrestrial ecosystems under elevated CO_2, both NPP and processing of soil organic matter (SOM), as well as the multiple links between them, must be examined. Within this context, both the quantity and quality of carbon flux from roots to soil are important, since roots produce specialized compounds that enhance nutrient acquisition (affecting NPP), and since the flux of organic compounds from roots to soil fuels soil microbial activity (affecting processing of SOM).

From the perspective of root physiology, a technique is described which uses genetically engineered bacteria to detect the distribution and amount of flux of particular compounds from single roots to non-sterile soils. Other experiments from several labs are noted which explore effects of elevated CO_2 on root acid phosphatase, phosphomonoesterase, and citrate production, all associated with phosphorus nutrition. From a soil perspective, effects of elevated CO_2 on the processing of SOM developed under a C4 grassland but planted with C3 California grassland species were examined under low (unamended) and high (amended with 20 g m^{-2} NPK) nutrients; measurements of soil atmosphere $\delta 13C$ combined with soil respiration rates show that during vegetative growth in February, elevated CO_2 decreased respiration of carbon from C4 SOM in high nutrient soils but not in unamended soils.

This emphasis on the impacts of carbon loss from roots on both NPP and SOM processing will be essential to understanding terrestrial ecosystem carbon storage under changing atmospheric CO_2 concentrations.

Abbreviations: SOM–soil organic matter, NPP–net primary productivity, NEP–net ecosystem productivity, PNPP–*p*-nitrophenyl phosphate.

Introduction

The potential for terrestrial ecosystems to sequester carbon from the atmosphere is governed both by their net primary productivity (NPP) and by their capacity for soil carbon storage. Though the flux of photosynthetically fixed carbon from shoots through roots to soil drives microbial activity in the rhizosphere, only recently has elevated carbon dioxide (CO_2) research expanded from a focus on photosynthesis and water use in plants (for recent reviews see Bazzaz, 1990; Bowes, 1993; Ceulemans, 1994) to an examination of direct and indirect effects of elevated CO_2 on ecosystem processes belowground. This expanded focus is essential to understanding net ecosystem productivity (NEP) under elevated CO_2. Microbial activity, fueled in part by rhizodeposition, affects the availability of nutrients in soils, which in turn influences the extent of plant response to elevated CO_2 (Field et al., 1992) and

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the processing of soil organic matter (SOM) (Lynch and Whipps, 1990). These two factors, plant productivity and SOM dynamics, determine carbon sequestration by the terrestrial biosphere.

In examining the effects of elevated atmospheric carbon dioxide concentrations on fluxes of carbon belowground in ecosystems, both the quantity of carbon moving from roots to soil and the form that carbon takes should be considered. Clearly, "loss" of carbon through production of particular compounds by roots which function to attract symbionts (e.g. luteolin, Bauer and Caetano-Anolles, 1990) or to aid with nutrient acquisition, cannot be viewed in the same light as more general carbon loss through sloughing of dead cells from growing roots or turnover of dead roots. In order to discuss these two roles of carbon release from roots of plants growing under elevated CO_2, the total loss of soluble and insoluble carbon into soil (including exudation, sloughing of live cells, loss of individual dead cells, and root turnover) is termed rhizodeposition in this paper. Root exudation refers to passive loss and active secretion of organic compounds by living roots. This agrees generally with Marschner's definitions (Marschner, 1995), but is more broad, for example, than the definitions used by Lynch and Whipps (1990) and Whipps (1990), who limit the term "exudation" to include only those compounds that diffuse out of roots into the soil.

There are a number of recent, excellent reviews of root and rhizosphere response to elevated CO_2 (Norby, 1994; O'Neill, 1994; Rogers et al., 1994; Stulen and den Hertog, 1993; Van Veen et al., 1991). In addition, ecosystem studies examining the feedbacks among belowground carbon storage, nutrient availability and atmospheric CO_2, have noted the interaction between rhizodeposition and microbial processes (Diaz et al., 1993; Wood et al., 1994; Zak et al., 1993). However, the role of symbionts in providing nutrients to plants, and the ability of roots to mobilize nutrients from soils, are also particularly important in unfertilized, natural ecosystems. As Norby (1994) points out, the relative importance of rhizodeposition as a whole vs. specifics of root physiology depends on the ecosystem of interest and the scale of inquiry. For example, studies of the capacity of annual grasslands to serve as sinks for atmospheric CO_2 should focus on soil carbon pools and the influence of rhizodeposition on processing of SOM (see section iii). However, when investigating effects of elevated CO_2 on individual plants or community dynamics within those same annual grasslands, the effects of CO_2 both on rhizodeposition and on production of particular root exudates (influencing nutrient availability and NPP) should be examined (see section ii).

