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Elevated atmospheric CO_2 increases microbial growth rates in soil: results of three CO_2 enrichment experiments

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Abstract

Increasing the belowground translocation of assimilated carbon by plants grown under elevated CO_2 can cause a shift in the structure and activity of the microbial community responsible for the turnover of organic matter in soil. We investigated the long-term effect of elevated CO_2 in the atmosphere on microbial biomass and specific growth rates in root-free and rhizosphere soil. The experiments were conducted under two free air carbon dioxide enrichment (FACE) systems: in Hohenheim and Braunschweig, as well as in the intensively managed forest mesocosm of the Biosphere 2 Laboratory (B2L) in Oracle, AZ. Specific microbial growth rates (μ) were determined using the substrate-induced respiration response after glucose and/or yeast extract addition to the soil. For B2L and both FACE systems, up to 58% higher μ were observed under elevated vs. ambient CO_2, depending on site, plant species and N fertilization. The μ-values increased linearly with atmospheric CO_2 concentration at all three sites. The effect of elevated CO_2 on rhizosphere microorganisms was plant dependent and increased for: *Brassica napus* = *Triticum aestivum* < *Beta vulgaris* < *Populus deltoides*. N deficiency affected microbial growth rates directly (N limitation) and indirectly (changing the quantity of fine roots). So, 50% decrease in N fertilization caused the overall increase or decrease of microbial growth rates depending on plant species. The μ-value increase was lower for microorganisms growing on yeast extract then for those growing on glucose, i.e. the effect of elevated CO_2 was smoothed on rich vs. simple substrate. So, the r/K strategies ratio can be better revealed by studying growth on simple (glucose) than on rich substrate mixtures (yeast extract). Our results clearly showed that the *functional characteristics* of the soil microbial community (i.e. specific growth rates) rather than *total* microbial biomass amount are sensitive to increased atmospheric CO_2. We conclude that the more abundant available organics released by roots at elevated CO_2 altered the ecological strategy of the soil microbial community specifically a shift to a higher contribution of fast-growing r-selected species was observed. These changes in functional structure of the soil microbial community may counterbalance higher C input into the soil under elevated atmospheric CO_2 concentration.

Keywords: *Beta vulgaris*, Biosphere 2, *Brassica napus*, elevated CO_2, FACE, glucose, microbial biomass, microbial growth rates, *Populus deltoides*, r and K strategy, rhizodeposition, rhizosphere microorganisms, substrate-induced respiration, *Triticum aestivum*

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Introduction

The mineralization activity of soil microorganisms is crucial in regulating CO_2 release into the atmosphere from soils, where about 60% of the carbon (C) stock of terrestrial ecosystems is sequestrated. An increased CO_2 concentration in the atmosphere of up to 450–600 ppm, as predicted for 2050 (Houghton *et al.*, 2001), cannot directly affect soil mineralization processes because the CO_2 concentration in soil is several orders of magnitude higher. Nonetheless, indirect plant-mediated effects of

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elevated CO_2 concentration – including more rhizodeposits and root exudates and changes in their composition (van Veen *et al*., 1991; Diaz *et al*., 1993; Hungate *et al*., 1997; Cheng, 1999) – can affect the amount, composition and functions of soil microorganisms (Montealegre *et al*., 2002; Carney *et al*., 2007; Kandeler *et al*., 2008). Surprisingly, recent studies have revealed only minor changes in soil microbial communities under elevated CO_2, especially in total prokaryotic and eukaryotic abundance (Lipson *et al*., 2006; Haase *et al*., 2008; Kanerva *et al*., 2008; Lesaulnier *et al*., 2008). Microbial biomass increased slightly (Ross *et al*., 1995; Insam *et al*., 1999, reviewed by De Graaff *et al*., 2006) or even decreased (Hungate *et al*., 1996) under elevated vs. ambient CO_2, and microbial respiration showed a more consistent increase – ca. 17% according to De Graaff *et al*. (2006). It remains unclear, however, which factors were responsible for the absence of strong effects of elevated CO_2 on microorganisms despite the greater quantity and availability of organic substrates released by roots (Zak *et al*., 2000b; Freeman *et al*., 2004). Increased input of available C by rhizodeposits does not activate SOM-mineralizing microorganisms (usually K-strategists): numerous long-term studies found no or a minor effect of elevated CO_2 on SOM content (Schlesinger & Lichter, 2001; Van Groenigen *et al*., 2003; Lichter *et al*., 2005; De Graaff *et al*., 2006 and references therein). Rapid decomposition of increased C inputs by more active microbial biomass under elevated CO_2 can potentially explain the unaffected content of recalcitrant soil organic carbon under such CO_2 conditions (Lichter *et al*., 2005; Taneva & Gonzalez-Meler, 2008). We therefore hypothesized that the increase of available C in the rhizosphere at elevated CO_2 will alter the structure and activity of the soil microbial community. The result is an increase of fast-growing r-strategists that quickly metabolize easily available substrates. In such a case, a greater input of available substrates by root exudates can be counterbalanced by faster microbial turnover, leading to higher rates of substrate mineralization.

Stimulation of fast-growing microorganisms by elevated CO_2 can be evaluated by examining the growth rates of the whole microbial community. Thus, *in situ* investigations of microbial growth rates are necessary to support the hypothesized increase of fast-growing microorganisms in the rhizosphere of different plant species under elevated CO_2. The lack of experimental studies relating specific microbial growth rates (μ) in soil with increasing atmospheric CO_2 is due to several difficulties: (1) direct measurements in pure cultures do not adequately reflect real rates of processes and microbial interactions in soil; and (2) until now there are no *direct* methods for satisfactorily estimating microbial growth *in situ*. An *indirect* approach suitable for estimating μ is based on the kinetics of substrate-induced respiration (KSIR) (Blagodatsky *et al*., 2000; Stenström *et al*., 2001). This approach characterizes the growth rates of whole microbial community according to microbial growth model proposed by Panikov (1995). The model reflects the transition of soil microorganisms from a 'sustaining' (Smith *et al*., 1986) to an active state, considering both the lag-phase and phase of exponential growth after substrate addition. Beyond estimating μ, the KSIR approach also reveals the sustaining and growing fractions of microbial biomass (Panikov & Sizova, 1996; Blagodatsky *et al*., 2000). If the available substrates in the rhizosphere increase under elevated CO_2, we expected more pronounced changes of microbial growth rates in the rhizosphere than in root-free soil.

