REVIEW

Climate change goes underground: effects of elevated atmospheric CO_2 on microbial community structure and activities in the rhizosphere

Barbara Drigo · George A. Kowalchuk · Johannes A. van Veen

Abstract General concern about climate change has led to growing interest in the responses of terrestrial ecosystems to elevated concentrations of CO_2 in the atmosphere. Experimentation during the last two to three decades using a large variety of approaches has provided sufficient information to conclude that enrichment of atmospheric CO_2 may have severe impact on terrestrial ecosystems. This impact is mainly due to the changes in the organic C dynamics as a result of the effects of elevated CO_2 on the primary source of organic C in soil, i.e., plant photosynthesis. As the majority of life in soil is heterotrophic and dependent on the input of plant-derived organic C, the activity and functioning of soil organisms will greatly be influenced by changes in the atmospheric CO_2 concentration. In this review, we examine the current state of the art with respect to effects of elevated atmospheric CO_2 on soil microbial communities, with a focus on microbial community structure. On the basis of the existing information, we conclude that the main effects of elevated atmospheric CO_2 on soil microbiota occur via plant metabolism and root secretion, especially in C3 plants, thereby directly affecting the mycorrhizal, bacterial, and fungal communities in the close vicinity of the root. There is little or no direct effect on the microbial community of the bulk soil. In particular, we have explored the impact of these changes on rhizosphere interactions and ecosystem processes, including food web interactions.

Keywords Elevated atmospheric CO_2 · Rhizosphere · Microbial community · Microfauna · Carbon dynamics

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Introduction

The concern over the possible consequences of increasing concentrations of atmospheric carbon dioxide (CO_2), largely as a result of the cumulative effects of emissions from fossil fuel combustion, land clearing, and the response of the oceans and biosphere to this anthropogenic perturbation, has created renewed interest in developing full understanding of the ecosystem responses to increased levels of CO_2 (IPCC 2007).

The rapid increase of CO_2 in the atmosphere over the last century has led to an increased global ecosystem carbon (C) storage (Schimel et al. 2000). Terrestrial ecosystems are intimately connected to atmospheric CO_2 levels through photosynthetic fixation of CO_2, sequestration of C into biomass and soil, and subsequent release of CO_2 through respiration and decomposition of organic matter. The amount of C stored globally in soils is much larger than that in the vegetation, with soil being the major organic C

pool in all terrestrial biomes. The CO_2 concentrations in the pore space of active soils is much higher, i.e., between 2,000 and 38,000 ppm, than in the atmosphere above the soil, and the predicted increases in the atmospheric CO_2 over the next century are small compared to the concentration of soil CO_2. Thus, direct effects of elevated atmospheric CO_2 concentrations on soil-borne communities would be expected to be negligible compared to potential indirect effects as modulated by plants. Indeed, decades of experimentation using growth chambers, glasshouses, open-top chambers, and recently free-air CO_2 enrichment (FACE) experiments have provided evidence that enrichment of atmospheric CO_2 can have severe effects on terrestrial ecosystems and interact with the C cycling belowground. The main cause of these effects is the changes in the organic C dynamics due to the impact on the primary source of organic C in soil, i.e., plant photosynthesis. As the majority of life in soil is heterotrophic and dependent on the input of plant-derived organic C, the activity and functioning of soil organisms may similarly be affected by changes in the atmospheric CO_2 concentration. In addition to photosynthesis, rising atmospheric CO_2 concentrations may also result in decreased evapotranspiration and, consequently, increased water use efficiency (Ainsworth and Long 2005). These effects are more pronounced in C_3 plants than C_4 plants (Long et al. 2004). Changes in photosynthesis under elevated CO_2 are likely to stimulate above-ground biomass production of C_3 plants, which will also have an effect on the C fluxes from the above-ground compartment into the roots and from the roots into the soil. Besides quantitative changes, also qualitative changes in the C that enters the soil as litter and root-derived products have been demonstrated (Cotrufo et al. 1998; King et al. 1997; Pendall et al. 2004; Zak et al. 1993, 2000).

Carbon input into the soil generally increases in response to elevated CO_2 concentrations even when there is no significant CO_2 stimulation of above-ground growth (Körner and Arnone 1992). Therefore, a major indirect response to an increase in atmospheric CO_2 consists in the greater below-ground C allocation through root exudation and turnover, which is likely to lead to changes in the size and the activity of soil microflora (Couteaux et al. 1999; Körner 2000; Rillig et al. 2001). As a result of greater biological activity, changes in the dynamics of major nutrients such as nitrogen (N) are also expected. Yet, the complexity in soil processes makes straightforward predictions difficult. For instance, Hu et al. (2001), Jongen et al. (1995), Van Ginkel and Gorissen (1998), and Van Ginkel et al. (2000) demonstrated a reduction in microbial decomposition in grasslands after exposure to elevated CO_2. Hu et al. (2001) suggested that elevated CO_2 results in a decrease of the amount of N available to microbes due to