Clearly, the direct effects of increasing CO_2 on plants, and the impact of plant responses on soil processes, will be essential in predicting carbon storage capacity in terrestrial ecosystems. A very simple scheme illustrates the influence that direct plant physiological responses to elevated CO_2 can exert on ecosystem carbon storage (Figure 1). At top, both stomatal conductance (Mott, 1988) and photosynthesis are affected by the CO_2 concentration inside the leaf; two interacting cascades ultimately influencing soil carbon storage and plant biomass accumulation combine to drive ecosystem carbon storage. Again, this scheme is simple; environmental influences other than elevated atmospheric [CO_2] are not taken into account. The scheme emphasizes, however, the interdependence of direct plant response to [CO_2], microbial processing of SOM, nutrient availability to plants, NPP and NEP.

This paper concentrates on new techniques and experiments addressing aspects of root/rhizosphere research not yet thoroughly explored. The question marks in Figure 1 indicate the foci of the several research projects discussed here. Experiments described in section ii explore changes in root physiology (e.g. exudation of high and low molecular weight compounds) under elevated CO_2 and the possible consequences of these changes for nutrient availability. Section iii highlights the plant-mediated influence of elevated CO_2 on mineralization of SOM.

ii. Carbon "loss" through exudation under elevated CO_2 – a plant perspective

The emphasis researchers have put on root responses to elevated CO_2 stems from the function of roots in acquiring both water and nutrients essential for plant growth. With more fixed carbon available for growth under elevated CO_2, plants may allocate more of their own resources toward acquiring limiting nutrients or water (Chapin, 1980; Garnier, 1991). Many studies have focused on changes in biomass allocation within the plant, examining root to shoot ratios, amount of fine roots, etc. (Rogers et al., 1994; Stulen and den Hertog, 1993). Roots may also adjust their physiology to acquire limiting nutrients through a variety of mechanisms: increasing uptake of particular nutrients per unit root surface (Garnier, 1991), producing enzymes capable of releasing nutrients from SOM and making

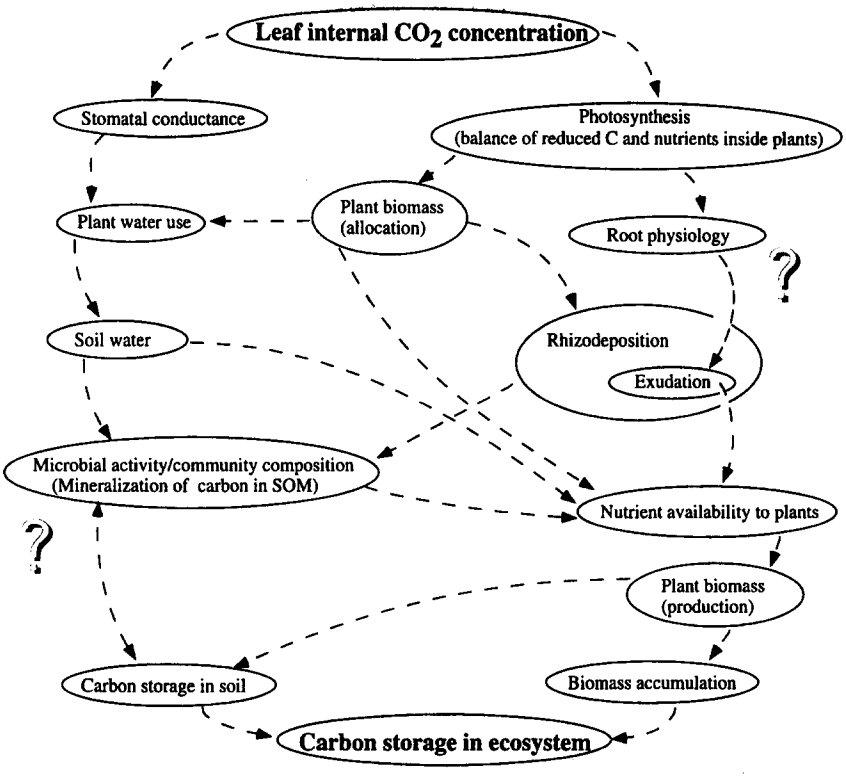

Figure 1. Diagram illustrating the many interlinked processes leading from direct effects of elevated [CO_2] on stomatal conductance and photosynthesis to two major components of ecosystem carbon storage: biomass accumulation and soil carbon storage. Question marks designate the portions of the diagram explored by experiments described in this paper.

them available for uptake (e.g. phosphatase, Tarafdar and Jungk, 1987; Kroehler and Linkins, 1988), altering patterns of exudation of compounds which mobilize particular nutrients from the soil (e.g. phytosiderophores to gather iron and other micronutrients, Treeby et al., 1989 and Römheld, 1991; citrate to mobilize P, Hoffland et al., 1992), or attracting symbionts capable of providing N or P (O'Neill, 1994). Exudation of particular compounds can also help protect roots from adverse environmental conditions; for example, exudation of organic acids, particularly citrate, can protect plants from Al toxicity (Kochain, 1995).

