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Issues and perspectives for investigating root responses to elevated atmospheric carbon dioxide

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Abstract

A thorough assessment of how plants and ecosystems will respond to increasing concentrations of atmospheric CO_2 requires that the responses of root systems and associated belowground processes be understood. Static measures of root-to-shoot ratio have not been satisfactory for describing the integrated responses of plants to CO_2-enriched atmospheres, but research with a process orientation has suggested that elevated CO_2 can stimulate root growth or root activity and provide a positive feedback on plant growth. There are, however, critical questions concerning the relevance of root data from short-term studies with potted plants when scaling to questions about plants in the field. Data on root responses to CO_2 enrichment in the field are fragmentary, but they allow us to more clearly define research questions for further investigation. Three perspectives for analyzing the significance of root responses as a component of the overall response of the terrestrial biosphere to increasing atmospheric CO_2 are suggested: (1) roots as a platform for nutrient acquisition and a mediator of whole-plant response to CO_2; (2) carbon storage in roots as a component of whole-plant carbon storage; and (3) effects of CO_2 enrichment on root turnover and the implications for carbon storage as soil organic matter. The relative importance of these different perspectives will vary depending on the ecosystem of interest and the larger-scale issues being considered.

Roots and global change issues

Analyses of plant responses to elevated concentrations of atmospheric CO_2 have focused largely on processes occurring above the ground. When questions are framed about plants in a CO_2-enriched atmosphere, root systems, being "out of sight", have often been "out of mind" as well. This is not surprising, since aboveground plant structures are generally of greater economic interest and are certainly easier to study than roots and belowground processes. Photosynthesis is particularly well studied, as it is the process by which the primary interaction between a green plant and atmospheric CO_2 occurs. But despite great advances in our understanding of photosynthetic responses to elevated CO_2 at levels ranging from molecular genetics to biochemistry to environmental physiology and micrometeorology, it is becoming increasingly clear that an understanding of photosynthesis alone is not sufficient to answer the important questions about terrestrial responses to a changing atmosphere. A whole-plant perspective is needed to understand the critical feedbacks and adjustments that occur within a plant and between plant and soil. Whether the questions concern carbon sequestration in vegetation and ecosystems or process level responses to a complex suite of environmental variables, root systems must be a significant component of the analysis.

The importance of understanding root responses has recently been highlighted by the challenge of the "missing carbon" within the global carbon cycle. Analyses of the annual exchanges of carbon between the atmosphere, terrestrial vegetation, and oceans (Post et al., 1990; Siegenthaler and Sarmiento, 1993; Tans et al., 1990) have failed to account for all of the carbon injected into the atmosphere each year by fossil fuel combustion. The physicist Freeman Dyson made the connection between the "missing carbon" and roots in a lecture at Oxford University in 1990 (Dyson, 1992):

"It is known from growth-chamber experiments that a common response of plants to an enrichment of carbon dioxide in the air is an increased root-to-shoot ratio ... Whether it occurs, or how much it occurs, in the world outside, we do not know. But it is at least plausible that the 15 percent increase of atmospheric carbon dioxide due to human activities has produced a worldwide shift in root-to-shoot ratios and a consequent growth of the carbon reservoir in soil. This, rather than accelerated growth of trees in undestroyed forests, may be the process that accounts for the bulk of the missing carbon".

There are many studies that have investigated at least some aspects of root responses to elevated CO_2. Recent reviews (Stulen and den Hertog, 1993; Rogers et al., 1994) have comprehensively compiled the data on those responses, and new field-oriented research projects are underway that will substantially augment that data base. It is the aim of this paper to review the responses of roots to elevated CO_2 while considering whether the data can be used to address the important questions concerning the terrestrial biosphere and atmospheric CO_2. Those questions, with an increased focus on biotic and abiotic interactions and feedbacks within ecosystems (Mooney, 1991), should direct the development of a new conceptual framework for studying root responses to elevated CO_2.

Responses of small plants

Root-to-shoot ratio

Most of the data on root responses to elevated CO_2 describe root mass of agronomic plants and, to a lesser extent, small tree seedlings and other perennials that had been grown in pots for weeks or months in ambient or elevated CO_2. The general conclusion that can be drawn from such studies is that root mass increases in elevated CO_2, as does shoot mass. This statement, however, does not specify whether there is a direct, specific effect of CO_2 on roots or rather a coordinated, whole-plant acceleration of ontogeny. The question then becomes whether root mass increases more or less than shoot growth. That is, does CO_2 enrichment affect carbon allocation patterns such that root growth specifically is altered. This question is most often answered by the simple calculation of the ratio of root dry mass to shoot (or stem) dry mass (root-to-shoot ratio) when the plants are harvested.

The data on effects of CO_2 concentration on root-to-shoot ratio are confusing and ambiguous, as evidenced by the wide range of generalizations present in review articles. Oechel and Strain (1985) state that "studies of root growth show that with increasing atmospheric CO_2 most plants allocate proportionately more of the extra carbon belowground, causing an increase in root-to-shoot ratios ..." Bazzaz (1990) also accepted that there generally is an increase in allocation to roots, but he suggested that the response was most apparent when nutrients and water are limiting. Eamus and Jarvis (1989) expressed the role of other resources more strongly: "... when nutrients have been supplied in adequate amounts sufficiently often, an increase in CO_2 concentration resulted in a decrease or in no change in the root:shoot ratio." From a horticultural point of view, Idso et al. (1988) concluded that for plants whose primary yield component is produced above-ground, "... it appears unlikely that atmospheric CO_2 enrichment has any significant effect on root:shoot ratios".