enhanced plant growth, thereby reducing the degradation capacity of the microbes. In turn, the greater immobilization of soil nutrients due to the increased C availability could lead to reduction or elimination of the plant's ability to respond to the increase in the atmospheric CO_2 concentration, ultimately resulting in a decrease in plant productivity. In line with this reasoning, several studies have shown that the C/nutrient ratios, such as the C/N or lignin/N ratios, as well as non-structural materials and C-based secondary compounds, such as phenolics and tannins of plant litter, change under elevated CO_2 concentrations and influence decomposition rates (Cotrufo et al. 1998; Gebauer et al. 1997; Körner 2000; Zak et al. 2000). These effects of elevated atmospheric CO_2 concentrations on soil organic matter dynamics may also indirectly affect soil structure by altering the processes controlling soil aggregation, for instance by affecting the concentration of binding agents in soil (e.g., glomalin-related proteins; Niklaus et al. 2001; Rillig and Allen 1999; Rilling and Mummey 2006). These changes have also the potential for a strong feedback effect on the responses of plants and soil microbes to elevated CO_2 (Niklaus et al. 2003).

Thus, while the responses of plants to elevated atmospheric CO_2 are fairly well understood, the responses of soil microbial communities are largely unpredictable. Yet, effects of global change on the microbial community in soil are potentially important, as microbes control the responses of terrestrial ecosystems through their effects on C and nutrient cycling, plant growth and vegetation development, and soil structuring processes. However, our understanding of soil-borne microbial communities remains rather poor, mainly due to their extraordinary complexity (Curtis et al. 2002) and difficulties associated with laboratory cultivation of most soil microbes. Nevertheless, this field of research has recently benefited from the combination of molecular techniques to describe microbial communities (Hugenholtz et al. 1998; Pace 1997), with more sophisticated data analysis methods for analyzing and comparing communities (Hughes and Bohannan 2004).

In this review, we will examine the current state of the art with respect to effects of elevated atmospheric CO_2 on soil microbial communities, with a focus on microbial community structure. The discussion is organized around a conceptual model which describes the fate of photosynthetically fixed C into the root and to the rhizosphere microbial communities (Fig. 1). The basic premise of the model is that at elevated atmospheric CO_2 concentrations, root exudation, and root turnover will increase and that the role of root symbionts, in particular mycorrhizal fungi, in translocating C into the soil is enlarged, thereby acting to funnel C to the rhizosphere where food web interactions subsequently mediate the ecosystem feedbacks that regulate the cycling of C and N.
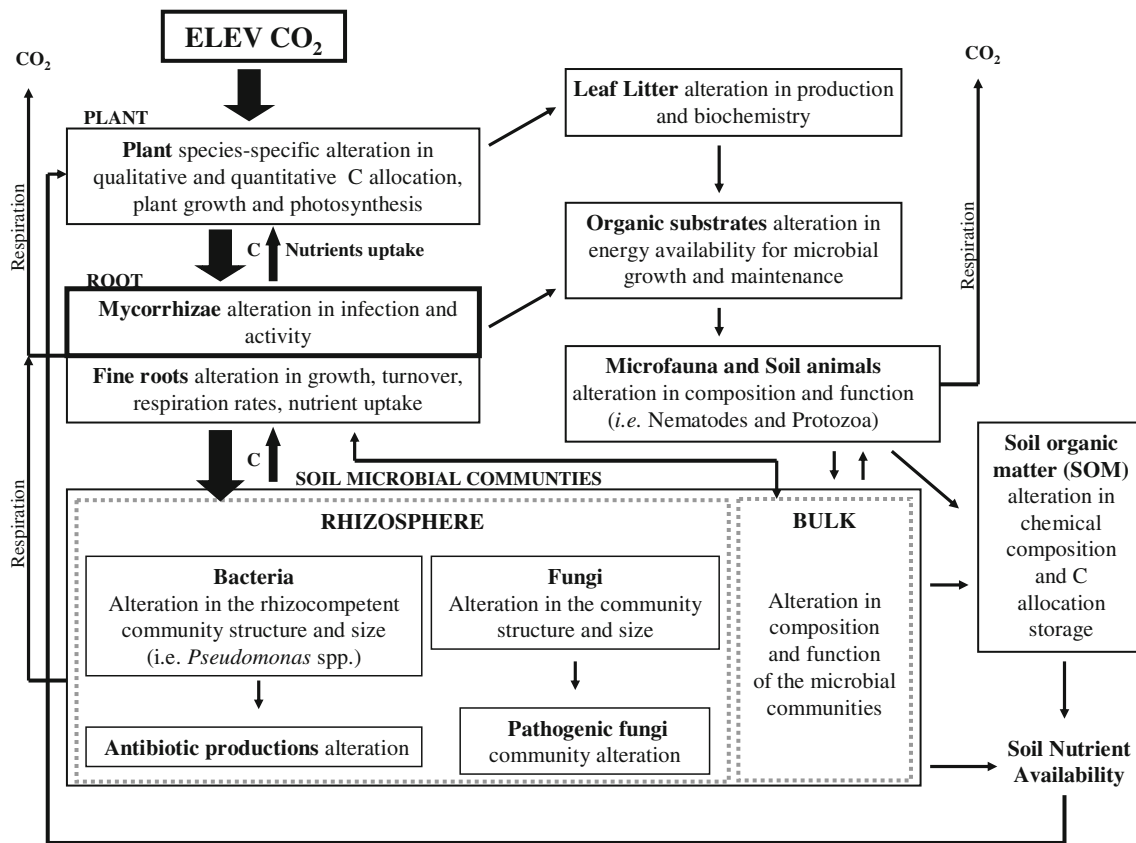
Fig. 1 Conceptual model which describes the response of dominant plant species to elevated atmospheric CO_2. The increased photosynthetically C allocation is initially directed mainly to mycorrhizae and root tissue. Mycorrhizae are translocating C into the soil microbial communities, thereby changing the structure, size, and activity of the rhizosphere microbial community (bacteria and fungi) to a larger extent than the community of the bulk soil. Soil microbial communities subsequently affect food web interactions and mediate the ecosystem feedbacks that regulate the cycling of C and N