Glucose is commonly used to induce the respiratory activity of soil heterotrophic microorganisms (Anderson & Domsch, 1978). Note that soil contains not only oligotrophic species capable of efficient growth on glucose, but also auxotrophic microorganisms, those that cannot grow in the absence of vitamins, amino acids, and growth factors (Alexander, 1991; Yadav, 2007). Accordingly, we applied – along with glucose and mineral salts – mixtures containing yeast extract in order to stimulate a broader spectrum of soil microorganisms with diverse nutrient requirements. Most of the C entering the soil as litter or rhizodeposits under elevated CO_2 consists of energy-rich, easily available carbon sources such as low molecular sugars, and dicarboxylic acids (Jones *et al*., 2004) of poor quality, e.g. with a wide C : N ratio caused by lower N availability (Grayston *et al*., 1998; Lesaulnier *et al*., 2008). We therefore hypothesized lower nutrients requirements of microbial communities under elevated CO_2.

Soil N availability controls the increase of above- and belowground plant biomass against the background of elevated CO_2. Depending on the intensity of N limitation under such conditions the root growth may decrease (Pregitzer *et al*., 2000) or, on the contrary, the root mass (especially that of fine roots) may increase (Hyvonen *et al*., 2007). Thus, the mineralization activity of microorganisms under elevated CO_2 is dependent on both interactions with the plant community and with N availability (Freeman *et al*., 2004). This causes the discrepant results and difficulties in investigating the effect of elevated CO_2 on microbial activity and mineralization processes in soil. Accordingly, the hypothesized shift in the soil microbial community from K- to r-strategists can be also affected by N availability.

We investigated the long-term effect of elevated CO_2 on microbial biomass and specific growth rates of microorganisms of root-free and rhizosphere soil in field experiments under three different CO_2 enrichment

systems at spatially remote locations. The following questions were investigated in detail: how does the effect of elevated CO_2 on microbial growth rates in soil depend on (1) the CO_2 concentration, (2) the plant species, and (3) N fertilization. In order to stimulate a broader spectrum of soil microorganisms, we investigated the shift in metabolic functioning of the soil microbial community using three organic-mineral mixtures of easily available substrates.

Materials and methods

Site descriptions and soils

Free air carbon dioxide enrichment (FACE) at Stuttgart-Hohenheim, Germany. The FACE facility in Hohenheim (FACE-H) is located south of Stuttgart, Baden-Württemberg, Germany (48°43′N, 9°13′E 100 m a.s.l.). The soil is a Gleyic Cambisol (WRB, 1998) without $CaCO_3$. The soil properties were identical under ambient and elevated CO_2 treatments (Table 1).

The CO_2 enrichment experiment, starting in 2002, included plots with elevated atmospheric CO_2 (540 ppm, with enclosures), ambient plots (380 ppm, with enclosures), and control plots (ambient CO_2 level, no enclosures) (Erbs & Fangmeier, 2006). Each treatment was replicated five times. Spring wheat (*T. aestivum* L. cv Triso) was annually planted on the plots from 2002 to 2006. In 2007, oilseed rape (*Brassica napus* L.) was grown on the plots for the first time. Beginning in 2003, inorganic NPK fertilizers were annually applied in equal amounts of 140 kg N ha^{-1}, 60 kg K ha^{-1}, 30 kg P ha^{-1} to each plot under ambient and elevated CO_2 treatments. No organic fertilizers were applied. Soil was sampled from the top 10 cm of each ambient and elevated-CO_2 plot using soil corers (inner diameters: 5 cm) in September 2007, 3 weeks after the rape harvest. Soil samples were taken from locations that adhered to the plant roots and stored at 7 °C for 1 week before analyses.

FACE at the Institute of Agroecology, FAL, Braunschweig, Germany. The second FACE facility (FACE-B) used in the study was located at the experimental farm of the Federal Agricultural Research Centre (FAL) in Braunschweig, Lower Saxonia, Germany (52°18′N, 10°26′E, 79 m a.s.l.). Investigations were carried out on plots under ambient (350 ppm) and elevated (550 ppm) atmospheric CO_2 concentrations, values which had been applied since May 2000 (Weigel *et al*., 2006). Two plant species, sugar beet (*Beta vulgaris* L.) and winter wheat (*T. aestivum* L.), grown in crop rotation were investigated. The soil type was loamy-sand Cambisol derived from loess (Table 1). N supply was restricted to

Table 1 Overview of the three CO_2 enrichment experiments and the relevant soil properties

Site location, acronym	Plant species	Atmospheric CO_2 (ppm)		Soil type	C (%)	N (%)	Sand (%)	Silt (%)	Clay (%)	pH_{KCl}	Reference
		Ambient	Elevated								
Braunschweig FACE-B	*Beta vulgaris, Triticum aestivum*	350	550	Cambisol, loamy sand	1.1	0.09	42	52.6	5.4	6.8	Weigel & Dämmgen (2000)
Hohenheim FACE-H	*Brassica napus*	380	540	Gleyic Cambisol	1.59	0.17	9	69	22	6.8	Erbs & Fangmeier (2006)
Oracle, AZ, B2L	*Populus deltoides*	400	800 or 1200	Silty loam	2	0.19	28	55	17	7.2	Torbert & Johnson (2001)

FACE, free air carbon dioxide enrichment; B2L, Biosphere 2 Laboratory.