Experimental data can be presented to support any of these differing generalizations. The compilation of all of the published data, however, suggests that on average, root-to-shoot ratio changes little in elevated CO_2. Among crop plants the range of response in root-to-shoot ratio with CO_2 doubling was an 8.5% decrease to a 6.4% increase, except in sweet potato (34.9% increase) (Cure, 1985). Wullschleger et al. (1994) compiled 398 observations of 73 tree species in elevated CO_2 and used this data set to examine trends in root-to-shoot ratio. From a subset of 224 observations, the log-transformed mean response in root-to-shoot ratio was only a 6% increase for plants grown in elevated CO_2 (S. D. Wullschleger, personal communication; Norby et al., 1994). Although the generalizations presented above might suggest that the availability of other resources could alter the response of root-to-shoot ratio to CO_2, the mean response in this data base was independent of soil water or nutrient status (S. D. Wullschleger, personal communication). Regardless of the average response of root-to-shoot ratio, large shifts in the ratio have been the dominant response to CO_2 enrichment in some experiments (e.g., Norby et al., 1986).

This analysis of root-to-shoot ratio of plants in elevated CO_2 does not lead to a simple or satisfactory conclusion. The statement that is best supported probably is that of Stulen and den Hertog (1993), as echoed by Rogers et al. (1994): "The assumption that a larger proportion of the extra dry matter produced under CO_2 enrichment is allocated to roots needs critical reconsideration." Stulen and den Hertog (1993) list several reasons why determination of root-to-shoot ratio is subject to experimental error, including problems in defining the root-shoot boundary, quantitative recovery of roots (especially fine roots) and accounting for root decomposition, control of the nutrient and water regime in a pot, ontogenetic effects, and accumulation of non-structural carbohydrates. In addition to evaluation of these experimental problems, part of any reconsideration of root-to-shoot ratio should include thought about why the parameter is of interest. Root-to-shoot ratio may well be a useful index of carbon partitioning within the framework of a specific experiment. But in better defining the research questions pertaining to global change issues, we may find that root-to-shoot ratio of small, potted plants provides little useful information for addressing the larger-scale issues concerning the integrated responses of plants and ecosystems to elevated CO_2.

A process orientation

In studies of annual plants or short-term studies of seedlings of woody perennials, our interest in the responses of roots to CO_2 enrichment derives primarily from their role in water and nutrient uptake. (An exception are those crop plants for which the roots have economic value.) Root systems of CO_2-enriched plants can provide critical feedbacks to whole-plant response by altering the capacity of the plant to acquire sufficient water and nutrients from the environment. For example, our research program at the Oak Ridge National Laboratory on responses of trees to elevated CO_2 centered around a hypothesis that elevated CO_2 would stimulate belowground processes, thereby circumventing nutrient limitations to growth (Norby et al., 1986, 1994). Static measures of root-to-shoot mass ratio do not help us understand the processes of resource acquisition by roots. A more useful framework considers CO_2 effects on root growth and soil exploration, as well as root activity and associated microbial activity (Zak et al., 1993). The concept is that nutrient availability could increase in elevated CO_2 either because of increased size of the root system or increased activity per unit root, although as will be discussed later, there are different implications for these two alternatives as the scale of interest increases. The relevant research topics include root length and branching, periodicity in root growth, distribution between different sizes or type of roots, nutrient uptake by roots, respiration, exudation, and symbiotic activity. This framework represents a process orientation that should prove more useful in understanding the role of roots in plant response to CO_2 enrichment.

Root growth

Experiments primarily with agronomic plants have shown that elevated CO_2 can cause significant changes in plant root morphology that can alter the capacity of a root system to explore the soil (Rogers et al., 1992). Several studies with woody plants have divided root systems by size class to determine the separate effects of CO_2 on fine roots and larger, coarse roots. Fine roots were the most responsive plant component to CO_2 enrichment in *Quercus alba* (Norby et al., 1986) and *Castanea sativa* seedlings (El Kohen et al., 1992), but in *Liriodendron tulipifera*, fine roots increased in mass with CO_2 enrichment to the same extent as coarse roots (Norby and O'Neill, 1991). In a model wet-tropical assemblage, the effect of CO_2 enrichment on root mass decreased with increasing root diameter. Mass of roots less than 1 mm diameter was 63% greater in elevated CO_2, roots from 1 to 3 mm diameter were enhanced by 37%, and larger roots were enhanced by 16% (Körner and Arnone, 1992). Separation of root responses by size class may be particularly important in tree species. The metabolically-active fine roots are of paramount importance in nutrient uptake and microbial interactions. The coarse, woody roots may represent most of the dry mass of the root system and are important in carbon and nutrient storage, but they are less important metabolically. In many experiments with herbaceous plants or small tree seedlings, the plants may be too small or the experiment of insufficient duration for any significant differentiation in root size to occur.