Though there is much speculation that elevated CO_2 may lead to increased root exudation into soils, which, in turn, may effect changes in microbial activity or community composition, processing of SOM, and availability of nutrients to plants, it is not yet known whether elevated CO_2 effects exudation per unit root, or, instead, whether exudation per unit root remains the same, while only root biomass is changed. The technical difficulties in measuring the flux of carbon (or, even worse, of particular organic compounds) into the soil from individual roots has made a definitive measurement of exudation per unit root length, mass, or surface area extremely difficult. If root physiology were altered under elevated CO_2, leading to a change in the quantity or quality of root exudation to the rhizosphere per unit root length, microbial processes would be effected, independent of whether or not more root biomass developed under elevated CO_2. Increased root biomass would further amplify this effect in an ecosystem. If, however, the amount of C flowing belowground were to increase *only* because of an increase in the amount of root under elevated CO_2, one might not expect rhizosphere processes around roots to be immediately affected by elevated CO_2, except through an increased probability of roots encountering one another during growth and each contributing C to a unit soil volume in the rhizosphere.

There are obvious difficulties in obtaining information about loss of particular compounds from roots to soil in natural ecosystems. Rhizosphere microbes can quickly utilize compounds exuded from roots, leaving nothing behind for identification. Also, the microbes

themselves can release compounds into soil that are identical to those released from roots. Because of these difficulties, researchers have tried to obtain information about the physiology of root "exudation" (including both the compounds that diffuse from (or are secreted by) live roots *and* compounds lost from lysed dead cells) by growing plants in sterile solution culture. In one such study, Norby et al. (1987) transferred *Pinus echinata* seedlings to sterile nutrient solution after an initial growth period in soil. They then analyzed loss of ^{14}C-labeled, water-soluble compounds from roots, and found that growth under elevated CO_2 drove an increase in the amount of soluble carbon lost from roots into the growth containers. This increased carbon loss under elevated CO_2 did not persist, and it was correlated with an increase in fine root biomass, implying that carbon-loss per unit fine root may not have changed under elevated CO_2. As Norby et al. (1987) note, however, it is known that exudation from roots in solution culture is decreased relative to that in soil (e.g. Barber and Martin, 1976), making extrapolation of these results to plants growing in soils difficult. Jones and Darrah (1993) suggest that this difference in net exudation from roots in sterile solution culture vs. in soil results from immediate bacterial use of root-released compounds in soil, whereas under sterile conditions, roots have the opportunity to re-absorb a significant amount of the C and N they have lost to the culture medium.

Another strategy for measuring carbon flux from roots to soil is exemplified in an earlier study of rhizodeposition under elevated CO_2. Whipps (1985) grew maize plants in soil under a continuous ^{14}C label at several concentrations of CO_2, and calculated that there was no difference in the percentage of the net fixed carbon that was lost from roots of plants grown at different CO_2 concentrations. Several other studies have examined rhizodeposition of ^{14}C-labeled compounds under elevated CO_2, particularly in agricultural species, using either pulse- or continuous labeling of new plant material with ^{14}C; they will be discussed in section iii. Though this approach provides valuable and ecologically relevant information about the total carbon flux from roots to soil, it cannot be used to quantify true exudation of particular compounds separate from other components of rhizodeposition.

Detection of the quantity and distribution of compounds lost from single roots to non-sterile soil

One new approach to detecting exudation of particular compounds from roots to soil relies on soil microbes to "report" the presence of the compound. Soil can be seeded with bacteria engineered to report the presence of particular compounds in their environment by producing ice-nucleation proteins inserted into the exterior of their outer cell membranes (Clark et al., 1992; Lindgren et al., 1989). Using these reporter bacteria, it is possible to determine zones of exudation along the axis of a root, along with the extent of exudation into the surrounding soil. The ice-nucleation reporter system is particularly well-suited to rhizosphere experiments; the assay consists of mixing a small amount of soil, harvested from around the root, into water, and then detecting the presence of ice-nucleating proteins using a droplet freezing assay (Lindow, 1990). Detection of report in these soil solutions is not problematic with this system, as it would be with, for example, a lux-linked reporter system relying on light production as the bacterial report from a soil slurry. Because the ice nucleation activity increases with approximately the second power of the concentration of ice protein in the cell membrane, this reporter system is extremely sensitive (Lindow, 1995). Engineered bacteria have already been used successfully in reporting Fe concentrations in the rhizosphere and on leaves (Loper and Lindow, 1994).