50% of adequate N in half of each of the FACE-B plots, and the soils were annually amended with mineral fertilizers at two different rates: 126 and 63 kg N $ha^{-1}\,yr^{-1}$. Soils were sampled after 3 and 4 years from the beginning of the fumigation with elevated CO_2.

Composite soil samples were taken in three replicates from the layer 0 to 10 cm during harvesting time (sugar beet) or 1 week after cropping the winter wheat. Rhizosphere soil was taken from locations that adhered to the plant roots, whereas root-free soil was taken from the space between rows of crops. Soil was stored field-fresh in aerated polyethylene bags for a maximum 8 weeks at 4°C. Samples taken from this experiment allow estimating the rhizosphere and N amendment effects along with the effect of elevated CO_2 on the SIR kinetics.

Intensively managed forest mesocosm, 'Biosphere 2,' in Oracle, AZ, USA. The Biosphere 2 Laboratory (B2L) is 1.27 ha virtually airtight environmental research facility located in Oracle, AZ (32°34′N, 110°51′W; 1200 m a.s.l.). Soil samples were taken from experimental plots belonging to the intensively managed forest mesocosm (Griffin *et al.*, 2002) with eastern cottonwood (*Populus deltoides* Bartr. ex Marsh.) grown under ambient (400 ppm) and under two levels of elevated (800 and 1200 ppm) atmospheric CO_2 concentrations. The design and operation of B2L are described in detail elsewhere (Zabel *et al.*, 1999). The soil is a silt loam (Torbert & Johnson, 2001), not fertilized, with properties presented in Table 1. Rhizosphere soil adhering to the plant roots was taken in three replicates (i.e. composite samples from three different trees) during root sampling, whereas root-free soil was taken in three replicates from the space under the trees, but >3–5 cm away from the root surface.

Not later than 1 day after sampling, the soils were sieved (<2 mm), fine roots and other plant debris were carefully removed, and the soils were stored at 4 °C during 1–3 months. Before the analyses, soil moistures were adjusted to 60% of water holding capacity (WHC) and samples were preincubated for 24 h at 22°C.

Microbial biomass and the kinetics of substrate-induced respiration

Parameters of microbial growth were determined according to the dynamics of the CO_2 emission rate from soil amended with glucose and/or yeast extract. Mixtures containing yeast extract were applied to soil in order to stimulate a broader spectrum of soil microorganisms and to estimate the metabolic activity of microbial guilds other than glucose-responding heterotrophs.

Samples of 10 g (dry weight) soil were amended with a powder-mixture (I) containing 10 mg g^{-1} glucose, 20 mg g^{-1} talcum, and mineral salts: $(NH_4)_2SO_4$ – 1.9 mg g^{-1}, K_2HPO_4 – 2.25 mg g^{-1}, and $MgSO_4 \cdot 7H_2O$ – 3.8 mg g^{-1}. Mixtures II and III were prepared as in mixture I with the following modifications: 0.3 mg g^{-1} of yeast extract LD (Difco Manual, 1998) was added to glucose (10 mg g^{-1}) in mixture II as a combined C source; 4 mg g^{-1} of yeast extract was used in mixture III instead of glucose. Optimal concentrations of substrates were found in preliminary experiments, so that the soil amendments were sufficient to enable unlimited exponential growth of soil microorganisms during the initial period. Mineral salts included in mixtures were selected so that the substrate changed the pH of soil for <0.1.

The CO_2 production rate was measured hourly at 22°C using an automated infrared-gas analyzer system (Heinemeyer *et al.*, 1989).

Soil microbial biomass C (C_{mic}) was determined using the initial rate of substrate-induced respiration when soil was amended with mixture I according to the equation of Anderson & Domsch (1978):

$$C_{mic}(\mu g g^{-1}\,\text{soil}) = (\mu L\,CO_2\,g^{-1}\,\text{soil}\,h^{-1}) \times 40.04. \quad (1)$$

Specific growth rate (μ) was determined by fitting of equation parameters to the experimental data on CO_2 evolution rate (v) according to:

$$v(t) = A + B \times \exp(\mu \times t), \quad (2)$$

where A is the initial rate of uncouple (nongrowth) respiration, B is the initial rate of couple (growth) respiration, t is the time (Blagodatsky *et al.*, 2000; Panikov & Sizova, 1996). The parameters of Eqn (2) were determined by fitting MODELMAKER © Version 3.0.3 software (Modelmaker, 1997). Fitting was restricted to the part of the curve that corresponded better to applied model Eqn (2), as indicated by maximum values of statistic criteria: r^2, the fraction of total variation explained by the model defined as the ratio of the model weighted sum of squares to the total weighted sum of squares.

Statistical analysis

All data presented are mean values from three replications. Nonparametric tests (Mann–Whitney and Wilcoxon) were used for estimating the significant effects ($P<0.05$) of the following factors: ambient vs. elevated CO_2 (CO_2); root adhered vs. root-free soil (Rhi); and different N amendment (N). Three-way analysis of variance (ANOVA) was applied to split the effects of the three mentioned factors and their interactions for two blocks of data: soil under sugar beet or under winter

wheat (FACE-B). Such analysis was appropriate only for FACE-B experiment where effects of three factors could be measured without interference of time and plant species. Two-way completely randomized ANOVA was applied for FACE-B experiment in order to characterize effects of elevated CO_2 and grown crop. When significant effects were found a multiple comparison using Duncan's multiple range test (significance level 5%) were performed. Regression analysis was used to check the effect of CO_2 concentration on microbial specific growth rates. All variables treated passed normality and equal variance tests.

Results

Microbial biomass and KSIR under elevated CO_2

Microbial biomass ranged from 204 to 429 $\mu g\,C\,g\,soil^{-1}$ under ambient CO_2 concentrations (Table 2). Significantly larger microbial biomass C was measured in FACE-H soil compared with that in B2L and FACE-B soils. No significant effect of elevated CO_2 on total microbial biomass C was observed in any soil if direct comparison of means or two-way ANOVA was applied, although in all cases the microbial biomass at elevated CO_2 tended to increase (Table 2). If influence of all three studied factors (atmospheric CO_2, plant roots, N application rate) is considered for FACE-B experiment, than elevated CO_2 effect on total microbial biomass under sugar beet is significant at $P<0.05$ level according to three-way ANOVA (Table 3).