Fine root production is of particular interest to considerations of physiological adjustments to increased CO_2 concentrations because of the presumption that plants with more fine roots can exploit a larger volume of soil, perhaps increasing the supply of water and nutrients to the plant. While the direct effects of elevated CO_2 on stomatal aperture (Eamus and Jarvis, 1989) may reduce whole-plant water use, water shortages will continue to be an important limitation to plant growth in many habitats. The balance between fine roots and leaf area may be particularly interesting. In *Liriodendron*, the ratio of fine root mass to leaf area increased with increasing CO_2, and this was interpreted as a compensatory response that could increase whole-plant water-use efficiency (Norby and O'Neill, 1991).

Increased fine root mass, however, does not necessarily imply a larger volume of soil occupied or exploited by the root system. A more direct assessment is provided by measures of root length and branching patterns. Del Castillo et al. (1989) reported that

CO_2 increased the number of soybean roots but not their elongation rate, from which they inferred that the soil volume explored by the root system would not increase, but a given volume of soil would be explored more thoroughly. Berntson and Woodward (1992) reached a different conclusion by measuring in situ the root system architecture of *Senecio vulgaris* (Fig. 1). Elevated CO_2 resulted in more highly branched, longer root systems that foraged through larger volumes of soil. Furthermore, the reductions in branching and root length in ambient CO_2 resulting from low water availability were compensated for by CO_2 enrichment. While there were few aboveground measurements from which to assess whether changes in root system structure were a specific response to CO_2 concentration or an indirect response to altered ontogeny, the authors concluded that under elevated CO_2 the intensity of foraging root systems might be unchanged while the extent of foraging is increased.

Root activity

An increased supply of photosynthate to roots could stimulate various metabolic or microbial activities in the roots or associated rhizosphere. The first question to address, then, is whether there is increased supply of photosynthate to roots of plants grown in elevated CO_2. Norby et al. (1987) used $^{14}CO_2$ to determine C allocation in *Pinus echinata* seedlings growing in ambient or elevated CO_2. Proportionately more ^{14}C was allocated to fine roots in the CO_2-enriched seedlings. Lekkerkerk et al. (1990), however, reported no differences in ^{14}C distribution to roots in wheat plants. Elevated CO_2 increased carbohydrate concentrations in *Pinus taeda* seedlings by 68% and compensated for the decline in carbohydrates caused by water stress (Tschaplinski et al., 1993). The concentration of non-structural carbohydrates (mainly starch) in roots of cotton plants was two- to four-fold higher in elevated CO_2 (Wong, 1990). While these increased carbohydrate concentrations are not necessarily indicative of increased photosynthate allocation to roots, they may be important as carbon reserves (Chomba et al., 1993) or in the response of roots to water stress (Tschaplinski et al., 1993).

If there is excess carbohydrate allocated to roots, the expected result could include higher rates of respiration or increased exudation of soluble organic compounds. Whipps (1985), however, reported that the percentage of ^{14}C translocated to maize roots that was subsequently released through respiration or exudation was not significantly affected by the CO_2 concentration in which the plants were grown. In the *Pinus echinata* study (Norby et al., 1987) exudation was marginally higher in elevated CO_2 and root respiration was lower. The apparent effect on exudation was attributed to a greater fine root mass rather than increased exudation per unit root. Lekkerkerk et al. (1990) concluded that as a percentage of the ^{14}C translocated to roots, relatively less was retained by the roots in elevated CO_2. The remainder was respired by roots or by the soil microbial community, presumably after exudation or sloughing of ^{14}C material. Körner and Arnone (1992) assumed that fine root turnover or exudation was higher in elevated CO_2 in their model tropical systems because soil xylanase and protease activities were higher. The possible effects of increased carbon supply to roots on microbial biomass or activity, as well as on mycorrhizal and nitrogen fixing relationships, are discussed by Norby et al. (1994) and O'Neill (1994).

Ion uptake across root membranes is an energy-demanding process that might respond to an increased supply of carbohydrate to root systems, but there have been few investigations on responses of ion uptake processes to elevated CO_2. Luxmoore et al. (1986) used a growth analysis approach to show that in *Pinus virginiana* seedlings specific nutrient uptake (i.e., nutrient uptake per unit root and time) was not altered by CO_2 enrichment. There have also been detailed mechanistic studies of ion uptake in response to soil CO_2 partial pressure (Thibaud et al., 1990).

Relevance of short-term experiments

Evidence from short-term experiments with small plants supports the suggestion that elevated CO_2 concentrations can in some cases stimulate root growth or root activity, providing a positive feedback on plant growth by improving resource availability. For example, in *Quercus alba*, the increase in fine root mass and associated mycorrhizal and rhizosphere activity was thought to be the mechanism by which P availability in the soil increased, thereby avoiding a P deficiency in the CO_2-enriched seedlings (Norby et al., 1986). Similar increases in N availability have not been observed, except in the case of N_2-fixing systems (Norby, 1987; O'Neill, 1994). Increased root and associated belowground activity in the CO_2-enriched tropical systems of Körner and Arnone (1992) apparently increased nutrient availability, but this resulted in nutrient loss from the system rather than in increased uptake by the plants. Stimulation of root growth in

