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REVIEW

Effects of Elevated Atmospheric CO_2 Concentrations on Soil Microorganisms

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Effects of elevated CO_2 on soil microorganisms are known to be mediated by various interactions with plants, for which such effects are relatively poorly documented. In this review, we summarize and synthesize results from studies assessing impacts of elevated CO_2 on soil ecosystems, focusing primarily on plants and a variety the of microbial processes. The processes considered include changes in microbial biomass of C and N, microbial number, respiration rates, organic matter decomposition, soil enzyme activities, microbial community composition, and functional groups of bacteria mediating trace gas emission such as methane and nitrous oxide. Elevated CO_2 in atmosphere may enhance certain microbial processes such as CH_4 emission from wetlands due to enhanced carbon supply from plants. However, responses of extracellular enzyme activities and microbial community structure are still controversy, because interferences with other factors such as the types of plants, nutrient availabilitial in soil, soil types, analysis methods, and types of CO_2 fumigation systems are not fully understood.

***Key words*:** elevated CO_2, microbial process, trace gas emission, soil enzyme activity, microbial community structure

Anthropogenic activities have caused the concentration of atmospheric CO_2 to increase from about 280 parts per million (ppm) at the beginning of the industrial revolution to over 370 ppm at the present time. Current estimates suggest that the atmospheric CO_2 concentration range will lie between 450 ppm and 600 ppm by the year 2050 (Kattenburg *et al.*, 1995). More than two decades of studies of the effects of CO_2 enrichment have provided a plethora of data and an improved understanding of a wide variety of plant responses such as net primary production, species abundance, community composition and soil respiration (root plus microbial respiration) in terrestrial ecosystems (Poorter, 1993; Curtis and Wang, 1998; Ball and Drake, 1998; Edwards and Norby, 1999; Mooney *et al.*, 1999; Zak *et al.*, 2000a). In addition, the chemical and physical composition of plant material and decomposability of plant litter have drawn much attention (Cotrufo *et al.*, 1994; Cotrufo and Ineson, 1995; King *et al.*, 1997). However, belowground processes in soils have received scant attention. In particular, the effects of elevated CO_2 on soil microbial communities and activities remain largely unknown, severely constraining our knowledge of whole ecosystem responses to global climatic change. Moreover, those soil microorganisms are considered to represent potential bio-monitors of the effects of global change or other changes in ecosystems (Foissner, 1999, Kennedy, 1999).

As microorganisms in soils regulate the dynamics of organic matter decomposition and plant nutrient availability, they play a key role in the responses of ecosystems to global climate changes. Elevated CO_2 would affect soil microorganisms indirectly through increased root growth and rhizodeposition rates (Rogers *et al.*, 1994; Rouhier *et al.*, 1994; Paterson *et al.*, 1997; Sadowsky and Schortemeyer 1997) because CO_2 concentration in soil is much greater than the atmospheric CO_2 (van Veen *et al.*, 1991). As such, responses of plants such as root dynamics, root exudates, and litter production and decomposition are of great importance in understanding microbial responses.

In this review, we aim to summarize and synthesize the results from studies assessing impacts of elevated CO_2 on soil ecosystems, focusing primarily on plants and a variety of microbial processes. The processes considered include changes in microbial biomass of C and N, soil

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enzyme activity, microbial community composition, organic matter decomposition, and functional groups of bacteria mediating trace gas emission in terrestrial and wetland ecosystems. In addition, we present the recent analysis technique for obtaining information of changes in microorganisms and discuss possible reasons for the often divergent responses of microbes to increasing CO_2.

Plant Responses to Elevated CO_2

The direct effects of increasing CO_2 on plants include quantitative and qualitative changes in above- and belowground production (Norby, 1994). For example, C_3 plant photosynthetic rates increase under a CO_2-enriched atmosphere (Paterson *et al.*, 1997; Robinson and Conroy, 1999). In addition, changes in plant chemistry have been noticed such as C/N ratio, concentrations of starch, sugars, and total nonstructural carbohydrates (Cotrufo *et al.*, 1994, Lewis *et al.*, 1994).