For all soils the increase in the respiratory response after glucose addition was steeper under elevated vs. ambient CO_2 (Fig. 1). The steepness of the respiration curves increased considerably with increasing CO_2 concentration (Fig. 1, bottom right). For each soil type, specific microbial growth rates (μ) estimated by Eqn (2) were significantly higher under elevated CO_2 (Table 2). Regression analysis clearly revealed significant positive relationships between increasing atmospheric CO_2 concentration and μ-values for all soils and treatments (Fig. 2).

Plant species and root vicinity effect on microbial growth rates

Significant differences ($P<0.05$) in specific microbial growth rates were observed between the three experimental sites for soils under ambient CO_2. Within the same FACE system and the same soil type (FACE-B), the differences between μ-values under different plant species *B. vulgaris* (root crop) and *T. aestivum* (cereal) were insignificant at ambient CO_2 concentration (Table 2). Thus, under ambient CO_2 the effect of soil type on μ-values was higher than the effect of plant species. The same tendency was also observed under elevated CO_2 (Table 2).

In root-free soil as well as in the rhizosphere of all plant species, the μ-values were always higher under elevated than under ambient CO_2 (Fig. 3), i.e. all ratios presented in Fig. 3 are higher than 1.0. The effect of elevated CO_2 on μ in the rhizosphere of different plants increased in the following order: *B. napus* (540 ppm) = *T. aestivum* (550 ppm) < *B. vulgaris* (550 ppm) < *P. deltoides* (800 ppm) (Fig. 3). In the last case, both the plant type and higher CO_2 concentration likely affected the microbial response. The effect of elevated CO_2 on μ tended to be more pronounced in the rhizosphere than

Table 2 Soil microbial biomass, and specific growth rates (μ) of rhizosphere microorganisms in the three CO_2 enrichment experiments

Site location	Plant species	Atmospheric CO_2 (ppm)	Microbial biomass, ($\mu g\,C \times g^{-1}$)	μ (h^{-1})
Braunschweig, FACE-B*	*Beta vulgaris*	350	303 Ab	0.246 Aa
		550	337 Ab	0.284 Ba
	Triticum aestivum	350	204 Aa	0.255 Aa
		550	206 Aa	0.297 Ba
Hohenheim, FACE-H*	*Brassica napus*	380	429 A	0.194 A
		540	464 A	0.207 B
Oracle, AZ, B2L†	*Populus deltoides*	400	296 A	0.301 A
		800	385 BC	0.393 B
		1200	322 AB	0.475 C

Treatments followed by the same letters are not significantly different between elevated and ambient CO_2 (uppercase letters) and various plants (FACE-B, lowercase letters) at $P \leq 0.05$.

*Data for rhizosphere soil with full rate of N application.

†Data for rhizosphere soil.

FACE, free air carbon dioxide enrichment; B2L, Biosphere 2 Laboratory.

Table 3 Input (%) of atmospheric CO_2 level (CO_2), N application rate (N), and plant roots (Rhi) into variation of microbial specific growth rates (μ) and total biomass estimated by three-way ANOVA for FACE-B experiment

Crop	Source of variation	Specific growth rate (μ)	Total microbial biomass
Beta vulgaris	CO_2	58*	28*
	Rhi	12*	48*
	N	16*	10*
	$CO_2 \times$ Rhi	6*	1
	$CO_2 \times$ N	0	1
	Rhi $\times$ N	1	0
	$CO_2 \times$ Rhi $\times$ N	4*	3
	Unexplained	4	11
Triticum aestivum	CO_2	35*	4
	Rhi	0	4
	N	25*	2
	$CO_2 \times$ Rhi	0	0
	$CO_2 \times$ N	0	0
	Rhi $\times$ N	3	28*
	$CO_2 \times$ Rhi $\times$ N	18*	5
	Unexplained	17	56

*Input significant at $P \leq 0.05$.
FACE, free air carbon dioxide enrichment; ANOVA, analysis of variance.

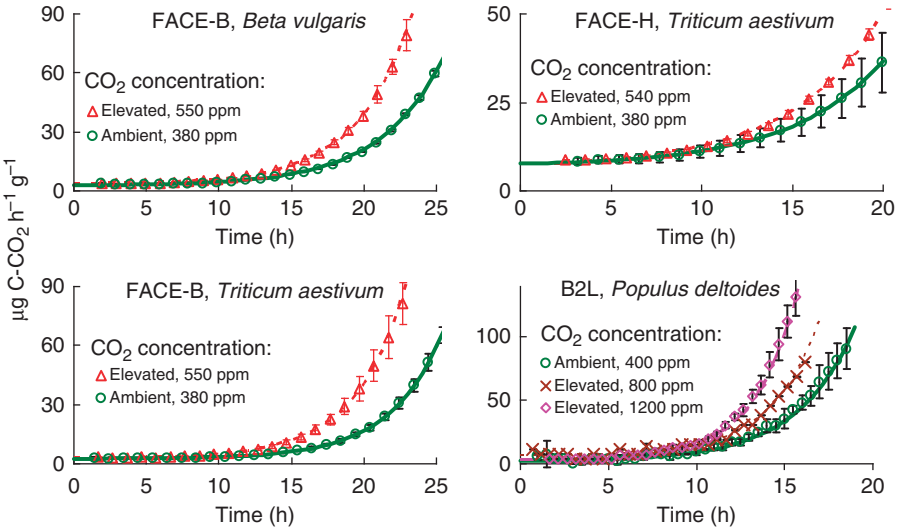

Fig. 1 Kinetics of substrate-induced respiration for soil under three plant species grown at different levels of atmospheric CO_2 in three different CO_2 enrichment experiments. Measured data are presented as symbols, simulation results as lines [Eqn (2)].

in the soil sampled between the rows. This tendency was significant only for soils under *B. vulgaris* (FACE-B) and under *P. deltoides* (B2L) at highest CO_2 concentration (Fig. 3).