This system is being used to investigate the roles of amino acid release from roots in influencing microbial activity in the rhizosphere. In particular, bacteria reporting the presence of tryptophan in 1-10 μmolar concentrations in the soil (developed by Clark, 1995) have been used to explore loss of amino acids from *Avena barbata* roots into soil (Jaeger et al., 1996). *Avena barbata* was chosen for the assays because it is a dominant grass in the California sandstone grassland, and because its roots are large, allowing easy harvest of soil at discrete distances from the root. Beyond these characteristics, a significant amount of information has been gathered about the physiology of *Avena* plants, both shoots and roots, growing under elevated CO_2 as part of the Jasper Ridge Elevated CO_2 Experiment (Field et al., 1996). Especially relevant is the fact that photosynthesis apparently is not substantially downregulated in the field under elevated CO_2 (Jackson et al., 1995). Some of the carbohydrates fixed by leaves at elevated CO_2 could be transported belowground and lost to the soil. In preliminary experi-

ments with hydroponically-grown *Avena barbata*, Cardon has found that carbohydrate "exudates" (both true exudates and compounds leaked from lysed cells) lost from roots into sterile solution and analyzed by HPLC (DX500system with CarboPac PA-1 column, sodium hydroxide eluent gradient, and pulse amperometric detection; Dionex Corp., Sunnyvale, CA) consist of several mono- and disaccharides, with fructose, glucose, and sucrose being prominent constituents. Jaeger, Cardon and Lindow are currently expanding the initial scope of the rhizosphere project to include examination of exudation of amino acids and sugars from roots of plants growing under ambient and elevated CO_2.

This technique provides a first glimpse into fluxes of particular compounds from roots into non-sterile soils. However, several cautions and limitations to the technique for ecological work should be noted. First, in most natural ecosystems, distinct soil profiles develop through time. It is known that root exudation can be strongly influenced by abiotic soil properties, including bulk density, moisture, pH, clay content, etc. (Oades, 1988; Van Veen and Kuikman, 1990). In considering the amount of exudation from roots of plants to soils in these ecosystems, it is necessary to recognize that roots growing through well-mixed soils will encounter less resistance to growth, and their patterns of exudation may be changed. Second, a limited suite of root-specific compounds can be detected using this reporter technique, since bacteria produce many compounds in common with plants. In complex cases, both the root and the microbial community may be contributing to the pool of a compound "reported" by engineered bacteria. In ecosystem studies, however, it is the existence of the pools in soil, their distribution, and their availability to microbes and roots that is of interest. Finally, damage by root grazers could release compounds that would trigger the bacterial report, giving an incorrect indication of exudation. However, again, it is the amount of compound lost per unit root that is of interest in examining rhizoshere processes, so this detection of both true exudation and loss of root compounds through damage could be viewed as an advantage in interpreting effects of altered C flux to soil compartments under elevated CO_2.

Root production of molecules associated with phosphorus nutrition

Many ecosystems, both tropical and temperate, are deficient in available phosphorus (Buol et al., 1980; Sanchez, 1976; Vitousek and Sanford, 1986). Of the phosphorus that is present in soils, over half in the A horizon is usually in an organic form (Barber, 1984). This P is linked by ester bonds to SOM, and is not immediately available for uptake by plants. It can be made available, however, by the action of phosphatases secreted by roots, mycorrhizae, or bacteria. Exudation of acid phosphatase enzyme by roots may constitute a very small portion of the total C lost to the rhizosphere, but this activity is extremely important in releasing P from phosphate esters in SOM for plant uptake, particularly in P-limited environments. In considering plant growth within a resource balance framework (that plants will adjust their allocation, structural or physiological, for capture of scarce resources), it is possible that acid phosphatase activity associated with roots of plants growing under elevated CO_2 might be enhanced if plants perceive the relative imbalance of P and C available for growth.

As part of the Jasper Ridge Elevated CO_2 Experiment (detailed in Field et al., 1996), California sandstone and serpentine grassland communities were grown in microcosms on their respective soils in open-top chambers under four treatments: elevated (700 ppm) CO_2 and ambient (350-400 ppm) CO_2, and high (soil supplemented with 20 g m^{-2} NPK as osmocote) and natural nutrient levels. Microcosms were 20 cm in diameter, 1 m deep, and a 15 cm layer of topsoil covered 75 cm of rocky subsoil. The phosphatase activity associated with roots of dominant species in these communities was measured through the growing season in a series of destructive harvests in 1993-94 (Cardon and Jackson, 1995). Phosphatase activity was measured as described by Silberbush et al. (1981) except that entire newly-washed *Avena barbata* and *Bromus hordeaceus* roots from the topsoil were assayed. For *Avena barbata*, elevated CO_2 did not consistently alter the surface-area-specific phosphatase activity through the season (Figure 2), though amendment of soil nutrients with NPK did drive an increase in phosphatase activity. A similar insensitivity of phosphatase activity (expressed on a unit root weight basis) to the elevated CO_2 treatment was observed for *Bromus hordeaceus* (data not shown). There are several possible explanations for these results. The plants may not have perceived a stronger imbalance in C and P in tissues growing under elevated CO_2, they may have adjusted some other root physiological parameter to enhance P availability, or regulatable phosphatase activity per unit root surface area may simply have been at a maximum at each nutrient level.