Of the various responses of plants to elevated CO_2, the following observations have implications for the soil microflora. First, numerous studies were performed focusing on changes in root dynamics and nutrient availability (Hungate *et al.*, 1999; Day *et al.*, 2000; Griffiths *et al.*, 2000; Schortemeyer *et al.*, 2000; Johnson *et al.*, 2001; Wiemken *et al.*, 2001). Most studies have found that root growth was stimulated under elevated CO_2 (Jongen *et al.*, 1995; Fitter *et al.*, 1997; Hebeisen *et al.*, 1997; Paterson *et al.*, 1997; Rogers *et al.*, 1998; Zak *et al.*, 2000a) and it was hypothesized that root production would increase as CO_2 accumulates in the Earth's atmosphere (Pregitzer *et al.*, 2000).

Secondly, elevated CO_2 may affect soil organisms indirectly through increased availability of labile C through exudation (Cheng, 1999). Curtis *et al.* (1994) and Diaz *et al.* (1993) showed that the elevated CO_2 led to an increase in carbon flux from plants to the soil. van Veen *et al.* (1991) suggested quantitative and qualitative changes in rhizodeposition linked to CO_2 enrichment. Lekkerkerk *et al.* (1990) showed that the root-derived, easily biodegradable compounds of wheat roots increased under a CO_2-enriched condition. This increased input of C in the soil may in turn stimulate mineralization (Baggs *et al.*, 2003) or N_2 fixation (Diaz *et al.*, 1993).

Finally, elevated CO_2 could alter the biochemical composition of plant tissue above and belowground (Cotrufo *et al.*, 1994; Cotrufo and Ineson, 1995), which then affect soil microorganisms (Curtis *et al.*, 1994; Ball, 1997). For example, root decomposition was retarded in plants grown under elevated CO_2 (Gorissen *et al.*, 1995; Arnone and Hirschel, 1997). Similarly, it has been found that litter decomposition of plants grown on NPK amended plots were retarded by elevated CO_2. It was thought that changes in chemical quality of plant matter caused retardation of its decomposition (Cotrufo and Ineson, 1995).

Effects of Elevated CO_2 on Soil Microbes

Various responses of soil microorganisms affected by the changes described above have been noticed. In this section, we review the responses in terms of soil microbial biomass, microbial numbers, activities, and several functional processes.

Microbial biomass C

Numerous studies have failed to find a significant response of microbial biomass C to elevated CO_2 (Jones *et al.*, 1998; Kampichler *et al.*, 1998; Niklaus, 1998; Insam *et al.*, 1999; Hungate *et al.*, 2000, Wiemken *et al.*, 2001; Larson *et al.*, 2002; Montealegre *et al.*, 2002; Mitchell *et al.*, 2003). For example, Wiemken *et al.* (2001) showed that the amounts of carbon (a general marker for microbial biomass) and chitin (a marker for fungal biomass) did not respond significantly to the treatments with elevated CO_2 or nitrogen fertilizer. The results of Niklaus and Körner (1996) and Rouhier *et al.* (1994) suggest that responses of microbial C to elevated CO_2 are unlikely to develop in nutrient deficient ecosystems. Schortemeyer *et al.* (1996) reported that the size of the total heterotrophic microbial populations, in the form of microbial C in the rhizosphere of white clover or perennial ryegrass, did not change under elevated CO_2. In a more recent study, Schortemeyer *et al.* (2000) reported that microbial biomass did not increase in a natural Florida scrub ecosystem after 2 years of CO_2 enrichment. In an artificial tropical ecosystem with low nutrient availability, Insam *et al.* (1999) reported that microbial biomass C, ergosterol contents, and fungal hyphal lengths were not significantly altered by high CO_2 concentration, although total bacterial counts were significantly higher. Zak *et al.* (2000b) found that microbial biomass remained unchanged in bulk soils under elevated CO_2 after 2.5 growing seasons. Niklaus (2001) analyzed ecosystem C partitioning and soil C fluxes in grassland exposed to elevated CO_2 for 6 years. They showed that C pools increased in plants (+23%) and surface litter (+24%), but were not altered in microbes and soil organisms.