Combined effect of elevated CO_2 and nitrogen fertilization on microbial growth rates

Both at ambient and at elevated CO_2 concentrations, the μ-values at plots with *B. vulgaris* tended to be higher at half vs. the full rate of N fertilization (Fig. 4, top, FACE-B). The opposite effect was observed in soils under *T. aestivum* (FACE-B, data not shown), but in general, N fertilization significantly affects specific growth rates under both cultures as is confirmed by three-way ANOVA results for FACE-B experiment (Table 3). Under *B. vulgaris* at half N rate, the effect of elevated CO_2 on μ-values (presented as ratio of $\mu_{elevated}$ to $\mu_{ambient}$) was significantly higher in rhizosphere than in root-free soil (Fig. 4, bottom, FACE-B). However, there were no

differences in the elevated CO_2 effect between rhizosphere and root-free soil at the full rate of N fertilizers (Fig. 4, bottom). The $2 \times 2 \times 2$ ANOVA (FACE-B data) showed no significant ($P < 0.05$) interactions between paired factors affecting the μ and total microbial biomass, i.e. $CO_2 \times N$ and $Rhi \times N$ for both crops. The only exception was the μ increase for $CO_2 \times Rhi$ interaction under sugar beet. In latter case significant rhizosphere effect in respect to μ was enhanced by elevated CO_2. Three factors interaction $CO_2 \times Rhi \times N$ influences significantly specific growth rates under both plant species.

Effect of elevated CO_2 on microorganisms with different nutrient requirements

The comparison of microbial growth on glucose and on yeast extract was used to estimate the response to elevated CO_2 of soil microorganisms with different nutrient requirements (FACE-B experiment). The lag-period before the start of exponential growth was 4–9 h shorter (Table 4) and the increase in respiration rate was steeper (Fig. 5, top) for microorganisms grown on yeast extract vs. glucose both under ambient and elevated CO_2. The maximal specific-growth rate was 2.1–2.7-fold higher on yeast extract (Table 4). Thus, microorganisms utilizing yeast extract differ (at least partly) from those metabolizing glucose in their metabolic abilities. Applying yeast extract along with glucose helps to estimate the effects of elevated CO_2 on a more diverse metabolic spectrum of soil microorganisms.

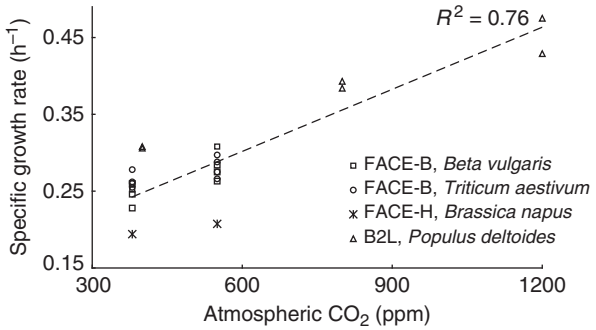

Fig. 2 Specific growth rate of microorganisms on glucose at different levels of atmospheric CO_2. Data for three experimental sites are shown.

The differences in steepness of microbial respiration curves at ambient and elevated CO_2 (Fig. 5, top) were maximal at growth on mixture I (glucose with mineral salts) and were less pronounced by increasing the amount of added yeast (mixtures II and III). Similarly to glucose (Fig. 1, Table 2), the growth on yeast extract had significantly higher μ-values at elevated vs. ambient CO_2 (Table 4). However, the increase at elevated (vs. ambient) CO_2 was stronger for growth on glucose than on yeast extract (Fig. 5, bottom). This shows the lower effect of elevated CO_2 on auxotrophic microorganisms compared with microorganisms capable of growth on glucose with mineral nutrients.

Discussion

Effect of elevated CO_2 on microbial growth rates

The microbial growth rates were significantly higher under elevated vs. ambient CO_2 in the three

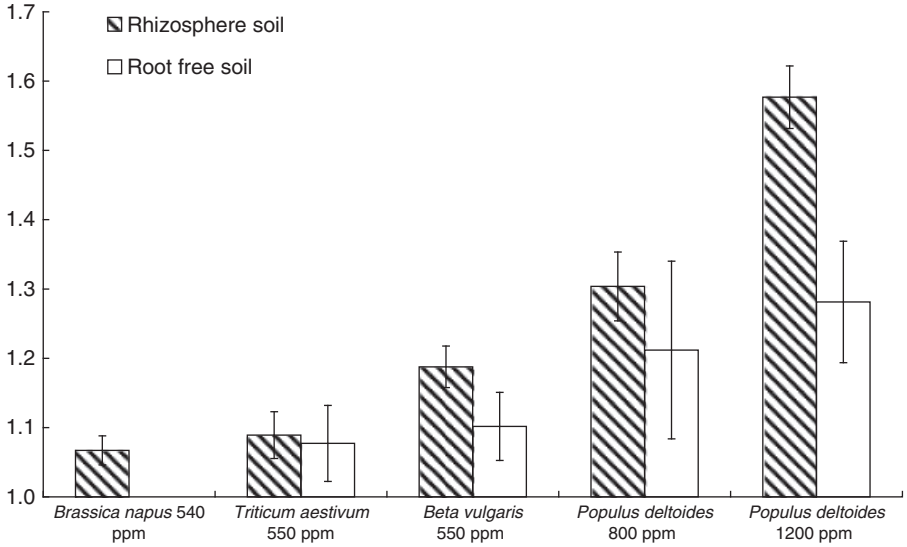

Fig. 3 Relative increase of microbial specific growth rate (μ) caused by CO_2 enrichment in root-free and rhizosphere soil of three CO_2 enrichment experiments. Bars show standard errors, $n = 3$.