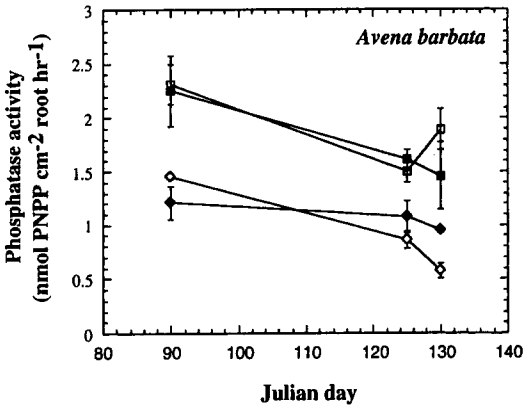

Figure 2. Acid phosphatase activity associated with *Avena barbata* roots. Phosphatase activity of roots was assayed following the method utilizing *p*-nitrophenyl phosphate (PNPP) in Silberbush et al. (1981). Roots were then stained pink with sulforhodamine dye and their length and surface area scanned and calculated.

When considering mineralization of P from SOM at the ecosystem level, however, it is important to note that though phosphatase activity per unit root may not change, the potential per plant for cleaving P from organic matter may increase simply because of increases in root biomass (e.g. Jackson and Reynolds, 1995). Such an increase in root-associated acid phosphatase activity (whether of root or microbial origin) could have large consequences for cycling of P in ecosystems. Phosphatase activity can be very long-lived, and phosphatases are produced both by microbes and by plant roots. Given that roots are the major carbon sources for microbial activity in soils, it is likely that a larger root mass will drive more phosphatase activity in a given volume of soil, whether the roots or microbes or both are responsible for that activity.

Seasonality of the phosphatase activity associated with roots is particularly noteworthy from the ecosystem viewpoint (Figure 2). As *Avena* plants senesced earlier in the year, the phosphatase activity associated with their roots decreased. (Phosphatase activity associated with the roots of *Bromus* growing with *Avena* continued at a high level even in early May; data not shown). At the ecosystem level, shifts in plant community structure could drive changes in the dynamics and extent of P release from SOM through the year.

Beyond acid phosphatase activity, there are other root physiological mechanisms also dedicated to phosphorus capture (Marschner, 1995). Gifford et al. (1996) explored root physiology of the native Australian grass *Danthonia richardsonii*, and they report that under phosphorus deficiency, phosphomonoesterase activities increased under elevated CO_2. Citrate production also increased consistently in these plants grown at elevated CO_2, though increases were not statistically significant. Exudation of citrate is important in releasing inorganic P from Al/Fe complexes in soils in highly P-limited systems (Marschner, 1995). As another example, *Eucalyptus rossii* grows on weathered skeletal soils in eastern temperate Australia, where soluble phosphate concentrations of the soil are very low, too low in fact to support crop growth without phosphate addition. However, *E. rossii* forests can achieve substantial above-ground C pools (Attiwell and Leeper, 1987). In a glasshouse study, Barrett (pers. com.) also studied production of citrate under elevated CO_2 by *E. rossii* growing on sand supplied with soluble and insoluble organic and inorganic phosphate. Observed increases in biomass under elevated CO_2 were accompanied by increases in citrate production (on a unit root dry weight basis) in plants growing under inorganic P regimes. (Citrate was assayed following the procedure of Dagley, 1974). It is not just the acidity of citrate, nor the loss of C per se from roots, that drives the release of P for the plants. Instead, it is specifically the regulatable production of citrate or phosphomonoesterase that is important for plant P nutrition and NPP (and NEP) in such P-limited ecosystems. These studies highlight interesting examples of possible changes in exudation from roots under elevated CO_2, that, given the increased C available in the plant, are consistent with the evolutionary history of *Danthonia richardsonii* and *Eucalyptus rossii* in P-poor soils.

iii. Rhizodeposition under elevated CO_2–a soil perspective

Over the last decade, there has been an emphasis on effects of elevated CO_2 on decomposition through the quality of leaf and root litter, with a number of researchers examining C:N and lignin:N ratios in plant tissues as an index for litter decomposability (Norby, 1994; Rogers et al., 1994). There have also been several recent papers examining other indirect effects of elevated CO_2 on microbial activity, nutrient cycling, and carbon storage in ecosystems, beyond the litter quality of individual plants (Diaz et al., 1993; Leavitt et al., 1994; Lekkerkerk et al., 1990; Van Veen et al., 1991; Wood et al., 1994 ; Zak et al., 1993). There is not as much information, however, about the effects of complex communities of plants on the rhizosphere processes they drive belowground (Hungate et

al, 1996a; Korner and Arnone, 1992; Rice et al., 1994; Van de Geijn and Van Veen, 1993). Finally, though there are several papers focusing on effects of available soil nutrients on breakdown of SOM (Liljeroth et al., 1990, 1994; Merckx et al., 1987; Van Veen et al., 1991), the interactive effects of atmospheric CO_2 and soil nutrient status on carbon storage and nutrient availability have not been extensively explored in communities of plants growing under natural light and rainfall conditions throughout their entire life cycle.