However, several studies conflict with such observations, and have reported an increase in soil microbial biomass under elevated CO_2 (Diaz *et al.*, 1993; Zak *et al.*, 1993; Dhillion *et al.*, 1996; Pregitzer *et al.*, 2000, Williams *et al.*, 2000; Klamer *et al.*, 2002). Zak *et al.* (1993) observed that microbial biomass C in the rhizosphere and bulk soil of *Populus grandidentata* was greater under elevated than ambient CO_2. In an acidic grassland herbaceous community an, increase of up to 80% in microbial biomass C occurred under elevated CO_2 (Diaz *et al.*, 1993). Dhillion *et al.* (1996) reported that microbial biomass C was significantly higher in root region of soil from monocultures of *Bromus madritensis*, a comman and sometimes dominant annual grass in Mediterranean model ecosystem plants under elevated CO_2. In tallgrass prairie exposed to elevated CO_2 for 8 years, soil microbial biomass C tended to be greater under elevated C compared to ambient treatment (Williams *et al.* 2000). Montealegre (2002) reported that bacterial populations increased about 1.4 fold under white clover after 3 years of CO_2 fumigation in pasture ecosystem. In addition, several research groups found an increase in mycorrhizal short roots and extra-radical mycelium in response to elevated CO_2 (Ineichen *et al.*, 1995; Lewis and Strain, 1996; Runion *et al.*, 1997; Walker *et al.*, 1997; Wiemken *et al.*, 2001).

Microbial biomass N

Understanding the effects of elevated CO_2 on microbial biomass N is of great importance, as such N plays a key role in plant productivity in N limited ecosystems (Diaz *et al.*, 1993; Zak *et al.*, 1993).

Elevated CO_2 can have a positive effect (Diaz *et al.*, 1993; Zak *et al.*, 1993; Niklaus, 1998) or no effect (Berntson and Bazzaz, 1998; Niklaus, 1998; Zak *et al.*, 2000b) on soil microbial N. For example, Niklaus (1998) reported that microbial biomass N was increased by 18%, although microbial biomass C was not influenced by elevated CO_2. In tallgrass prairie exposed to elevated CO_2 for 8 years, soil microbial biomass N tended to be greater under elevated CO_2 compared to ambient treatment (Williams *et al.* 2000). Billings *et al.* (2004) examined the effects of elevated CO_2 on soil nitrogen dynamics in the Mojave Desert. They showed elevated CO_2 increased microbial biomass N in dry soils under a perennial grass. However, Barnard *et al.* (2004a) showed that microbial biomass N was not affected by elevated CO_2 in four European grassland ecosystems after several years of treatment. Insam *et al.* (1999) found that microbial biomass C was increased by 27% under high CO_2 under low nutrient conditions in an artificial tropical ecosystem, but microbial biomass N was decreased slightly.

Microbial number

O'Neill *et al.* (1987) and Whipps (1985) were unable to find differences in the total number of bacteria between ambient and elevated CO_2 treatments. In studies of nitrifiers, the elevated CO_2 had no effect on population of nitrifiers (O'Neill *et al.*, 1987; Schortemeyer *et al.*, 1996). However, several authors have observed an increase in bacterial numbers under elevated CO_2 (Rogers *et al.*, 1992, Runion *et al.*, 1994; Insam *et al.*, 1999; Marilley *et al.*, 1999). In addition, Schortemeyer *et al.* (1996) showed that number of specific species increased twofold in a natural Florida scrub ecosystem after 2 years of CO_2 enrichment while no effect was found for total population.

Microbial respiration

Many studies have found that microbial respiration was significantly greater in elevated CO_2 conditions (Rogers *et al.*, 1992; O'Neill, 1994; Runion *et al.*, 1994; Dhillion *et al.*, 1996; Williams *et al.*, 2000). For example, Williams *et al.* (2000) observed that microbial respiration was higher in tallgrass prairie exposed to elevated CO_2 for 8 years. However, Tuchman *et al.* (2003) reported microbial community respiration decreased significantly by 36.8% in the stream ecosystems with *Populus tremuloides* seedling grown in elevated CO_2 conditions. Similar results were also noted by Larson *et al.* (2002).