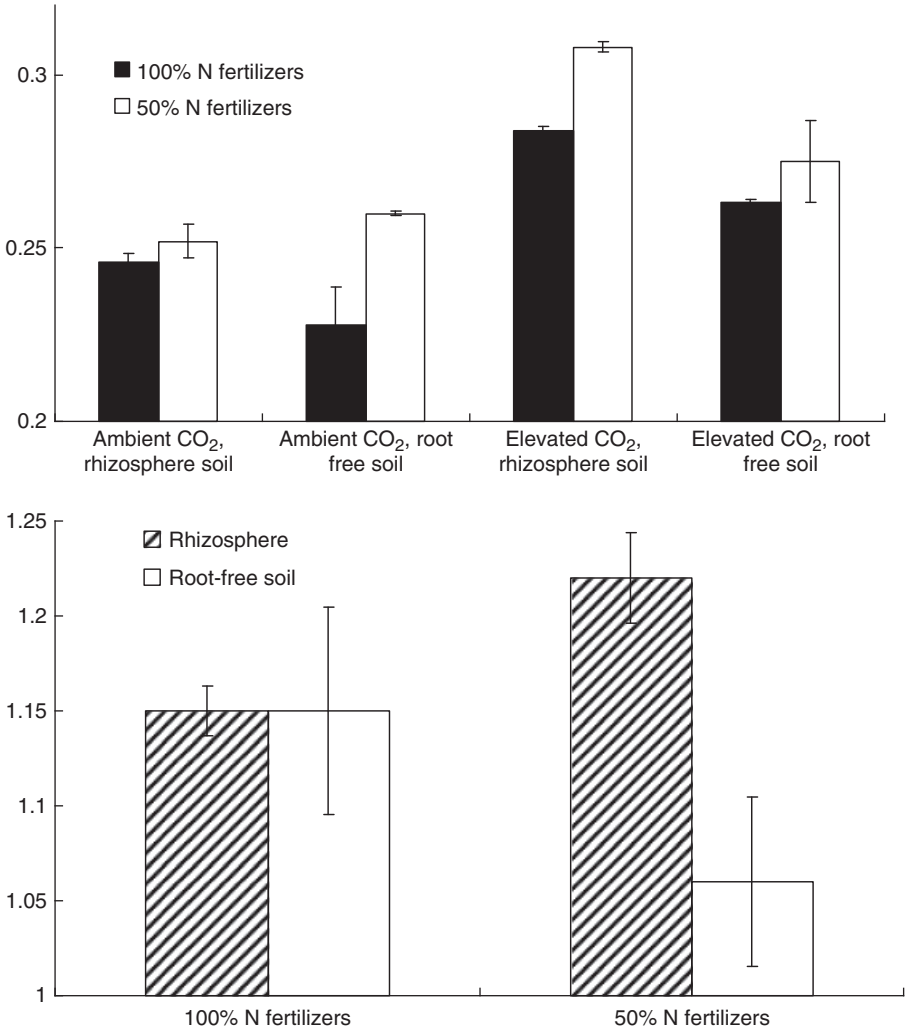

Fig. 4 Effect of nitrogen fertilization on microbial specific growth rate μ (top) and its relative increase (bottom) under sugar beet (*Beta vulgaris*) grown at ambient and elevated CO_2 in free air carbon dioxide enrichment-B experiment. Bars shows standard errors, $n = 3$.

Table 4 Specific growth rates (μ) of microbial community growing on yeast extract and index of auxotrophy for FACE experiment in Braunschweig

Crop	Atmospheric CO_2 (ppm)	μ_{max} (h^{-1})	Lag-time (h)	Index of auxothrophy (μ) glucose/μ yeast extract
Beta vulgaris	350	0.608 A	6.1 A	0.405 A
	550	0.637 B	6.2 A	0.446 B
Triticum aestivum	350	0.588 A	6.4 B	0.434 A
	550	0.64 B	5.7 A	0.464 A

Treatments followed by the same letters are not significantly different between elevated and ambient CO_2 at $P \leq 0.05$.
FACE, free air carbon dioxide enrichment.

investigated CO_2 enrichment systems, for every studied soil type, plant species and N fertilization rate. According to the ecological theory of life strategies, under nonlimiting conditions, microorganisms with r-strategy are characterized by higher μ-values and less efficient substrate utilization than K-strategists (Andrews & Harris, 1986). Hence, plants growing in an elevated CO_2 atmosphere selectively increased the fraction of r-strategists in soil microbial communities. A strong increase in CO_2 concentration (from 400 to 1200 ppm in

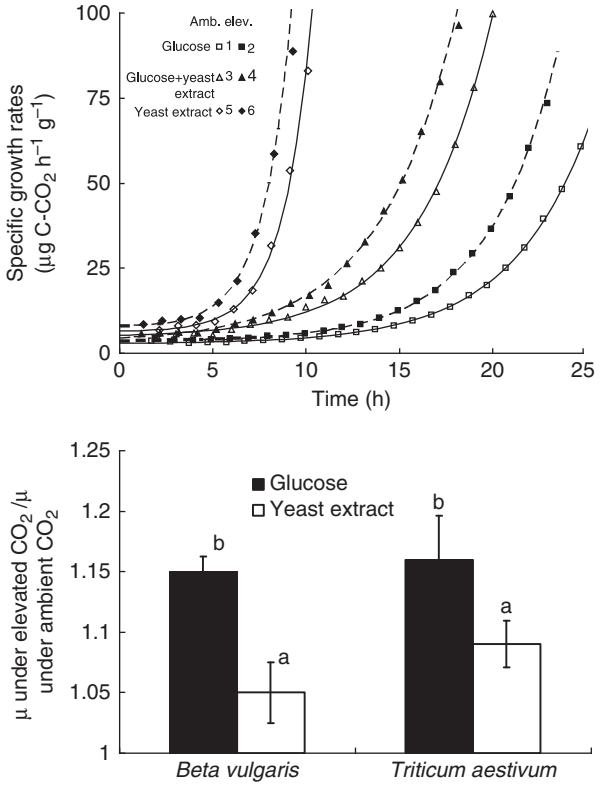

Fig. 5 CO_2 evolution rate (top) and relative increase of microbial specific growth rate μ (bottom) for free air carbon dioxide enrichment-B soil under sugar beet (root-free, full N application rate) after different substrate addition: odd numbers (filled symbols) – plots under elevated atmospheric CO_2, even numbers (empty symbols) – plots under ambient CO_2. Substrates added: 1, 2 – glucose (mixture I); 3, 4 – glucose + yeast extract (mixture II); 5, 6 – yeast extract (mixture III). Treatments followed by the different letters are significantly different at $P < 0.05$.