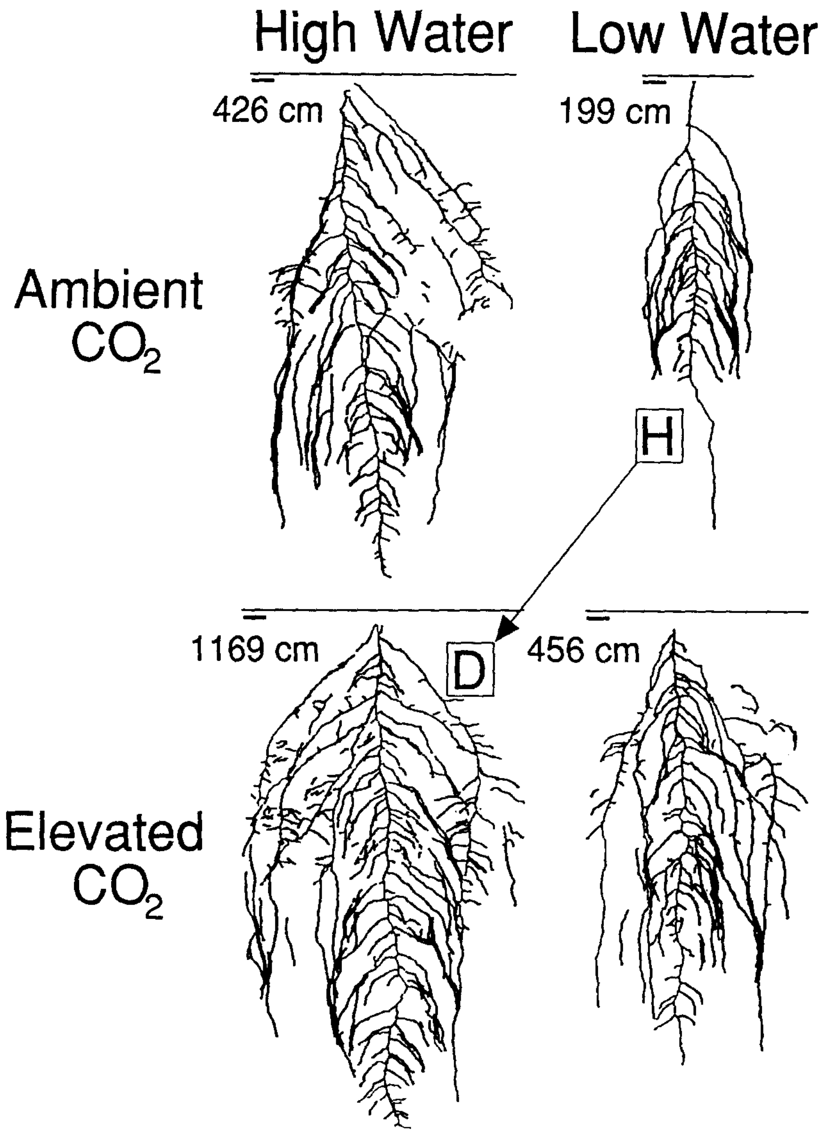

Fig. 1. Representative tracings of root systems of *Senecio vulgaris* plants grown in ambient of elevated CO_2 and high or low water availability (Berntson and Woodward, 1992). The long horizontal bar above each root is the maximum horizontal spread of the root system, with the shorter bar representing 1 cm. The number below the bars gives the total root length. CO_2 enrichment resulted in a more dichotomously branched (D) and longer root system that foraged through larger volumes of soil. Low water availability reduced branching and root length, resulting in a herringbone pattern (H), but this was compensated for by CO_2 enrichment. Figure courtesy of Glenn Berntson, Harvard University.

CO_2-enriched *Liriodendron tulipifera* seedlings, particularly the ratio of fine roots to leaf area, was associated with higher whole-plant water-use efficiency, which occurred in the absence of consistent effects of CO_2 on leaf-level stomatal conductance and transpiration efficiency (Norby and O'Neill, 1991).

Can the data from short-term experiments and our interpretations of those data be applied to plants growing in the field? In most cases the primary rationale for conducting the experiments was the hope that they would provide insight — if not direct predictions — about the responses of plants growing in the field in the CO_2-enriched atmosphere of the future. (The exceptions are considerations of responses to CO_2 in greenhouses for commercial production; Porter and Grodzinski, 1985). Hence, it is particularly important to consider whether the typical design of the short-term experiment produces relevant and appropriate data for larger-scale perspectives. The first consideration is whether the use of an artificial rooting medium contained in a pot creates an artifact large enough to prevent extrapolation to the field. Small pots can alter root growth and morphology, and CO_2 effects on root deployment can interact with pot characteristics (Berntson et al., 1993). Photosynthesis and other aboveground responses to elevated CO_2 are altered when root growth is restricted by small pots (Thomas and Strain, 1991). The effect of root restriction on growth and reproductive yield may be reversed by the addition of supplemental nutrients (McConnaughay et al., 1993), but nutrient additions to an inert rooting medium can create serious artifacts in plant nutrition and growth if growth rate and nutrient flux are mismatched (Brown and Higginbotham, 1986). Sophisticated approaches can maintain steady-state plant nutrition by matching nutrient flux density to relative growth rate (Pettersson and McDonald, 1992), but in the field steady-state nutrition is achieved by root growth (and concomitantly exploited soil volume and nutrient flux density) increasing in relation to shoot growth (Ingestad, 1982). Substituting nutrient additions for increased soil exploration may allow for a detailed examination of particular plant metabolic processes in response to CO_2 enrichment, but the responses of root growth and whole-plant carbon allocation cannot then be applied directly to a field situation in which root-soil interactions are important.