Consequences of increasing CO_2 concentrations on root production and turnover

Our conceptual model (Fig. 1) is based on the idea that elevated atmospheric CO_2 modifies plant C allocation and initiates a series of physiological and biochemical changes, particularly in fine roots. These changes ultimately affect the structures of rhizosphere food webs as well as rates of C and N cycling. Fine roots thus play an important role in controlling the flow of C through several trophic levels in soils. Fine roots and mycorrhiza are important for plant and nutrient uptake, soil C input, and soil microbial activity (Norby 1994). It is estimated that 33% of global annual net primary productivity is used for the production of fine roots (Jackson et al. 1997). With their high turnover rate, fine roots are thought to be especially sensitive to elevated atmospheric CO_2 and may influence sequestration of CO_2 atmospheric on annual to decadal timescales (Pendall et al. 2004). Elevated CO_2 concentrations can enhance fine root growth (Curtis 1996; Curtis and Wang 1998; Pendall et al. 2004; Rilling et al. 1997), nutrient uptake rates, and

mycorrhizal infection or activities, thereby altering the dynamic equilibrium between plants and microbes in the rhizosphere (Hu et al. 1999; Klironomos et al. 1996). Root turnover and respiration rates are positively correlated with fine root N concentration (Pendall et al. 2004; Pregitzer et al. 1998, 2000; Zak et al. 2000), which is expected to decrease by 10–25% under elevated CO_2 (Bernston and Bazzaz 1997; Curtis et al. 1990; Cotrufo et al. 1998; King et al. 1997; Pregitzer et al. 2000; Rogers et al. 1994; Wan et al. 2004).

Effects of increasing atmospheric CO_2 on C dynamics in the rhizosphere

It is generally accepted that increases in CO_2 concentration quantitatively and qualitatively alter the release of root derived compounds. An increase in C flux and a higher C/N ratio of rhizodeposition have been observed even when no increase in plant biomass is evident (Hu et al. 1999; Paterson et al. 1997). In other words, plants under elevated

CO$_2$ decrease their allocation of N-rich metabolites and increase the allocation of C-rich metabolites to root exudates (Tarnawski and Aragno 2006). However, in situ study of the quantity and chemical composition of rhizodeposition, particularly the exudation of low-molecular-weight soluble organic compounds, is inherently difficult. Such analyses are often performed either in hydroponic solutions, of which, the conditions differed completely from the rhizosphere conditions, or in artificial soils, which do not constitute a reservoir of rhizosphere-adapted microorganisms (Van Veen et al. 2007). Analysis of root-derived compounds in natural soils is hampered by the continual uptake and degradation of such easily metabolized compounds by rhizosphere microorganisms. Thus, such measurements often actually represent the flux between release and uptake, which itself depends on the biomass, affinity, and consumption rate of consumers (Hu et al. 1999). Furthermore, roots are also capable of reabsorbing exudates, as often observed in hydroponic conditions, and it is not yet known how important this process is in the rhizosphere (Tarnaski and Aragno 2006). In addition, active microbial cells exudates many of the same compounds as plants, making it difficult to distinguish the source of detected chemicals. In summary, numerous factors complicate the accurate description of root exudates, and instantaneous measurement of root exudate concentrations and composition may bear little reflection to the actual flows of secreted compounds.

Recent results emphasize the important role of microorganisms as both passive (Owen and Jones 2001; Jones et al. 2004) and active (Phillips et al. 2004) promoters of root exudation under elevated CO$_2$ (Phillips et al. 2006a, b). Phillips et al. (2006a, b) have quantified influx and efflux of amino acids in maize, annual ryegrass, and medic seedlings grown at 425 versus 850 ppm CO$_2$ in the atmosphere. They proposed two mechanisms to explain the enhanced rhizodeposition at elevated CO$_2$. For the C$_3$ crops, wheat, and medic, they suggested that higher CO$_2$ promoted root growth without altering amino acid efflux rates (nmol g^{-1} root fresh weight), with a larger root surface area allowing for more total exudation. For the C$_4$ plant, maize, elevated CO$_2$ concentrations did not stimulate root or shoot growth, but there was a 44% increase in total efflux rate of 16 amino acids. These studies used axenic seedlings to examine the innate efflux and influx capacities of plant growing in the absence of microorganisms. Roots of the three plant species studied, under both ambient and elevated CO$_2$ concentrations, took up the 16 amino acids at rates 94–374% higher than they were exuded. However, in soil, the absorption of amino acids to soil particles may prevent reuptake by roots and microorganisms, which may lead to a net increase in the rhizodeposition under elevated CO$_2$ conditions.