Carbon storage in ecosystems is influenced not only by net primary production (and storage in accumulated biomass), but also by accumulation and loss of SOM. As Van de Geijn and Van Veen (1993) point out, it is not clear whether SOM pools will grow or decline under elevated CO_2. There have been a number of experiments examining changes in litter quality under elevated CO_2, and the effects of these changes on the ability of microbes to mineralize SOM (Cotrufo et al., 1994; Coteaûx et al., 1991; Kemp et al., 1994). Certainly, this is one major pathway through which elevated CO_2 concentrations will affect carbon storage in ecosystems. Beyond litter quality, however, there are other probably equally important ways that changes in atmospheric CO_2 concentrations may drive altered carbon storage in soils. Elevated CO_2 may influence dynamics of SOM processing by causing altered rhizodeposition (O'Neill, 1994; Stulen and den Hertog, 1993; Van de Geijn and Van Veen, 1993; Van Veen et al., 1991), by changing soil water content via altered water use by plants (Hungate et al., 1996a), or both (Figure 1). Altered decomposition will, in turn, affect nutrient availability to plants either positively (Zak et al., 1993) or negatively (Diaz et al., 1993), ultimately feeding back to influence net primary productivity. As Hungate et al. (1996b) and Harrison et al. (1995) illustrate, because SOM pools are so large in comparison to annual inputs from vegetation, it is difficult to detect changes in pool sizes in the short time period of most elevated CO_2 experiments. However, by using carbon isotope tracers in both ambient and elevated CO_2 treatments, it will be possible to estimate both the flux of carbon from roots to the soil, and the amount of carbon mineralized from new root inputs and from older SOM.

The use of carbon isotope tracers to follow deposition of new and respiration of new and old carbon in this way is not new. Over 20 years ago, Warembourg and Paul (1973) labeled grassland plants in the field with a pulse of ^{14}C, collected soil atmosphere CO_2 through buried tubes, and were able to quantify translocation of C belowground and respiration of labeled carbon. More recently, a number of papers have used ^{14}C pulse or ^{14}C or ^{13}C continuous labeling to follow fluxes of C through plant and soil pools, and to quantify contributions of old and new carbon to these pools (e.g. Ineson et al.,1996; Liljeroth et al., 1990, 1994; Merckx et al., 1987; Swinnen et al., 1994). Following this approach, Lekkerkerk et al. (1990) grew wheat plants in growth chambers under ambient and elevated (2 × ambient) CO_2, using ^{14}C in the atmosphere to continuously label fixed C in the plants. Because of the ^{14}C tracer, they were able to quantify the amount of the total soil respiration from their columns that was derived from newly fixed (^{14}C-labeled) carbon, and from older more resistant native SOM. They found that decomposition of older SOM accounted for a higher percentage of respired CO_2 at ambient than at elevated CO_2, and that decomposition of more resistant, native, SOM had been depressed under elevated CO_2. Based upon this and a measured increased flux of easily decomposable compounds into the soil at elevated CO_2, Lekkerkerk et al. (1990) suggest that microorganisms may have turned to using more easily decomposable and readily available substrates from roots instead of more resistant native SOM under elevated CO_2. Ultimately, a decrease in SOM (relative to unplanted soil) resulted from wheat growth at ambient CO_2, whereas an increase in SOM resulted from wheat growth at elevated CO_2.

Similar experiments exploring the effects of mineral nutrients and active roots on decomposition of SOM have also yielded interesting results. Using continuous ^{14}C labeling, Liljeroth et al. (1994) grew wheat and maize at different nutrient levels and explored the breakdown of organic matter in the soil. Previous experiments have suggested that differences in quality or quantity of rhizodeposition from the two species might cause quite different responses in the rhizosphere (Liljeroth et al., 1990; Merckx et al., 1987). Liljeroth et al. (1994) found that at high nutrients, relatively more ^{14}C was released from wheat roots to soil than at low nutrients, and decomposition of native SOM was depressed in the high relative to the low mineral nutrient treatment. They surmised that microbes preferentially utilized new root material, turning away from decomposition of more resistant, native SOM. This decrease in decomposition of native SOM, however, was not apparent when maize was grown at high nutrients, though the authors noted increased flux of ^{14}C to roots of maize at high N, as was observed in wheat. Clearly, some difference in the physiology of

maize vs. wheat roots during growth (whether quality of exudates, alteration of rhizosphere pH, nutrient uptake capacity, etc.) drove substantial differences in rhizosphere responses to rhizodeposition and subsequent processing of native SOM (Liljeroth et al., 1994).

In a recent synthesis considering possible effects of elevated CO_2 on carbon dynamics in soil, Van Veen et al. (1991) suggest that elevated CO_2 may lead to enhanced rhizodeposition, a switch of microbial preference to these new rhizodeposits, and, finally, a decrease in decomposition of older more resistant carbon. Carbon storage in soils might increase both because of decreased decomposition of old SOM, and because of decreased degradability of new litter inputs from roots and shoots grown under elevated CO_2. Van Veen et al. (1991), however, suppose that sufficient nutrients will be made available for plant growth by symbionts in this scenario. Diaz et al. (1993), on the other hand, argue that increased microbial populations may compete with plant roots for nutrients, immobilizing N and P and reducing plant response to elevated CO_2. Clarholm et al. (1985), however, suggest that increased microbial populations (growing on rhizodeposits as a carbon source) initially immobilize nutrients, but that ultimately, when populations of soil fauna increase and prey on the bacteria, more nutrients are made available to plants. If this "priming" of mineralization of SOM occurs under elevated CO_2, nutrient availability to plants might increase under elevated CO_2 because of rhizosphere microbial activity.