Some artifacts can be avoided by using unamended native soil as the rooting medium (e.g., Norby et al., 1986; Norby and O'Neill, 1989, 1991; El Kohen et al., 1992), although soil heterogeneity, microbial processes, and aeration are still disrupted, and maintenance of appropriate water supply is difficult. While the responses to CO_2 in unamended soil may be confounded with nutrient interactions, this is not necessarily a problem when the research questions are specifically focused toward responses in nutrient-limited habitats. Regardless, no pot can contain a tree for several growing seasons without affecting root growth when the roots of the field-grown trees extend for a meter or more in every direction. The results of CO_2 enrichment experiments with potted plants can suggest useful hypotheses about root responses to be tested in a more natural setting, but the responses should be used cautiously if the purpose is to predict the responses in the field.

Another problem is the difficulty in interpreting static measures of root mass when root growth is episodic or when significant root death and sloughing occur. While harvests of plant shoots can be scheduled in relation to observable phenological events (e.g., completion of leaf expansion), temporal patterns of root growth remain invisible without specialized equipment. The relative effects of CO_2 enrichment on root *versus* shoot growth are likely to be different just prior to a flush of root growth compared to after the completion of the growth flush. The difference between *Cedrus atlantic* and *Pinus nigra* in their chronological patterns of root growth in relation to shoot growth was an important determinant of the overall response to CO_2 (Kaushal et al., 1989). Poorter et al. (1988) also emphasized the importance of ontogenetic shifts in relative root growth. Root-to-shoot ratio of *Plantago major* was at first higher and later lower in elevated CO_2 compared to the ratio in plants in ambient CO_2, with the shift attributed to the increase in self-shading of leaves with increasing plant size. With this sort of variation, extrapolations from static measures of relative root mass to the field situation have little meaning.

A related complication in using short-term studies to predict responses of plants over several years is the role of roots in storage and remobilization of carbohydrates and nutrients. These issues usually are not relevant and therefore not considered in most short-term experiments. The possibility of remobilization of stored reserves complicated the analysis of CO_2 effects in *Quercus alba* seedlings because of the species' cyclic, episodic growth pattern (Norby and O'Neill, 1989). Wong (1990) argued that differential accumulation of non-structural carbohydrates in different CO_2 concentrations obscures the effect of CO_2 on growth and could lead to misinterpretations, particularly of the root-to-shoot ratio. Sasek and Strain (1988) suggested that their 60-day experiment with *Pueraria lobata* (kudzu) underestimated the effect CO_2 enrichment might have had on root-to-shoot ratio over a full growing season, since photosynthates are allocated to root growth and storage in the latter part of the growing season. Increased storage in overwintering roots could be expected to support increased shoot growth in the next growing season, a response to CO_2 not readily observable in short-term experiments with potted plants. The most difficult issues to address in relating the short-term pot experiment to the field situation are those requiring scaling across levels of ecological complexity. Patterns of resource acquisition within a competitive stand may differ from that under non-competitive conditions (Bazzaz and McConnaughay, 1992), but little is known about how CO_2 affects root growth in the presence of interspecific competition for soil resources. An increase in fine root proliferation that was observed in a pot experiment, for example, may not occur in the field if the rooting space is already occupied by another root system. Hence, pot experiments may be most useful for identifying the responses of small-scale processes to CO_2 enrichment rather than for describing root growth itself.

In extrapolating process data from a pot to a complex ecosystem, it is important to consider whether the response was a result of a specific stimulation of a process, such as increased exudation per unit root, or a result instead of increased root growth providing a larger platform for the process to occur. Although an increase in total exudation may be important within the simple ecosystem defined by the pot, if the response occurred only because there were more roots, then we have learned nothing new that would alter our predictions of plant response in the field. That is, if exudation scales linearly with root growth (as apparently was the case in the studies cited above), only the effect of CO_2 on root growth need be known to evaluate the potential consequences of increased exudation. Furthermore, if root density reaches a maximum value in a developing ecosystem (similar to the maximum leaf area after canopy closure), then any effects of CO_2 on exudation, nutrient uptake, mycorrhizal density, symbiotic N_2 fixation, or other such process would not continue to be affected by CO_2 unless the rates per unit root were stimulated.