Nitrification and denitrification

Understanding of the effects of elevated CO_2 on processes such as nitrification and denitrification is of great concern because these processes regulate soil inorganic N concentrations, nitrate (NO_3^-) leaching and production of nitrous oxide (N_2O). The effects of CO_2 on denitrification has attracted particular attention because it is one of the most important mechanisms returning N from terrestrial or aquatic ecosystems to the atmosphere (Kaplan *et al.*, 1979), while also mediating release of the potent greenhouse gas N_2O (Smart *et al.*, 1997; Baggs *et al.*, 2003; Deiglmayr *et al.*, 2004). It has been reported that denitrifying activity increased significantly under CO_2 enrichment in both controlled environments and field conditions. For example, Inneson *et al.* (1998) found higher N_2O-N, metabolite of denitrification, and production beneath *Lolium perenne* growing under high N inputs and elevated CO_2. The higher denitrification rates under elevated CO_2 may be due to activation of denitrifiers by higher growth of fine roots and enhanced root exudation (Rogers *et al.*, 1998) and formation of anaerobic conditions induced by increased soil respiration and soil water content (Korner, 2000; Zak *et al.*, 2000a).

However, several researchers have reported that elevated CO_2 did not affect denitrifying enzymes activity (DEA) and nitrifying enzyme activity (NEA) (Barnard *et al.*, 2004a), or alternatively decreased them (Matamala and Drake, 1999; Barnard *et al.*, 2004b). Even in a single study, contrasting responses have been observed depending on sampling dates (Billings *et al.*, 2003). For example, Zak *et al.* (2000b) found that nitrification did not change in bulk soils under elevated CO_2 after 2.5 growing seasons. Barnard *et al* (2004a) showed that elevated CO_2 had limited effects on the amount of active nitrifying and denitrifying enzymes presented in four European grassland soils. In mono-specific grassland mesocosms (*Holcus lanatus* and *Festuca rubra*) grown under elevated CO_2, NEA decreased substantially, while DEA was less responsive to elevated CO_2 (Barnard *et al.*, 2004b). Matamala and Drake (1999) showed that potential denitrification rates were reduced in soil cores taken from *Scirpus olneyi* community exposed to elevated CO_2.

Methanogenesis

The concentration of atmospheric methane has increased at *ca.* 1% per year. Most of the atmospheric CH_4 is produced by bacterial activities in extremely anaerobic ecosystems such as natural and cultivated wetlands, sediment, sewage, landfills, and the rumen of herbivorous animals (IPCC, 1995).

In recent years, a number of studies have addressed the potential changes in trace gas emissions from wetlands exposed to elevated CO_2 (Table 1). For example, Drake (1992) reported CO_2 enrichment stimulated methane emissions by 80% in a salt marsh containing sedge *S. olneyi*. Hutchin *et al.* (1995) also found a similar effect for mire peat and vegetation exposed CO_2 enrichment treatment. Allen *et al.* (1994) reported the same results in the combined condition of increased CO_2 and temperature. Wang and Adachi (1999) also provided evidence that elevated atmospheric CO_2 concentrations could promote CH_4 production from flooded soils. Megonigal and Schlesinger (1997) who performed experiments with *Orontium aquaticum* reported CH_4 emissions increased by 136% under elevated CO_2.