Hungate and Chapin (1995) combine these theories to explain the conflicting results of Diaz et al. (1993) (decreased mineral nutrient availability to plants growing under elevated CO_2) and Zak et al. (1993) (increased mineral nutrient availability to plants under elevated CO_2). In their scheme, if mineral nutrients are abundant in soils, microbes easily utilize C-rich rhizodeposits and immobilize mineral N. Degradation of more resistant SOM is depressed in this case, as noted by Lekkerkerk et al. (1990). Overall, mineral nutrient availability to plants is decreased (as reported by Diaz, 1993). If, instead, mineral nutrients are scarce in soils, microbes utilize rhizodeposits as a carbon-source, but break down more SOM in order to obtain nutrients. More nutrients are then moved into the active N pool in soil where, eventually, they may be made available to plants (e.g. via "priming").

Clearly, the complexity of interactions between nutrient and carbon cycling in soils dictates that soil carbon storage under elevated CO_2 may increase or decrease, and only with better understanding of the soil processes involved can predictions be made in particular ecosystems. It should be noted that increased availability of mineral N can have variable effects on the decomposition of soil organic material (for a review, see Dormaar, 1990), depending, perhaps, on the ease of decomposition of soil substrates (Van Veen et al., 1989). Also, Robinson et al. (1989) calculate that the ability of a "priming" effect to contribute a meaningful amount of nutrient to plants depends strongly on the form of carbon released from roots, on the presence of bacterial predators, and the form of SOM already present.

In considering processing of SOM in natural ecosystems under elevated CO_2, results from growth chamber experiments or experiments with single plant species can not necessarily be extrapolated to communities of plants living their entire life cycles under elevated CO_2 concentrations. However, the expense of and significant control issues associated with using continuous (or even pulse) ^{14}C labeling in the field in open-top chambers are prohibitive. In order to begin examining *combined* effects of elevated CO_2 and soil nutrient status on SOM dynamics under communities of plants growing under natural light and rainfall in the field, Cardon et al. (1996) explored processing of SOM in unfertilized and fertilized soils as part of the Jasper Ridge Elevated CO_2 Experiment (Field et al., 1996). Instead of relying on an atmospheric label (^{14}C or ^{13}C) to mark new carbon fixed by plants under elevated CO_2, C3 California sandstone and serpentine grassland communities were grown on soil developed under C4 grasslands in Colorado (provided by Elisabeth Holland, NCAR, gathered from the Central Plains Experimental Range, and mixed 1:1 v/v with sand to improve drainage). This strategy has been successfully used in other studies examining dynamics of SOM breakdown (e.g. Balesdent et al., 1987; Schonwitz et al., 1986). SOM in these Colorado soils was naturally labeled with a C4 carbon isotope signature; $\delta^{13}C$ was approximately -18 per mil. Leaves and roots from the C3 California sandstone grassland dominants *Avena barbata* and *Bromus hordeaceus* growing at ambient and elevated CO_2 had $\delta^{13}C$ signatures of approximately -30 and -40 respectively. (Soil and leaf $\delta^{13}C$ were measured with a isotope ratio mass spectrometer coupled with a combustion system and gas chromatograph, Europa Scientific). Soil atmosphere CO_2 was collected with gas-tight syringes from hollow stainless steel probes buried in the C4 soil; syringe contents were emptied into evacuated glass ampules, and analyzed

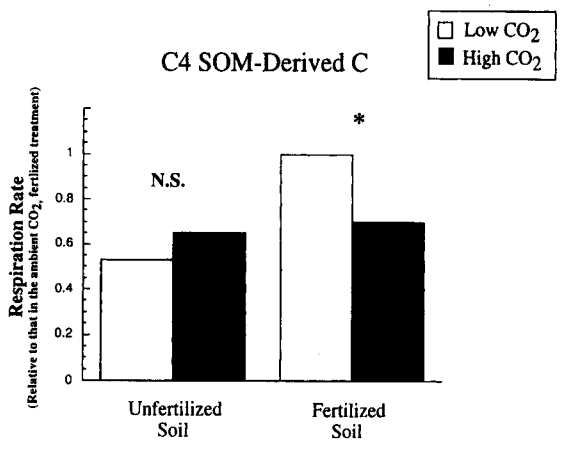

Figure 3. Rates of respiration of C4-derived CO_2 under four treatments, expressed relative to the highest absolute respiration rate (at high nutrients, low CO_2).

at SIRFER at the University of Utah.) By growing these C3 communities on this C4 soil and measuring soil respiration rate (with a LiCor 6200) and the $\delta^{13}C$ of soil atmosphere CO_2, it was possible to detect the dynamics of the amounts of respired C derived from current year's growth (labeled as C3 plant material) and of C from breakdown of C4-labeled SOM in the various treatments throughout the growing season.