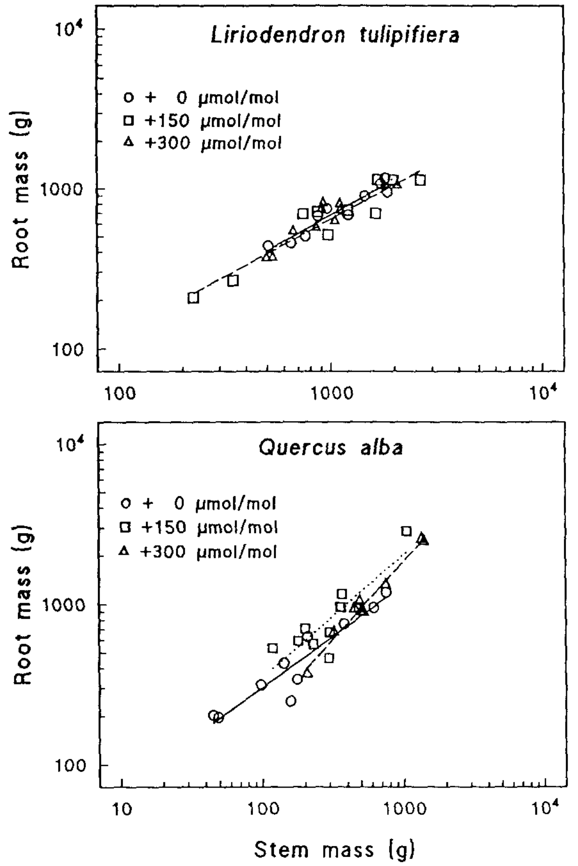

Fig. 2. Allometric analysis of root and stem mass of *Liriodendron tulipifera* and *Quercus alba* saplings grown for 3–4 growing seasons in open-top field chambers at different atmospheric CO_2 concentrations (Norby et al., 1992, 1993). In *Q. alba* the slopes of the log-log regression increased with increasing CO_2 concentration ($p < 0.09$).

Root responses in the field

Given the difficulty in applying the information about root responses from experiments with small, potted plants to answer larger-scale questions about plants and ecosystems in a CO_2-enriched atmosphere, it is especially important that root responses are measured in field experiments. Unfortunately, the number of experiments on elevated CO_2 conducted in the field is small, and none have focused specifically on root responses. The problems with studying roots in the field are well known: excavation is tedious, labor-intensive, and prone to error; non-destructive observations are difficult, so static measures cannot easily be related to root phenology; most approaches to measuring root processes such as respiration or exudation are likely to alter the process itself. Nevertheless, there exist enough data on root responses to CO_2 in the field to allow us to explore the important concepts and better define hypotheses and future research priorities.

The experiments that will be drawn upon in this discussion are the free-air CO_2 enrichment (FACE) of cotton in Mississippi and Arizona, in which Rogers et al. (1992) measured many root parameters and associated soil microbial responses; open-top chamber exposure of *Liriodendron tulipifera* (yellow-poplar) and *Quercus alba* (white oak) saplings for 3 to 4 years (Norby et al., 1992, 1993), in which root mass, fine root density, mycorrhization, and CO_2 efflux were measured; long-term exposure of *Citrus aurantium* (sour orange) trees under horticultural conditions (Idso and Kimball, 1991, 1992), in which fine root density was measured; and one-year exposure of *Populus grandidentata* (bigtooth aspen) (Zak et al., 1993), in which total root mass and associated soil responses were measured. New experiments with *Pinus taeda*, *Pinus ponderosa*, *Pseudotsuga menziesii*, and temperate grasslands have an increased focus on belowground responses, and should be providing valuable data in the future.

Root mass

Carbon stored in large, woody, perennial roots is a component of ecosystem carbon storage, just as is the carbon stored in woody stems, yet it is largely invisible and therefore largely ignored in analyses of global carbon budgets. To assess the potential of trees in a CO_2-enriched atmosphere to store additional carbon based only on their stem growth responses is implicitly to assume that roots and stems respond similarly. To address this problem, we excavated the tap roots of *Liriodendron* and *Quercus* saplings from the soil within the open-top chambers in which the trees were grown under different atmospheric CO_2 concentrations (Norby et al., 1992, 1993). The *Liriodendron* tap roots extended approximately 0.6 m deep, and the *Quercus* roots were as deep as 1.2 m. Lateral roots, which extended 1–2 m from the trunk, could not be excavated completely, so their mass was estimated from their diameter at the point of attachment to the tap root, using a regression analysis developed from a subset of lateral root systems that were completely excavated. *Liriodendron* root mass was 10% greater in trees grown in ambient + 150 μmol/mol CO_2, and 15% greater in ambient + 300 μmol/mol, compared to trees grown in ambient air. These differences were similar to the differences in whole-tree mass and were not close to statistical significance. In contrast, *Quercus* root mass was 77% greater in +150 and 136% greater in +300 compared to ambient-grown trees, significantly different at $p < 0.05$. Root-to-shoot ratio in these data sets is not particularly meaningful because the ratio decreased with increasing plant mass, thus confounding any specific effects of CO_2 on root growth with ontogenetic shifts coinciding with CO_2 effects on whole-plant growth. The allometric approach, in which root mass of individual plants is plotted against stem mass on log scales (Fig. 2), suggests that there were no effects of CO_2 on relative root growth in *Liriodendron*, but root growth was enhanced relative to stem growth in *Quercus*. The slopes of the regressions for *Quercus* are 0.64 in ambient CO_2, 0.76 in ambient + 150 μmol/mol, and 0.97 in ambient + 300 μmol/mol ($p < 0.09$). Specific effects of CO_2 enrichment on *Quercus* root mass are particularly important with regard to carbon storage because root mass was more than twice that of stems, whereas in *Liriodendron*, root mass was about 60% of stem mass.