B2L experiment) yielded a 58% increase in μ-values. However, at the smaller increase (from 350 to 550 ppm in both FACE experiments) the μ-values increased by 0.013–0.038 h^{-1}, i.e. 7–15% of the μ-values under ambient CO_2. Even such relatively slight increases in growth rates can have important consequences because exceedingly small differences in the specific growth rates (as small as 0.0055 h^{-1}) of two populations may be sufficient for the marginally faster growing organisms to be more competitive (Mason & Slater, 1979). Hodge *et al.* (1998) reported more cultivated bacteria (usually r-strategists) as along with more intensive rates of substrate utilization on BIOLOG-plates in soil with ryegrass grown under elevated vs. ambient CO_2 concentrations. These observations were supported by quantitative-PCR analyses that revealed more heterotrophic decomposers within a bacterial community at elevated CO_2 (Lesaulnier *et al.*, 2008). However, total bacterial and eukaryotic abundance as well as the abundance of denitrifying bacteria were not affected by elevated CO_2 (Haase *et al.*, 2008; Lesaulnier *et al.*, 2008). We suggest that the functional characteristics of soil microbial community (microbial growth or substrate mineralization rates) rather than total microbial biomass are sensitive to increased atmospheric CO_2.

Most studies using relatively conservative indexes of soil microbial communities – DNA content, total PLFA and diversity estimated based on PLFA analysis – registered no or only a subtle effect of elevated CO_2 (Griffiths *et al.*, 1998; Zak *et al.*, 2000b; Ebersberger *et al.*, 2004; Lipson *et al.*, 2006; Denef *et al.*, 2007; Kanerva *et al.*, 2008; Paterson *et al.*, 2008). The studies using community level physiological profiling (CLPP) and similar approaches (Rillig *et al.*, 1997; Grayston *et al.*, 1998; Mayr *et al.*, 1999), showed, on the contrary, distinct response of microbial community to elevated CO_2. Direct comparison of total (DNA-based) and active (RNA-based) bacterial communities (Jossi *et al.*, 2006) showed that pCO_2 increase mainly influenced active and root-associated bacterial components. After adding glucose, potential enzyme activities under elevated CO_2 were up to twofold higher than under ambient CO_2 (Dorodnikov *et al.*, 2009b). Supporting our observations, this indicates the increased activity of fast-growing microorganisms, which leads to accelerated turnover of available carbon in soil under elevated CO_2. The measurement of specific growth rates of microorganisms responding to substrate additions can therefore serve as robust and simple method to quantitatively estimate changes in whole microbial community caused by environmental changes (Monson *et al.*, 2006; Lipson *et al.*, 2009) including pCO_2 increase (Dorodnikov *et al.*, 2009a).

The inconsistency of effects of elevated CO_2 on microbial biomass and activity observed in different studies (Zak *et al.*, 2000b) can be related to interactions with other factors such as nutrient limitation and the effect of plant species.

Rhizosphere and plant species effects

Elevated CO_2 increases microbial specific growth rates both in rhizosphere and in bulk soil (Fig. 3). Though the rhizosphere effect was observed for the μ response to elevated CO_2 in several cases, the altered functional properties of microbial communities included the whole soil volume and were not merely transient or linked with the current input of root exudates.

We find no direct effect of plant species (*B. vulgaris* and *Triticum aestivum*) on specific microbial growth rates within the same FACE system (FAL). This agreed with Deiglmayr *et al.* (2004), who found no differences between *Lolium perenne* L. and *Trifolium repens* L. effects on the nitrate-reducing community in the rhizosphere

under long-term elevated CO_2. Similarly to our observations, inconsistent effects of different plant species on soil microbial populations were found in the rhizosphere of *L. perenne* and of *T. repens* (Schortemeyer *et al*., 1996; Montealegre *et al*., 2002). Such inconsistency can be due to nonuniform temporal and spatial responses of root systems. According to Haase *et al*. (2007), the C flux from plant roots of *Phaseolus vulgaris* L. was significantly increased only in apical root zones. Moreover, even in those zones, the elevated CO_2 effect was inconsistent and resulted in an increase or decrease in rhizodeposits within the plant development stages (Haase *et al*., 2008). Study of plant and soil lipid modification under elevated CO_2 argued against plant/soil turnover determinations for individual compounds (Wiesenberg *et al*., 2008). Thus, studies focused on the effects of root exudations on microbial growth rates are necessary to reveal the effect of higher CO_2 levels under different plant species during the vegetation season.

Combined effect of nitrogen and CO_2 enrichment

Addition of N stimulates plant and microbial growth, and these effects may be modified by elevated CO_2 (Hungate *et al*., 1996). Strongly increased plant growth by N fertilization may also increase the total amount of roots and rhizodeposits. However, this effect is frequently counterbalanced by a decreased percentage of C allocated belowground at higher N availability (Kuzyakov & Domanski, 2000; Kuzyakov *et al*., 2001; Henry *et al*., 2005).