However, Kang *et al.* (2001) found no significant differences for CH_4 emission on northern fen peat with *Juncus* and *Festuca* spp., although the mean value was higher under elevated CO_2 conditions. Saarnio *et al.* (1998) reported that the average release of CH_4 from *Sphagnum* samples exposed to the doubled concentration of CO_2 was significantly lower than that at ambient CO_2 at 9°C and 4.5°C. Saarnio and Silvola (1999) showed the release of CH_4 for each temperature condition (1.5°C-14°C), was on average only 6-23% higher under CO_2-fertilized conditions. More recently, Saarnio *et al.* (2000) found that elevated CO_2 (560 ppm) increased CH_4 efflux by only 15-20% in boreal mires over two years using mini-FACE rings. The increase was clearly weaker than that in previous reports from temperate or subtropical areas where CH_4 efflux increased by 80-150% during the growing season (Dacey *et al.*, 1994; Hutchin *et al.*, 1995, Megonigal and Schlesinger, 1997). The increases in CH_4 emission under elevated CO_2 conditions can be explained by two mechanisms. First, elevated CO_2 often results in ample supply of carbon into soil and hence larger amounts of organic carbon are available for methanogens. Secondly, elevated CO_2 concentration might indirectly enhance CH_4 emissions from wetlands by promoting net primary production (Guthrie, 1986; Dacey *et al*, 1994). Previous studies of natural and artificial wetlands have reported positive correlations between methane emission rates and plant aboveground biomass (Sass *et al.*, 1990; Whiting *et al.*, 1991; Whiting and Chanton, 1992).

However, a few studies have reported the opposite trend with CO_2 enrichment leading to the attenuation of methane production due to increased delivery of oxygen to the rhizosphere. For example, Schrope *et al.* (1999) reported methane emissions from rice grown in a sandy soil under doubled CO_2 were 4-45 times less. The increased root biomass due to elevated CO_2 may have more effectively aerated the soil, suppressing methane production.

Uncertainties in the response of CH_4 emission from wetlands exposed to elevated CO_2 arise due to a lack of long-term studies. In addition, changes in litter chemistry (Gorissen *et al.*, 1995; Hirschel *et al.*, 1997), nutrient deficiency (van de Geijin and van Veen, 1993; Niklaus and

Table 1. Effects of elevated CO_2 on CH_4 fluxes in wetlands. Changes in CH_4 flux under elevated CO_2 are presented as (elevated-ambient)/ambient ×100. No significant differences between ambient CO_2 and elevated CO_2 treatments are indicated by the letter, NS

Wetlands	Species	CO_2 level (μl/l) Ambient /elevated CO_2	Change of CH_4 flux (%)	Temp.	Facility	Reference
Bog	*Sphagnum*	360 / 560	NS†	17-20°C	Glasshouse	Saarnio *et al.* (1998)
			(–) ($p = 0.008$)	9°C		
			(–) ($p = 0.007$)	4.5°C		
	Sedge	360 / 560	(+) ($\alpha = 0.05$)	17-20°C	Glasshouse	
			NS	9°C		
			NS	4.5°C		
	Sphagnum	400-550 (night)/560 330-390 (day) / 560	+ 28 ($p = 0.016$)	Summer	Mini-FACE	Saarnio *et al.* (2000)
Emergent aquatic macrophyte	*Orontium aquaticum*	350 / 700	+ ($p = 0.06$)	ND	Glasshouse	Megonigal and Schlesinger (1997)
			+ 136 ($p \leq 0.01$)	ND	Growth chamber	
Rice on sand	*Oryza sativa*	350 / 700	(–) ($p \leq 0.01$)	ND	Greenhouse Tunnels	Schrope *et al.* (1999)
Marsh	*Scirpus olneyi*	345 / 690	+ 80 (F = 0.012)	Summer	Open-top chamber	Dacey *et al.* (1994)
Fen	*Juncus Festuca* spp	350 / 700	+ 74.5 NS	ND	Open-top chamber	Kang *et al.* (2001)

Körner, 1996), the height of water table (Roulet *et al.*, 1992) or peatlands area (Gorham, 1991) may also interfere with the capacity for methane emissions from wetland ecosystems under elevated CO_2 conditions.