Carbon storage in roots is probably not an important issue in the analysis of responses of agronomic systems to CO_2 enrichment (the root-to-shoot mass ratio of cotton is less than one-tenth that of *Quercus alba* saplings), but changes in rooting could influence other aspects of crop performance. In the FACE study of cotton in Arizona (but not as clearly in the similar Mississippi experiment), root length and mass density were greater in elevated CO_2, especially in the upper profile (Rogers et al., 1992). Taproot diameter, mass, and volume (but not length) increased, as well as the number, length, and mass of lateral roots. The responses were considered to be potentially important for seedling establishment, especially under stress conditions, but perhaps less significant under conditions of unlimited water and nutrient supply.

Fine roots

The importance of considering fine roots separately was emphasized in several previously discussed seedling experiments. In the field experiment with *Quercus* and *Liriodendron* saplings, fine root density of both species increased with increasing CO_2, and in *Liriodendron*, the increase greatly exceeded any effects of CO_2 on other plant components (Norby et al., 1992). However, the percentage of total root mass accounted for by fine roots was less than half the comparable percentage in seedlings (cf. Norby and O'Neill, 1991), and the increase in fine root mass had a minimal impact on whole-tree mass. Because of ontogenetic shifts in plant morphology, specific effects of elevated CO_2 on fine root mass will become a relatively less important component of whole-plant carbon storage as trees grow, and large effects of CO_2 on total root mass observed in seedlings may not translate into an equivalent effect in larger trees if the CO_2 effect is due primarily to increases in the fine root component. This analysis emphasizes the importance of separating functionally distinct components of root systems to increase the value of the data at different scales.

Regardless of the limited implications of fine root mass to carbon storage in the plant, fine roots are undoubtedly important physiologically for nutrient and water uptake, as a platform for interactions with soil microbes, and for carbon cycling in ecosystems. In addition to the *Liriodendron* and *Quercus* study, other field studies have shown fine roots to increase in elevated CO_2. In the cotton study, fine root density increased in elevated CO_2 at most depths to 90 cm, but was increased more significantly in the upper 45 cm of soil. The effect of CO_2 also tended to be larger as the distance from the row center increased, suggesting faster or more prolific spread of roots in elevated

CO_2 (Rogers et al., 1994). In *Citrus* trees fine root mass to a depth of 1.2 m increased 180% in elevated CO_2, identical to the increase in calculated aboveground mass (Idso and Kimball, 1991). Subsequent extractions of soil cores to 0.4 m led to an estimate of a 130% increase in fine root mass in the upper profile (Idso and Kimball, 1992). The relationship of fine root mass to trunk cross-sectional area through time was the same in ambient and elevated CO_2. Together these results suggest that CO_2 enrichment had no specific effect on fine root production in *Citrus* trees. Buried root ingrowth bags were used to measure root production in a tallgrass prairie ecosystem (Owensby et al., 1993b) and a brackish marsh ecosystem (Curtis et al., 1990). Root production in the tallgrass system was 130% greater in elevated CO_2 chambers in a wet year and 17% greater in a dry year, compared to that in ambient CO_2 chambers. Root production in the marsh community dominated by the C_3 sedge *Scirpus olneyi* was increased 83% in elevated CO_2.

None of these studies have been able to measure root mortality and decomposition in order to estimate total fine root production. Static measures of fine root mass at the end of a growing season probably underestimate the amount of carbon allocated by the plant to fine roots. An alternative, indirect measure is provided by the CO_2 efflux from the soil, which results from a combination of root respiration and soil microbial respiration. In the *Liriodendron system,* CO_2 efflux tended to be higher in elevated CO_2 (Norby et al., 1992), and this response was found to be a consistent and statistically significant one beneath the *Quercus* trees during the next year (R. J. Norby, unpublished data). Increased CO_2 efflux was interpreted as resulting from increased carbon allocation to fine roots in elevated CO_2 leading to an increase in carbon cycling through the soil. In the *Quercus* system, specific fine root respiration was lower in elevated CO_2, but the increase in fine root density more than compensated, and the proportionate increases in calculated total fine root respiration with increasing atmospheric CO_2 concentration were similar to the proportionate increases in CO_2 efflux from the soil (R. J. Norby, unpublished data).

Although it is easy to speculate that increased fine root production should be physiologically beneficial to the plant, the *Populus* study (Zak et al., 1993) has been the only one which attempted to quantify the linkages between CO_2 effects on root production and secondary responses. *Populus grandidentata* root sprouts were planted in nutrient-poor sand within open-top chambers and grown for 152 days in ambient or twice-ambient CO_2. Total plant biomass, of which about 80% was in roots, was significantly increased by CO_2 enrichment. The number of roots, root length, and root length production all were substantially greater at elevated CO_2. Associated with these root responses, labile carbon in the rhizosphere of CO_2-enriched plants was significantly greater than for plants in ambient air, and microbial biomass carbon was increased in both rhizosphere and bulk soil. The apparent increase in N mineralization was evidence for a positive feedback on soil C and N dynamics, effected through stimulation of belowground production. This experiment illustrates the importance of root production, mortality and metabolism, and their influence on soil microorganisms in understanding ecosystem responses to rising CO_2.