Consequences of elevated CO$_2$ on rhizosphere respiration

Several studies using C isotope tracers have demonstrated that the production of CO$_2$ in the rhizosphere by roots and microorganisms is significantly stimulated by elevated CO$_2$ plant growth conditions (Billes et al. 1993; Cheng and Johnson 1998; Cheng et al. 2000; Kuikman et al. 1991; Paterson et al. 1996). The stimulation of CO$_2$ respiration in the rhizosphere may be much higher than the enhancement of root biomass. Cheng and Johnson (1998), Hungate et al. (1997), and Lekkerkerk et al. (1990) demonstrated that although plants produced only 15–26% more biomass under elevated CO$_2$, rhizosphere-respired C increased by 56–74% as compared to ambient CO$_2$ treatments. A meta-analysis of 47 studies evaluating responses of soil microorganisms to elevated CO$_2$ found that soil respiration (root + microbial respiration) under plants exposed to higher CO$_2$ generally increased (Zak et al. 2000). The extent of changes in soil respiration varied from a 10% decline to a 162% increase, with average increases of 51% for grasses, 49% for herbaceous dicots, and 42% for woody plants (Zak et al. 2000). Calculated values for the microbial respiration component of soil respiration in the same report found mean increases at elevated CO$_2$ of 34% for grasses, 34% for herbaceous dicots, and 20% from woody plants, whereas estimates of microbial biomass showed increases with elevated CO$_2$ of 17% for grasses, 29% for herbaceous dicots, and 19% for woody plants. Other studies of these and related parameters, which were not included in the meta-analysis, show similarly divergent results (Bruce et al. 2000; King et al. 2004; Montealegre et al. 2000; Phillips et al. 2002; Ronn et al. 2002, 2003; Sowerby et al. 2000; Wiemken et al. 2001; Zak et al. 1996).

Based upon these results, Cheng and Gershenson (2007) proposed two potential mechanisms of elevated CO$_2$-induced soil respiration enhancement. First, roots grown under elevated CO$_2$ can exude more C (Cheng and Johnson 1998) while having higher turnover rates (reviewed by Pregitzer et al. 2007), thereby resulting in a more than proportional increase in total rhizosphere respiration under elevated CO$_2$. However, in reality, this mechanism may not operate, as we showed earlier that at elevated CO$_2$, the N concentration in roots may decrease and, proportionally to that, decrease the turnover of roots (e.g., Cotrufo et al. 1998; Pregitzer et al. 2000; Wan et al. 2004). Alternatively, rhizosphere microbial associations could be enhanced under elevated CO$_2$, resulting in higher rhizosphere microbial activities per unit of root growth. This latter hypothesis is supported by studies that showed an increased percentage of colonization of ecto- and arbuscular mycorrhizal infection (Alberton et al. 2007; Drigo et al. 2007; Staddon 2005) and enlarged N$_2$ fixation across several types of associations (Arnone and Gordon 1990; Thomas et al.

1991; Tissue et al. 1996) at elevated atmospheric CO_2 concentrations.

A major consequence of the increase in microbial activity and consequently in CO_2 production is a potential negative effect on the accumulation of organic C in soils and thus on potential sequestration of soils. Indeed, Hungate et al. (2003) concluded that the priming effect as a result of the enhanced microbial activity in soil at elevated atmospheric CO_2 concentration will have a significant negative feedback on global change processes and will reduce the sequestration potential of soils. Carney et al. (2007) concluded that elevated CO_2, by altering soil microbial communities, can change the soil as a potential carbon sink to become a carbon source.

Mycorrhizal responses at elevated atmospheric CO_2 concentration

The effects of elevated atmospheric CO_2 concentration on soil microbial community structure are often characterized by an increased mycorrhizal colonization due to the increased plant demand for nutrients, coupled with increased C assimilation rates (reviewed in Johnson and Gehring 2007). Functions mediated by mycorrhiza include plant nutrient foraging (especially P), plant C allocation and architecture, changes in soil structure, and soil C storage (Rillig et al. 2002; Staddon et al. 2002).

Hence, understanding mycorrhizal responses to anthropogenic environmental changes can help predict the trajectories of future communities and ecosystems in a changing world. Mycorrhizal fungi form symbiotic associations with plants and depend directly on photosynthetic products from their hosts. Therefore, it would follow that these organisms will be affected first, and most strongly, by any CO_2-induced changes in the C budget of their hosts (Hodge 1996; Soussana and Hartwig 1996). Enrichment of CO_2 is expected to alter the balance of trade between mycorrhizal fungi and their plant hosts. CO_2 enrichment should increase mycorrhizal biomass because plant demands for N and P will increase concurrently with C assimilation rates, and plants will allocate more photosynthates belowground to the roots and mycorrhizal fungi to help satisfy this increased nutrient demand. Greater fine-root mass and mycorrhizal infection promote enhanced P uptake in mycorrhizal plants grown under elevated CO_2 concentrations (Hodge 1996). In turn, increases in mycorrhizal colonization have strong feedback effects at the individual plant, community, and ecosystem levels, potentially modifying responses to global change factors. Changes in atmosphere CO_2 concentrations indirectly affect mycorrhizas through changes in C allocation from their host plants (Allen et al. 2005; Gamper et al. 2004, 2005;

Parrent et al. 2006; Sanders et al. 1998; Staddon et al. 2002; Treseder and Allen 2000).