Enzyme activities

Alterations in microbial mineralization and nutrient cycling may control the long-term response of ecosystem to elevated CO_2. Because microorganisms are regulators of decomposition, an understanding of microbial activity is crucial. Elevated CO_2 concentration can affect extracellular enzyme activities in several ways. Dhillion *et al.* (1996) reported that dehydrogenase, cellulose, phosphatase, and xylanase were increased by elevated CO_2 in the root region of soil from monocultures of *Bromus madritensis*, a common and sometimes dominant annual grass in Mediterranean model ecosystem. Of the four enzymes examined, dehydrogenase and xylanase activities were significantly higher in soils under elevated CO_2 than in ambient. Moorhead and Linkins (1997) suggested that elevated CO_2 altered the soil enzyme characteristics in a tussock tundra ecosystem. They found significantly higher phosphatase activities at 680 mol/mol CO_2 on the surfaces of plant roots, mycorrhizal surfaces, and in the shallowest organic horizons soil.

Conflicting results have also been reported. Moorhead and Linkins (1997) found lowering of endocellulase and exocellulase activities in the surface organic soil horizons of tussock tundra exposed to elevated CO_2. In alpine grassland, cellobiohydrolase and *N*-acetylglucosamidase activity was found to increase under elevated CO_2, while leucine-7-aminopeptidase activity decreased and β-D-glucosidase remained unaffected (Mayr *et al.*, 1999; Larson *et al.*, 2002). Kang *et al.* (2001) reported no significant differences in the soil enzyme activities (acid phosphates, β-glucosidase and *N*-acetylglucosaminidase) in a northern fen bulk soil exposed to CO_2 enrichment. When data from different wetlands were compared, phosphatase and *N*-acetylglucosaminidase activities varied according to nutrient availability in each wetland (Kang *et al.*, 2004). It is suspected that actively growing vegetation under elevated CO_2 may compete against microbes for nutrients, resulting in general decrease in microbial activity (Freeman *et al.*, 1998). Insam *et al.* (1999) showed that protease and xylanase activities were not significantly affected, while dehydrogenase activity was significantly lower under elevated CO_2 in an artificial tropical ecosystem.

Several studies have assessed CO_2 effects on enzyme involved in nitrogen fixation. For example, Zanetti *et al.* (1996) reported an increase in symbiotic nitrogen fixation activity for *T. repens* growth under enriched CO_2 atmosphere. Dakora and Drake (2000) also observed that elevated CO_2 stimulated greater N_2 fixation and nitrogenase activity in stands of the C_3 sedge, *Scirpus olneyi* of the Chesapeake Bay wetland. They also showed a significant increase in N_2 fixation in plant free marsh sediment exposed to elevated CO_2.

Microbial community composition

Although changes in soil microbial number, biomass, activity, and microbial C and N in response to elevated CO_2 have been demonstrated in several studies (Diaz *et al.*, 1993; Zak *et al.*, 1993; Rice *et al.*, 1994; Dhillion *et al.*, 1996), information on the effects on soil microbial community structure is highly limited (Zak *et al.*, 1996; Griffiths *et al.*, 1998; Insam *et al.*, 1999; Marilley *et al.* 1999; Wiemken *et al.*, 2001; Klamer *et al.*, 2002; Deiglmayr *et al.*, 2004). For microbial community structure, phospholipid fatty acid analysis (Zak *et al.*, 1996; Montealegre *et al.*, 2002), and several molecular methods have been employed (Griffiths *et al.*, 1998; Marilley *et al.*, 1998; Marilley *et al.*, 1999; Montealegre *et al.*, 2000; Klamer *et al.*, 2002; Deiglmayr *et al.*, 2004). For example, fungal community composition has been determined by terminal-restriction fragment length polymorphism (T-RFLP) analysis of the internal transcribed spacer (ITS) region (Klamer *et al.*, 2002). In addition, DNA hybridization, percent G+C base profiling, and PCR-based fingerprinting were used in other studies (Griffiths *et al.*, 1998; Montealegre *et al.*, 2000). Marilley *et al.* (1999) employed DNA restriction analysis (ARDRA) and colony hybridization, while PCR-RFLP with primers for the *narG* gene was used by Deiglmayr *et al.* (2004).