Root decomposition and soil organic matter

The mean residence time of live fine roots generally is about 1 year, although longer times have also been reported (Joslin and Henderson, 1987). Increased fine root production in elevated CO_2 cannot, therefore, represent an important mechanism for increasing long-term carbon sequestration by plants (except, perhaps, indirectly by improving nutrient or water status). However, the large annual production of fine roots coupled with their relatively slow decomposition rates after they are shed implies that a large portion of soil organic matter is derived from fine roots (McClaugherty et al., 1982). At least 25% of the soil organic matter in hardwood forests could be accounted for by fine roots (McClaugherty et al., 1984). If CO_2 enrichment causes a specific stimulation of fine root production or a decrease in fine root decomposition, ultimately there could be increased carbon sequestration by the ecosystem, even if not by the plant.

Root decomposition is in part a function of the chemical composition of the tissue in relation to the nutritional requirements of decomposer organisms, and is most commonly characterized as the C-to-N ratio. Carbon-to-N ratio (or N concentration) of live fine roots may be a reasonable predictor of root decomposition because N reportedly is not mobilized from fine roots prior to senescence (Nambiar, 1987). (In contrast, C-to-N ratio of green leaves is not a good predictor of leaf litter decomposition because N is withdrawn prior to leaf senescence; O'Neill, 1994.) Carbon-to-N ratio of roots of the marsh plant *Scirpus olneyi* was 22% higher in elevated CO_2 (Curtis et al., 1990). Nitrogen concentration in roots also tended to be lower in elevated CO_2 on the tallgrass prairie (Owensby et al., 1993a), in *Quercus alba* and dead *Liriodendron tulipifera* fine roots from field-grown saplings (R. J. Norby, unpublished data), and in fine roots from potted *Quercus* and *Liriodendron* seedlings (Norby et al., 1986; Norby and O'Neill, 1989).

Decomposition of fine roots produced in elevated CO_2 has not been measured. The possibility of changes in fine root production or decomposition leading to increased carbon sequestration as soil organic matter, while supported by ecosystem models (Hunt et al., 1991; Post et al., 1992; Thornley et al., 1991), is wholly a matter of speculation. The link between relatively short-term plant responses to CO_2 enrichment and any longer-term consequences at the ecosystem level will be very difficult to measure, but the possibility should not be ignored when attempting to extrapolate plant physiological data to predict ecosystem responses.

The need for better questions

The discussion above about the potential significance of CO_2 effects on fine roots illustrates an important need in global change research. It is imperative that the issues of interest be clearly defined so that the research questions are asked correctly and the data are interpreted in the right context. Three perspectives for analyzing the significance of root responses as a component of the response of the terrestrial biosphere to increasing atmospheric CO_2 are apparent:

(1) Roots as a platform for nutrient acquisition and a mediator of whole-plant response to CO_2.

(2) Carbon storage in roots as a component of whole-plant carbon storage.

(3) Effects of CO_2 enrichment on root turnover and the implications for carbon storage as soil organic matter.

The relative importance of these different perspectives will vary depending on whether the primary issue of interest is the role of vegetation in the global carbon cycle or rather plant and ecosystem responses for their own sake (i.e., environmental quality, economic benefit). For analyses of the global carbon cycle, carbon storage in roots and contributions of fine roots to soil organic matter are of paramount importance, but the role of roots in mediating whole-plant response is only important indirectly insofar as whole-plant carbon storage is increased. For analyses of plant and ecosystem responses, physiological responses of roots and associated microbial responses increase in importance (O'Neill et al., 1991). Implicit in all of these issues is the recognition that root responses cannot be considered apart from the rest of the system.

These issues will also vary in importance depending on the ecosystem of interest. In agricultural systems the only perspective of interest is the physiological role of roots. (Root crops are again an exception since carbon storage in roots has an economic attribute). In heavily fertilized and irrigated agrosystems, as distinct from dryland and less intensive systems, even CO_2 effects on nutrient acquisition might be of limited importance. In grasslands, both the physiological role of roots and the linkage between root production and soil organic matter could be of interest, but without woody roots, carbon storage in the living root system might have limited direct bearing on ecosystem carbon storage. In forest systems, all three perspectives could be critical, depending on the temporal and spatial scale of interest.

If the root response data from short-term experiments with potted seedlings are to be relevant to the larger-scale issues of interest, there must be some recognition that different perspectives are needed for different situations. Clearly, data describing CO_2 effects on root-to-shoot ratio are insufficient to address all of the critical questions concerning ecosystem responses to increasing atmospheric CO_2.

There is increasing recognition that responses below the ground to the CO_2 environment above the ground may be a critical missing link in our analyses of ecosystem responses to a changing atmosphere. Those belowground responses begin with changes in root growth and root processes. The extant data set that is relevant to the larger-scale issues of interest is partial, fragmentary, and sometimes ambiguous. Nevertheless, that data set has helped to provide us with sufficient understanding of the important and potentially responsive processes of root systems to study in CO_2 enrichment experiments, as well as providing the proper perspectives for analyzing those processes. It is no longer appropriate to state simply that "we need to know more about belowground responses." We can now better define our questions about roots and formulate specific testable hypotheses. A primary objective of the new generation of field-oriented experiments should now be to test and refine those hypotheses and evaluate the important role of roots and associated belowground responses in ecosystem responses to elevated atmospheric CO_2.

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