It seems reasonable to expect that at elevated CO_2 levels, mycorrhizal biomass will increase as C becomes relatively less limiting and soil nutrients become more limiting to plant growth. However, information available in literature is not always consistent on this point. For example, Rillig and Allen (1999) and Treseder and Allen (2000) showed that at elevated atmospheric CO_2 levels, the extraradical hyphal lengths of both arbuscular mycorrhizal (AM) and ectomycorrhizal (EM) fungi increased, but, in contrast, Staddon et al. (1999) and Walker et al. (1997) did not find any effects of CO_2 on EM or AM root colonization when accounting for plant size. It is likely that these apparent contradictions arise from differences in the experimental systems applied and, in particular, in differences in the plant and fungal species studied.

The species composition of EM in a spruce forest in northern Sweden responded dramatically to CO_2 enrichment (Fransson et al. 2001). Similarly, AMF communities have been shown to change in response to CO_2 enrichment (Wolf et al. 2003). Klironomos et al. (2005) demonstrated that when the atmospheric CO_2 levels were abruptly increased from 350 to 550 ppm in one single step, the species composition and functioning of AMF communities changed sharply. However, they also observed that responses did not occur when the CO_2 level was increased gradually over 21 generations. This is an interesting observation, as most studies look at very sudden changes in CO_2 concentration, and this may have a major impact on how results are extrapolated to the real world.

Increasingly, studies of relationships between mycorrhizal fungi and global change focus on interactions among multiple factors rather than single factors (Johnson and Gehring 2007; Rillig et al. 2002). This seems to be a realistic approach as few, if any, natural environments will experience only one change, and interactions among multiple factors may generate complex outcomes (Johnson and Gehring 2007). For example, increased availability of photosynthetic C may reduce the consequences of drought and also change the balance between costs and benefits in mycorrhizal symbioses. This was shown in a mesocosm experiment by Johnson et al. (2003). Elevated CO_2 increased the species richness of the plant community when AMF were present, but not when they were absent. The survival of slow-growing mycotrophic forbs was increased at elevated CO_2 levels, suggesting that the treatment ameliorated the C cost of the symbiosis (Johnson and Gehring 2007; Johnson et al. 2003).

Although some recent progress has been made in our understanding of overall C fluxes from the plant through AMF into the rhizosphere communities and the soil food web (Carney et al. 2007; Kreuzer-Martin 2007), knowledge

is still rather scarce with respect to the relative flow of C to different functional groups of the plant–soil ecosystem.

Effects of elevated CO_2 on the microbial communities in the rhizosphere

The response of soil microorganisms to changes in plant production under elevated CO_2 is highly variable due to very different patterns of plant C allocation in different plant–soil systems. Microbial biomass, gross N mineralization, microbial immobilization, and net N mineralization under elevated CO_2 show a high degree of variability within and between plant life forms (Zak et al. 2000). However, rates of soil and microbial respiration are generally more rapid under elevated CO_2, indicating that enhanced plant growth under elevated CO_2 increases the amount of C entering the soil, thereby stimulating soil microbial activity.

Effects of elevated CO_2 on the microbial biomass

Soil microorganisms are often C-limited; therefore, increased C availability should stimulate microbial growth and activity (Cotrufo and Gorissen 1997; Diaz et al. 1993; Paterson et al. 1997; Sadowsky and Schortemeyer 1997; Zak et al. 1993, 2000). Nevertheless, microbial biomass measurements based on cell component ATP, phospholipid fatty acid analyses (PLFA), chloroform fumigation assays, or total cell counts have yielded mixed results. Quantitative alterations in C supply have been shown to decrease (Diaz et al. 1993; Ebersberger et al. 2004), increase (Hungate et al. 2000; van Ginkel and Gorrisen 1998; van Ginkel et al. 2000; Williams et al. 2000; Zak et al. 1993), or not affect (Chung et al. 2006; Hungate et al. 2000; Kandeler et al. 1998; Lussenhop et al. 1998; Randlett et al. 1996; Richter et al. 2003) microbial biomass and activities (e.g., decomposition and nutrient cycling (Hu et al. 1999; Jones et al. 1998). Indeed, after 3 months of fumigation with 700 ppm $^{14}CO_2$, a 42% increase in microbial biomass was measured in *Lolium perenne* soil (van Ginkel and Gorrisen 1998; van Ginkel et al. 2000). In contrast, in soils under *L. perenne* and *Trifolium repens*, Richter et al. (2003) did not detect any changes in microbial biomass after 7 years of fumigation with 600 ppm CO_2 compared to current conditions. In addition, no changes in microbial biomass were detected in the rhizosphere and soils of poplar (Lussenhop et al. 1998). In tall grass prairie system exposed to elevated CO_2 for 8 years, Williams et al. (2000) showed a slight increase (not significant) in microbial C and N biomass, whereas there was a significant increase in overall microbial activity. Similarly, an increase in microbial activity, together with unchanged microbial biomass, was observed in sandstone grasslands (Hungate et al. 2000).