Several studies have suggested that elevated atmospheric CO_2 could alter the composition of soil microbial communities due to changes in the amount and/or composition of plant material input into the soil (Mayr *et al.*, 1999; Mitchell *et al.*, 2003). For example, the bacterial substrate utilization assay by Dhillion *et al.* (1996) reported that components or assemblages of bacterial communities might be susceptible to shifts or change by elevated CO_2 in root region soil from monocultures of *Bromus madritensis*, a common and sometimes dominant annual grass in Mediterranean model ecosystem. Jones *et al.* (1998) showed that the composition of soil fungal species changed in an artificial system under elevated CO_2. Polymerase Chain Reaction (PCR) fingerprinting of genomic DNA by Montealegre *et al.* (2000) showed that the isolates (*Rhizobium* strains) from plants grown under elevated CO_2 were genetically different from those isolates obtained from plants grown under ambient conditions in a pasture ecosystem of Swiss FACE experiment. These results indicate that elevated atmospheric CO_2 may shift community composition of soil microorganisms.

In addition, elevated atmospheric CO_2 affects the competitive ability of root nodule symbionts, most likely leading to a selection of these particular strains to nodulate white clover. Recently, Montealegre *et al.* (2002) used PLFA analysis to examine microbial community composition in the rhizosphere soil of white clover plants grown under ambient or elevated CO_2. They reported that the change in soil microbial community composition occurred after 3 years of CO_2 fumigation. Lekkerkerk *et al.* (1990) reported that specific species responded significantly to changes in CO_2 concentration.

However, some studies have shown no alteration of microbial community composition by elevated CO_2 concentrations. Zak *et al.* (1996) did not find any significant changes in microbial community composition in soil. Ringelberg *et al.* (1997) reported that elevated CO_2 caused only subtle changes in gram negative bacteria and actinomycetes. Griffiths *et al.* (1998), using broad-scale DNA techniques, showed that the rhizosphere microbial communities of ryegrass and wheat (*Triticum aestivum* L.) were 86% similar under ambient and elevated CO_2. In an artificial tropical ecosystem, Insam *et al.* (1999) found that elevated CO_2 did not affect the shift in bacterial community employing phospholipid fatty acid analysis (PLFA) patterns and community level physiological profiles (CLPP).

Factors Modifying the Responses of Soil Microorganisms to Elevated CO_2

The patterns of microbial response to elevated CO_2 are influenced by several factors such as the types of plants examined (Sadowsky and Schortemeyer, 1997), nutrient status, soil type, the analysis method (Zak *et al.*, 1996; Griffiths *et al.*, 1998), experimental system (O'Neill *et al.*, 1987), and diversity of microbes in the ecosystem (Montealegre *et al.*, 2000).

Types of vegetation and microbial responses to elevated CO_2

Montealegre *et al.* (2002) found that the microbial population associated with white clover (*Trifolium repens* L.) under elevated CO_2 increased, while no effect on total or metabolically active bacteria in bulk soil of perennial ryegrass (*Lolium perenne* L.) was noted. Schortemeyer *et al.* (1996) also found a positive effect of the elevated CO_2 on the bacterial numbers in the rhizosphere of ryegrass, but a negative effect in the rhizosphere of *T. repens*. However, Deiglmayr *et al.* (2004) found that plant species had no apparent effect on microbial responses.

Nutrient status

According to Cardon (1996), the influence of elevated CO_2 is linked to the nutrient status of the soil. Under nutrient (mostly N) limited conditions, effects of elevated CO_2 on plants were generally found to be much smaller (Körner *et al.*, 1997) and it was suggested that poor N supply limited the microbial utilization of C compounds (van Veen *et al.*, 1991). For example, Arnone and Körner (1995) found that under severe N limitation, the C contents of any ecosystem compartment (above- and below-ground biomass, soil organic matter) remained unchanged. Rice *et al.* (1994) and Niklaus and Körner (1996) did not find CO_2 fertilization effects on the soil microbiota without N fertilization, while they found an increased respiration under elevated CO_2 with N supply. Likewise, an increase in microbial activity was found under N limited conditions in a study on prairie soils, but the response was larger when fertilizer was added (Rice *et al.*, 1995). In addition, it has been hypothesized that an optimal soil nitrogen concentration exists for the functioning of the symbiosis that depends on the combination of the tree and fungal species (Wallenda and Kottke, 1998). Accordingly, nutrient status, particularly N supply, is thought to be an important factor controlling the magnitude of microbial response to elevated CO_2.