The quantity of carbon respired from roots and microbes utilizing newly fixed C3 carbon vs. the quantity respired from older C4-labeled SOM was calculated in each of the four treatments (high and low nutrients, high and low CO_2) from January through May. Distinct shifts in the percentage of total respired carbon derived from C4 SOM were found under different CO_2 and nutrient treatments. This information was combined with soil respiration rates measured at the same time that isotope samples were collected, and a profile of respiration of SOM- and plant-derived C through the season was produced. Preliminary results from the high nutrient treatment during peak vegetative growth in February indicate that elevated CO_2 caused a 30% decrease in the contribution of C4 carbon to the soil atmosphere CO_2 relative to ambient CO_2 ($p<0.05$), whereas in the low nutrient treatment, no significant difference in contribution of respired C from SOM under ambient and elevated CO_2 was observed (Figure 3). These results extend the ideas proposed by Lekkerkerk et al. (1990), Merckx et al. (1987), Van Veen et al. (1991) that nutrient availability in soils strongly influences processing of SOM (and thus carbon storage) under elevated CO_2.

Several assumptions must be made in order to assign the proportion of respired CO_2 to the "new" or "old" pools in this (or any tracer) experiment, and these assumptions may not always hold. First, isotopic composition of different components of SOM are different, and the components break down at distinct rates, leading to measured respired CO_2 (and remaining SOM) being of slightly different $\delta^{13}C$ than original total SOM (Balesdent et al., 1987). In fact, even the CO_2 collected from respiration of microbes growing on simple substrates such as glucose can exhibit a carbon isotope composition different from the original substrate's isotopic composition (Blair et al., 1985; Mary et al., 1992). This fractionation presents a problem with trying to assign an exact proportion of respired CO_2 to the SOM or the C3 source in tracer experiments. Second, as CO_2 diffuses through soil, it is fractionated, with $^{13}CO_2$ diffusing more slowly (Cerling et al., 1991). The effect of this fractionation is most pronounced at extremely low soil respiration rates. The C4 soils, however, had relatively high respiration rates, diminishing the importance of this fractionation in our experiment. Even given these limitations to assigning exact proportions of C4-derived vs. C3-derived respiration of carbon, we see statistically significant changes in the carbon isotope signature of soil atmospheric CO_2 in elevated vs. ambient CO_2 treatments in the high soil nutrient case, implying altered decomposition under elevated CO_2. Since the flux of carbon from plant communities to soil can be quantified using the C3 tracer under both elevated and ambient CO_2, and since soil carbon can be analyzed and the amounts of C4 SOM and new C3 SOM quantified, it will be possible to get a much more sensitive measure of soil carbon storage than would be possible by only measuring small changes in huge, unlabeled soil carbon pools.

Conclusions

With the remarkable interplay between C and nutrient dynamics in ecosystems, it is clear that an understanding of ecosystem carbon storage will only emerge through a new, integrated view of the physiology of roots in concert with the activities of microbes. Plant species differ both in their phenology and in the compounds their roots exude, and it is not difficult to imagine that shifts in community composition (driven through altered competitive interactions under elevated CO_2) could lead to different dynamics of rhizosphere soil organic matter processing in a variety

of ecosystems. Beyond this, not only could changes in root exudation of specific compounds in specific plants influence plant nutrition and mineralization of nutrients, but also shifts in plant community composition in ecosytems can strongly influence the character of even the bulk soil. Elevated CO_2 research is moving toward a more comprehensive understanding of processes linked belowground, and it is becoming clear that the effects of elevated CO_2 on these processes may well determine whether terrestrial ecosystems accumulate carbon, buffering increasing atmospheric CO_2 concentrations, or release carbon from stored pools, exacerbating the steady increase of atmospheric CO_2.

Acknowledgments

Thanks to Manuel Lerdau, Bruce Hungate, Robert Jackson, Damian Barrett, and two anonymous reviewers for comments on the manuscript, to Margret van Vuuren for discussion of isotope fractionation during decomposition, and to F S Chapin for support of much of this work in his lab. Particular thanks also to Damian Barrett and to Jason Jaeger and Steven Lindow for providing preliminary data cited in this article. This research was supported in part by a Department of Energy Distinguished Postdoctoral Fellowship for Global Change to ZGC, administered by the Oak Ridge Institute for Science and Education. Work associated with the Jasper Ridge CO_2 Experiment was supported by National Science Foundation grants DEB 90-20135 to the University of California, Berkeley, DEB 90-20134 to the Carnegie Institution of Washington, and DEB 90-20347 to Stanford University.

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Section editor: H Lambers