It is generally assumed that the CO_2-induced increases in soil C availability will increase fungal biomass more than bacterial biomass (Klironomos et al. 1996; Rillig et al. 1998). Indeed, Jones et al. (1998) observed increases in fungal abundance in response to elevated CO_2. In their study, changes observed in soil fungi at elevated CO_2 were thought to be related to increased concentrations of dissolved organic C in the rhizosphere and to increases in soil–water dissolved organic N. In one of our studies (Drigo et al. 2007), we also demonstrated that elevated CO_2 increased the fungal biomass, but not the size of the bacterial community. Given the important roles played by fungi in organic matter degradation, nutrient cycling, plant nutrition, and soil aggregate formation, shifts in fungal communities might have a strong impact on soil functioning. In line with this reasoning, Carney et al. (2007) found that the ratio of fungi to bacteria was higher in soils from elevated as opposed to ambient CO_2 sites and that increased fungal abundance not only promoted lignolytic enzyme activity but also reduced soil C storage. This finding supports the "priming effect" theory in which the increased influx of labile C may stimulate microbial degradation of soil organic matter due to microbial mining of soil organic matter for nutrients or changes in microbial activity or community composition (Hungate et al. 2003). Many other studies across different ecosystems have found that elevated CO_2 increased fungal abundance in soils (Janus et al. 2005; Klamer et al. 2002; Lipson et al. 2005). Furthermore, lower N availability at elevated CO_2 may, in part, explain these increases in fungi, as fungi tend to have a higher C/N ratio than bacteria and so have a lower demand for nitrogen than bacteria have (Hu et al. 2001).

Effects of elevated CO_2 on the dynamics of rhizosphere microbial populations

No general tendency has emerged with respect to the response of rhizosphere bacterial biomass to elevated CO_2 conditions (Hu et al. 1999; Zak et al. 2000). For example, an increase in bacterial biomass was observed in fertile pastures after 5 years under elevated CO_2 (Hu et al. 2001). However, Montealegre et al. (2000) did not detect any effect of CO_2 increase on the size of the bacterial community in the rhizosphere soil of *L. perenne* and *T. repens*. Griffiths et al. (1998) noticed that under *L. perenne*, the amount of photosynthetic C allocated to bacterial biomass was not influenced by atmospheric CO_2 concentration, whereas the amount of non-microbial C in the rhizosphere increased by a factor of 2.6 within 28 days.

Increased rhizodeposition might also induce shifts within the bacterial community composition linked to the different growth strategies. An increase in culturable bacteria and a simultaneous decrease in total bacterial cells (with a

dominance of oligotrophic, "non-culturable" K-strategists) under elevated CO_2 was observed by Hodge et al. (1998) in the rhizosphere of *L. perenne* and by Insam et al. (1999) in artificial tropical systems. The number of culturable bacteria was significantly higher in *L. perenne* rhizosphere after 2 years under 600 ppm CO_2 as compared to current conditions, whereas no changes were observed in the bulk soil (Marilley et al. 1999). This was confirmed 7 years later on the same plots by Fromin et al. (2005). Except for a short period in spring, no such change occurred under *T. repens.*

Tarnawski and Aragno (2006) hypothesized that at elevated atmospheric CO_2 conditions, fast-growing r-strategists, which are adapted to feed on easily utilizable substrates, are favored. This may result in a decreased soil N availability due to higher sequestration by the plant and the bacteria (Hu et al. 2001). As a result, according to these authors, the growth and activity of the slow-growing K-strategists, which are more adapted to degrade less labile, more recalcitrant, substrates, might be disadvantaged, and, consequently, these substrates may accumulate. Another contrasting hypothetical mechanism suggested for the shift from K- to r-strategists implies that increased growth of r-strategists may induce increases in bacterial grazer populations. If grazers do not discriminate between fast- and slow-growing bacteria, such an increase would decrease the relative numbers of slow-growing K-strategists (Tarnawski and Aragno 2006). It is, however, rather unlikely that such mechanisms will be operational under natural conditions, as abundant and fast growing populations will certainly be preferred by grazers over single starving cells of K-strategists.

Response of the rhizosphere microbial community structure to elevated CO_2

It has become evident that general biomass and activity measures are inadequate to describe specific soil-borne microbial community responses to elevated atmospheric CO_2 concentrations, which are needed to understand the consequences of global change effects on soils (Janus et al. 2005). Molecular-based methodologies (Hugenholtz et al. 1998; Pace 1997) now allow for more detailed and comparative analyses of microbial community structure (Hughes and Bohannan 2004). Using a variety of molecular community analysis methods, mixed results have been reported regarding the effects of increased atmospheric CO_2 levels on the structure of soil and rhizosphere microbial communities. Observations range from pronounced effects (Janus et al. 2005; Jossi et al. 2006; Mayr et al. 1999; Montealegre et al. 2000; Rillig et al. 1997) to subtle or undetectable effects (Bruce et al. 2000; Ebersberger et al. 2004; Griffiths et al. 1998; Insam et al. 1999; Klamer et al. 2002; Montealegre et al. 2000; Zak et al. 2000). However,

these contrasting results have to be viewed in the light of the different systems studied and the different methods applied to assess community structure and diversity. Moreover, most of the relevant studies conducted under field conditions (Janus et al. 2005; Jossi et al. 2006; Marilley et al. 1999; Sonnemann and Wolters 2005) focused on specific bacterial communities and showed CO_2-related shifts in community composition of *Pseudomonas* spp. (Marilley et al. 1999), of *Rhizobium* species (Schortemeyer et al. 1996; Montealegre et al. 2000), or stimulation of *Proteobacteria* (Jossi et al. 2006).