Higher microbial specific growth rates under plots with 50% N supply (*B. vulgaris*, FACE-B, Blagodatsky *et al*., 2006) coincide with larger fine root production in these conditions (Weigel *et al*., 2006). Presumably, a trade-off in N uptake between plants and microbes took place in the rhizosphere of *B. vulgaris* (reduced N fertilization), leading to differences in specific growth rates of microbial communities under ambient and elevated CO_2. Thus, N enrichment smoothed the effect of elevated CO_2 on μ-values in the rhizosphere soil under *B. vulgaris*.

The significant effect of $CO_2 \times Rhi \times N$ interactions along with the direct N effect on microbial specific growth rates (Table 3) supports the two-way influence hypotheses: N deficiency can affect microbial growth rates directly (by decreasing C use efficiency, Blagodatskiy *et al*., 1993) and indirectly (through changing the quantity of fine roots). In the latter case plant species effect and root vicinity (rhizosphere effect) modify the effect of N fertilization. Thus, the effect of elevated CO_2 (higher μ-values) can be counterbalanced by N amendment. Similar to our observations, Chung *et al*. (2007) reported that the response of microbial communities to elevated CO_2 was counterbalanced by N deposition in a study on the effect of plant species diversity on microbial biomass and fungal abundance in a BioCON-FACE experiment in east-central Minnesota. However, in another FACE experiment with *Populus*, N amendment stimulated C utilization rates estimated by the Microresp-CLPP method (Lagomarsino *et al*., 2007). A 30% increase in soil heterotrophic respiration caused by elevated CO_2 in the same FACE experiment was not affected by N fertilization (Lagomarsino *et al*., 2009), so the real availability of C and N in this case does not correspond to microbial growth rates obtained by the CLPP approach. Increased N availability reduced microbial respiration in a pine forest under elevated CO_2 at the Duke Free Air Carbon Enrichment site (Billings & Ziegler, 2008). These changes in total heterotrophic activity were apparently connected with the shift in microbial community structure estimated by δ^{13}C-PLFA analysis. Interestingly, Gram-positive actinomycetes, typical soil bacteria with features of K-strategists, i.e. lower maximal-specific growth rates, were abundant under elevated CO_2 and full N fertilization (Billings & Ziegler, 2008). Such findings agree well with our results showing lower μ under full N supply and elevated CO_2.

Thus, our study confirms a plant species effect on microbial response to N availability under elevated CO_2 (Hungate *et al*., 1996). Contradictory N effects on microbial activity under different plant communities can be explained by the different N requirements of dominant microbial species in the rhizosphere of various plant species. Different effects of N fertilizers on root biomass as well as on the rhizodeposition of various plant species (Kuzyakov *et al*., 2001, 2002) are additional potential explanations for inconsistent interactive $N \times$ elevated CO_2 effects on microbial growth parameters.

Effect of elevated CO_2 on auxotrophic microorganisms

Applying three different organic-mineral mixtures to estimate microbial growth better mirrors metabolic diversity and the auxotrophic requirements of soil microorganisms than applying glucose only. The higher μ-values during growth on yeast extract vs. glucose indicated that yeast extract offers selective advantages to microorganisms that are able to grow rapidly on rich media but require growth factors. The greater the increase in μ-values on yeast extract vs. glucose, the greater the auxotrophic requirements of the microbial community. Thus, the ratio between maximal specific growth rates on glucose/yeast extract (μ_{gl}/μ_{ye}) may serve as an auxotrophy index of the soil microbial community (Blagodatskaya *et al*., 2003). High auxotrophy indices correspond to communities with low auxotrophic requirements. For both crops studied in

the FACE-B experiment (*B. vulgaris* and *T. aestivum*), this index was higher under elevated vs. ambient CO_2 (Table 4). Thus, the portion of the microbial community lacking growth factors and vitamins seems to be lower in soils under elevated vs. ambient CO_2. Lower auxotrophic requirements at higher CO_2 concentrations can be due to an increased C input in the form of rhizodeposits with a higher C/N ratio (Cotrufo *et al.*, 1994, 2005). This results in the selection of microorganisms capable of rapid growth on energy-rich, simple substrates (Freeman *et al.*, 2004). This observation was indirectly confirmed by a strong decrease in the diversity of fungal species as well as of nitrate-reducing bacteria and archaea under elevated CO_2 (560 ppm), as estimated by ribosomal DNA sequences (Lesaulnier *et al.*, 2008).

The microbial respiration response induced by glucose was more sensitive to changes in atmospheric CO_2 concentration than the response induced by yeast extract (Fig. 5). This result agrees with Lipson *et al.* (2006), who also found glucose-induced SIR to be the best parameter reflecting the effect of elevated CO_2. We suppose that higher CO_2 levels stimulate rhizodeposition with a more uniform composition and high carbohydrate content; this benefits the development of r-strategists, whose respiration response is more distinct upon glucose amendment. Thus, the shift in specific growth rates reflecting the ratio between r- and K-strategists depends on the choice of test substrate. This r–K ratio can be better revealed by studying growth on simple than on rich substrate mixtures.

Conclusions

The significant increase of specific microbial growth rates was observed in three CO_2 enrichment experiments with different soils, plants, CO_2 levels, and N fertilization. This shift was probably mediated by increased rhizodeposition under elevated CO_2, resulting in a higher input of easily available substrates into the rhizosphere. N fertilization affects microbial growth rates both directly and through the modification of root biomass and thus, exudation amount. This bidirectional N influence may stimulate or counterbalance effect of elevated CO_2 depending on plant type, vegetation stage and general nutrient limitation. Thus, the functional characteristics of the soil microbial community (microbial growth and substrate mineralization rates) – rather than total microbial biomass amount – are sensitive to higher CO_2 concentrations in the atmosphere.

Elevated CO_2 decreased metabolic diversity and auxotrophy requirements of the soil microbial community. The higher (20 to 60%) specific growth rates reflect faster turnover of soil microorganisms under elevated CO_2. We therefore explain the absence of soil organic C and microbial biomass accumulation in soil under elevated CO_2 (as might be expected because of higher C input into the rhizosphere) by faster C mineralization, confirmed by the faster turnover rates of soil microorganisms.

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