In a study by Insam *et al.* (1999), the small effects on the soil microbiota were probably due to low nutrient supply and low organic matter content of the soil. However, Deiglmayr *et al.* (2004) reported that fertilizer treatment had no apparent effect.

Microhabitats in soil

The extent of changes in microbial processes often depends on the location of soil microorganisms. In particular, the distance between microbes and plant root surface is of great importance (Montealegre *et al.*, 2002). For example, elevated CO_2 affected bacteria colonized in the rhizosphere and the rhizoplane-endorhizosphere most substantially (Marilley *et al.*, 1999; Schortemeyer *et al.*, 2000; Wiemken *et al.*, 2001; Montealegre *et al.*, 2002). Likewise, Wiemken *et al.* (2001) found that the microbial communities in bulk soil or rhizosphere were clearly less responsive to elevated CO_2.

Other factors

In a tallgrass prairie, Rice *et al.* (1995) found a positive effect of elevated CO_2 on the microbial biomass under dry, but not under humid conditions, and they attributed the response to better moisture retention due to more efficient CO_2 uptake under elevated CO_2. Deiglmayr *et al.* (2004) reported that the structure of the nitrate-reducing community was primarily affected by season and pH of the sampling site.

Conclusions and Future Directions

The overall mechanisms and responses of soil microorganisms to elevated CO_2 are summarized in Fig. 1. Soil microorganisms are known to be affected by elevated CO_2 through various interactions with plants, including increased root exudation, altered leaf chemistry, and competition for resources. As microbial responses to elevated CO_2 are indirectly mediated by plant responses, results of studies considering CO_2 effects on microorganisms are often relatively unclear. Higher carbon supply from plants caused by elevated

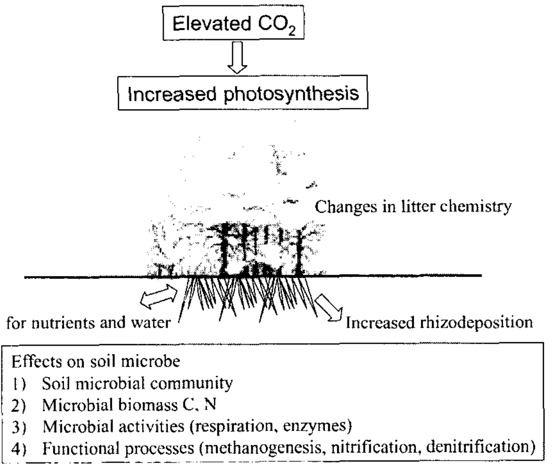

Fig. 1. Summary of mechanisms by which soil microorganisms can be affected by elevated CO_2 in the atmosphere.

CO_2 may enhance certain microbial processes such as CH_4 emission from wetlands. However, other microbial properties such as extracellular enzyme activities and microbial community structure are connected partially with other factors (e.g., nutrient availability, vegetation type, or microhabitat), and hence unequivocal conclusions about the effects of elevated CO_2 on soil microorganisms are still lacking.

An improved understanding of microbial responses to elevated CO_2 could be obtained in further studies. For example, recent techniques such as micro-arrays and other molecular tools could be applied more effectively to this field. Compared to other fields of microbiology, information gathering using this approach is relatively lacking. Secondly, better experimental design and sampling techniques appear warranted if we are to account for interferences from other factors as well as artifacts from heterogeneity of soil media. Thirdly, there are likely to be many advantages to be gained from the simultaneous application of multiple techniques to a single or small set of experiments, so that various aspects of microbial structure and functions (and their interactions) can be considered. Finally, appropriate statistical techniques and modeling approaches are required to extrapolate microbial data to ecosystem or global scales.

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