Griffiths et al. (1998), Kandeler et al. (1998), Tarnawski and Aragno (2006), and Zak et al. (1996) did not observe significant differences in the structure of microbial communities associated with the growth of *L. perenne* under elevated CO_2, as revealed by a global characterization of soil DNA (thermal denaturation and G + C content) and PLFA profiles. However, using similar approaches, Montealegre et al. (2000) did detect changes in white clover rhizosphere communities in response to CO_2 enrichment. Using molecular fingerprinting approaches of total (DNA-based approach) and active (RNA-based approach) bacterial communities associated with two perennial grasses, *L. perenne* and *Molina coerulea*, Jossi et al. (2006) showed that elevated CO_2 had a greater influence on the active bacterial communities than on the total community. Specific bacterial communities showed specific responses. For instance, Actinobacteria populations were especially active in bulk soil, but were little affected by elevated CO_2, whereas δ-Proteobacteria were stimulated by elevated CO_2 only in the vicinity of the root. These results were supported by Drigo et al. (2007) in greenhouse experiments conducted at current and elevated CO_2. They showed that the bacterial community structure in the rhizosphere was most affected by elevated CO_2, whereas the bacterial bulk and fungal community structure in bulk soil were less influenced.

Regarding "functional" or "metabolic" communities, Denef et al. (2007) attributed the higher ^{13}C enrichment of fungal PLFAs biomarkers to the faster utilization of new plant-C by the fungal community and much later by the bacterial community. Elhottova et al. (1997) showed changes in the composition of bacterial nutritional groups at elevated CO_2. Hodge et al. (1998) linked the faster utilization of Biolog C-sources by bacteria isolated from the rhizosphere soil of perennial grasses grown under elevated CO_2 to a higher number of culturable bacteria. However, Insam et al. (1999) did not observe changes in community level physiological profiles with Biolog plates nor in PLFA profiles of isolates from an artificial tropical plant–soil ecosystem at elevated atmospheric CO_2 levels.

Rhizosphere bacteria are known to have a range of possible interactions with plants, and it is not yet known

how these interactions will be affected by changes in plant traits resulting from elevated CO_2. For instance, several *Pseudomonas* species have been shown to behave as plant-growth-promoting bacteria (PGPR), and it is of great interest to understand if such activities are affected by rising atmospheric CO_2 concentrations. The proportion of HCN-producing *Pseudomonas* strains, considered as potential inhibitors of root parasitic fungi isolated from bulk and rhizosphere soil and from root fractions of two perennial grassland systems (*L. perenne* and *M. Coerula*), reduced when the plants were grown at elevated (600 ppm) CO_2 concentrations as compared to plants grown at current concentrations of 360 ppm (Tarnawski and Aragno 2006). Interestingly, Drigo et al. (unpublished data) showed that the abundance of 2,4-DAPG, pyrrolnitrin, and phenazine antibiotic producing *Pseudomonas* strains as probable inhibitors of the root infecting *Trichoderma* sp. and *Fusarium* sp. increased in the rhizosphere of plants grown at elevated CO_2 concentrations.

Effects of elevated CO_2 on the rhizosphere microfauna

Bacteria and fungi, the initial consumers of soil organic matter, are themselves substrates for a multitude of tiny predators and grazers, including protozoa, nematodes, and arthropods, which comprise the soil food web (Brussaard et al. 1997). Therefore, an increase in bacterial growth due to an increasing C allocation at elevated atmospheric CO_2 levels may be followed by an increase in grazing, resulting in a higher turnover of the microbial biomass. Increased grazing thus results in faster recycling of nutrients from the microbial biomass, which would increase the flux of nutrients to the plant. Several authors, e.g., Hungate et al. (2000), Williams et al. (2000), and Yeates et al. (1997), have observed an overall increase in the activities of bacteria and fungi as well as their grazers, protozoa, and microarthropods in the rhizosphere at elevated CO_2. Yet, studies of elevated CO_2 effects on soil food web structure show a complex picture. Available data indicate that increased CO_2 generally stimulates mycorrhizal fungi (i.e., Denef et al. 2007; Olsrud et al. 2004; Rilling and Field 2003; Staddon 2005). However, in one study where fungal grazers were examined, the number of collembolans, which prefer non-mycorrhizal fungi, increased with additional N and elevated CO_2, as their favored fungal food sources proliferated more than the mycorrhizal fungi (Klironomos et al. 1997). Elevated atmospheric CO_2 levels altered the mixture of species present in assemblages of nematodes (Hoeksema et al. 2000; Yeates et al. 2003), protozoans (Treonis and Lussenhop 1997; Yeates et al. 2003), and collembolans (Jones et al. 1998). In most cases, the changes in the species present occurred without altering the total

abundance of organisms. Such changes may not always reflect varying availability of C resources because other parameters, such as soil moisture, could also have changed (see review by Phillips et al. 2006a, b).

Soil food webs are typically dominated by either bacteria or fungi, which are viewed as separated channels for energy flows (De Ruiter et al. 1993; Moore and Hunt 1998; Phillips et al. 2006a, b). Measurements at the ETH FACE site showed clear effects of elevated CO_2 and N fertilization on these two energy channels (Phillips et al. 2006a, b). Saprophytic and mycorrhizal fungi declined with increased N fertilization, which is consistent with their sensitivity to mineral fertilizers (Tenuta and Ferris 2004). These changes would favor shifts toward the bacterial energy channel, which occur with increasing amounts of N derived from mineralization. In addition, shifts toward the fungal energy channel at elevated CO_2 were suggested, in which case the potential for C storage and N immobilization would increase. Understanding these interactions and how they relate to concurrent N limitations (Luo et al. 2004) are requirements for predicting the effects of climate change on soil food web functions and so on plant growth and ecosystem responses.

Predator feeding specificity and changes therein may also be an important mechanism for the observed responses in microbial community structures at elevated atmospheric CO_2 levels. Hoeksema et al. (2000), Neher et al. (2004), and Yeates et al. (2003) suggested that effects of elevated CO_2 on soil nematode communities would not necessarily have a simple functional relationship with rhizosphere carbon allocation. Neher et al. (2004) observed that elevated CO_2 can lead to shifts in nematode community structure, increasing the bacterivores and decreasing fungivores. Based on preliminary nematode identification data for samples collected from *C. arenaria* soil, Drigo et al. (unpublished data) confirmed this theory. They showed that elevated CO_2 influenced the nematode community of different soils planted with *C. arenaria* and *F. rubra* directly through changes in plant C allocation to the belowground environment and indirectly via changes in microbial communities that responded to plant C inputs.

Future perspectives

Future studies on the effects of elevated CO_2 on soil and rhizosphere microbiota should dedicate more attention to the polyphasic use of modern molecular tools for the description of microbial communities at the phylogenetic as well as functional levels. Such polyphasic approaches must also necessarily be coupled with proper statistical/numerical tools to analyze and combine these results. The use of isotopes, especially ^{13}C and ^{14}C, will continue to be

indispensable in this field of research. Particular focus should be placed by the combined use of isotopes and molecular detection and identification techniques, so-called stable isotope probing (SIP) approaches (Radajewski et al. 2000).

High-throughput technologies, such as the emerging "omics" technologies (e.g., genomics, transcriptomics, proteomics, metabolomics), will no doubt allow for the examination of soil-borne microbial community dynamics and functions in unprecedented detail. The integration of such data will improve our predictions of plant responses to global change. This requires the application of adequate bioinformatics tools, including conceptual and predictive models, to be able to deal with the huge datasets and to couple them with data on environmental conditions.

Unresolved questions on the effects of elevated CO_2 on plant–soil microbial community interactions

An emerging view in the elevated CO_2 research is that root–microbial interactions are likely to play an important role in controlling ecosystem-scale responses to global change (Phillips 2007). This would argue for a more rhizocentric view of the interactions between plants and soil microbes, whereby mycorrhizal fungi are just extensions of the host plant's root system (Fitter et al. 2000). Recently, it has been argued that the mycorrhizal fungal partner needs to be considered as an organism in its own right with its own interests, especially in terms of C use (Fitter et al. 2004; Staddon et al. 2005). Also based on our own work, we feel that in the future, more attention should be given to the central role of mycorrhiza in the context of global change, as they appear to be a keystone in the CO_2-related response.

Disparate changes in soil microorganisms and complex adjustments in the food web structure reported under higher elevated CO_2 concentration in a multitude of experiments suggest that a better understanding of the C flow from the plant, through the mycosphere, to the rhizosphere and into the soil food web is strongly needed. Moreover, attention should be paid to the response to elevated CO_2 concentration of the microflora of the rhizosphere, particularly PGPR and their production of metabolites such as antibiotics.

Conclusions

During the past two decades, a wealth of information on the impact of global change on terrestrial ecosystems has been produced by researchers from a variety of disciplines. However, trying to put all this information together still

results in "patchwork", making it difficult to draw general conclusions about the full effects of elevated CO_2 on terrestrial ecosystems. Besides the highly disputed changes in climate changes as a result of the greenhouse effect, the more discrete, but perhaps equally important, impact on photosynthesis could greatly modify the relationships between plants and their microbial cohabitants.

According to the present state of knowledge, the main effects of elevated atmospheric CO_2 on soil microbiota occur via plant metabolism and root secretion, especially in C_3 plants. Many studies agree that there is little or no direct effect on the microbial community of the bulk soil and that the mycorrhizal, bacterial, and fungal communities in the close vicinity of the root do play major roles in coping with the increased C allocation, whereby r-strategists are favored as compared to the K-strategists at elevated CO_2 conditions. Consequently, also the grazing activity by protozoa and nematodes may be altered by elevated CO_2.

Much remains to be investigated in our quest to understand the nature and the magnitude of microbial interactions with plants in response to global changes induced by human activity. We believe that short-term experiments, combined with long-term and incremental experiments of elevated CO_2 effects, using function-driven approaches (e.g., SIP) and emerging "omics" technologies will help deliver the information necessary to predict global consequences of climate change-induced effects on terrestrial ecosystems.

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