RESEARCH ARTICLE

Stimulation of *r*- vs. *K*-selected microorganisms by elevated atmospheric CO_2 depends on soil aggregate size

Maxim Dorodnikov[1,2], Evgenia Blagodatskaya[1,3], Sergey Blagodatsky[1,3], Andreas Fangmeier[2] & Yakov Kuzyakov[1]

[1]Department of Agroecosystem Research, University of Bayreuth, Bayreuth, Germany; [2]Institute of Landscape and Plant Ecology (320), University of Hohenheim, Stuttgart, Germany; and [3]Institute of Physico-Chemical and Biological Problems in Soil Science, RAS, Puschino, Russia

Correspondence: Sergey Blagodatsky, Institute of Physico-Chemical and Biological Problems in Soil Science, RAS, Institutskaya 2, Puschino 142290, Russia. Tel.: +74 96 77 30 840; fax: +74 96 73 30 595; e-mail: sblag@mail.ru

Present address: Maxim Dorodnikov, Institute of Botany and Landscape Ecology, University of Greifswald, Grimmer Str. 88, 17487 Greifswald, Germany

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Abstract

Increased root exudation under elevated atmospheric CO_2 and the contrasting environments in soil macro- and microaggregates could affect microbial growth strategies. We investigated the effect of elevated CO_2 on the contribution of fast- (*r*-strategists) and slow-growing (*K*-strategists) microorganisms in soil macro- and microaggregates. We fractionated the bulk soil from the ambient and elevated (for 5 years) CO_2 treatments of FACE-Hohenheim (Stuttgart) into large macro- (> 2 mm), small macro- (0.25–2.00 mm), and microaggregates (< 0.25 mm) using 'optimal moist' sieving. Microbial biomass (C_{mic}), the maximum specific growth rate (μ), growing microbial biomass (GMB) and lag-period (t_{lag}) were estimated by the kinetics of CO_2 emission from bulk soil and aggregates amended with glucose and nutrients. Although C_{org} and C_{mic} were unaffected by elevated CO_2, μ values were significantly higher under elevated than ambient CO_2 for bulk soil, small macroaggregates, and microaggregates. Substrate-induced respiratory response increased with decreasing aggregate size under both CO_2 treatments. Based on changes in μ, GMB and lag period, we conclude that elevated atmospheric CO_2 stimulated the *r*-selected microorganisms, especially in soil microaggregates. Such an increase in *r*-selected microorganisms indicates acceleration of available C mineralization in soil, which may counterbalance the additional C input by roots in soils in a future elevated atmospheric CO_2 environment.

Introduction

Increased concentrations of atmospheric CO_2 have a pronounced effect on terrestrial plants, resulting in enhanced carbon (C) assimilation (Melillo *et al.*, 1993; Schimel, 1995). Along with the higher net primary productivity of plants (Melillo *et al.*, 1993; Schimel, 1995) or changes in plant chemistry (Cotrufo *et al.*, 1994; Henry *et al.*, 2005; Wiesenberg *et al.*, 2008) under elevated CO_2, stimulation of root growth (Arnone & Korner, 1995; Paterson *et al.*, 1997; Rogers *et al.*, 1998; Hamilton *et al.*, 2002) will affect many belowground processes. Increased root growth should enhance rhizodeposition, including root exudation (van Veen *et al.*, 1991; Cheng, 1999; Phillips *et al.*, 2006). In turn, root exudates could stimulate the activity and growth of the soil microbial biomass (Korner & Arnone, 1992) because of the increased availability of such substrates for decomposition by soil microorganisms (reviewed by Kuzyakov, 2002). However, the reviews by Zak *et al.* (2000) and Freeman *et al.* (2004) stated that the response of soil microorganisms to elevated CO_2 is highly variable, regardless of whether the response is measured as total biomass, microbial activity, or microbial transformations of N. The reported inconsistencies in the effects of elevated atmospheric CO_2 on soil organic matter turnover could reflect various factors, one of which, soil aggregation, might play the key role because (1) the accessibility of plant residues for microbial decomposition can be decreased by occlusion in aggregates (reviewed in Jastrow *et al.*, 2007) and (2) aggregates of different sizes could provide contrasting environments for microorganisms (Chenu *et al.*, 2001). Thus, fungi were found to occupy macroaggregates (> 250-μm diameter) because

their hyphae could not penetrate small pores of soil microaggregates (Gupta & Germida, 1988; Guggenberger *et al.*, 1999; De Gryze *et al.*, 2005). In turn, the location of bacteria in small pores ($< 10\,\mu m$) or within aggregates enhances the survival of microorganisms by protecting them from predation (Vargas & Hattori, 1986; Postma & Van Veen, 1990; Wright *et al.*, 1993) and desiccation (Nishiyama *et al.*, 1992).

The combined effects of elevated atmospheric CO_2 and soil macro- and microaggregates on the activity and structure of soil microbial communities have been investigated in only two studies, which used phospholipid fatty acid (PLFA) profiles or enzyme activity assays (Eviner & Chapin, 2002; Niklaus *et al.*, 2007). Neither study reported significant effects of elevated CO_2 and soil aggregation on the composition and activity of the soil microbial biomass.

Rather than using PLFA profiles or enzyme activity to understand how elevated CO_2 and soil aggregation affect the microbial community, the current study used the substrate-induced growth respiration (SIGR) method (Panikov, 1995; Stenström *et al.*, 2001). SIGR characterizes growth rates of the soil microbial community on available substrate revealing the growth strategy of soil microorganisms (Andrews & Harris, 1986). The *r*-strategists grow rapidly on easily available substrates, whereas the *K*-strategists use the resources slowly but more efficiently, even in the absence of limitation (Pianka, 1970). *K*- and *r*-strategists can be differentiated by their maximum specific growth rate under conditions with excess substrate (Andrews & Harris, 1986). Because both types of microorganisms, *r*- and *K*-strategists, are abundant in soil, changes in growth rates of the whole microbial community after amendment with easily available substrates can reflect the shift toward *r* or *K* types (Blagodatskaya *et al.*, 2007).

In a previous study, the microbial growth strategies were determined for bulk soil under agricultural crops (winter wheat and sugar beet) exposed to elevated CO_2 for 3–4 years using free air CO_2 enrichment (FACE, Braunschweig) (Blagodatsky *et al.*, 2006). In that study, the microbial community contained a higher proportion of *r*-strategists under elevated atmospheric CO_2 than under ambient atmospheric CO_2. Because of these promising results, we chose to focus on microbial growth strategies to investigate the effects of elevated CO_2 on the relative proportions of *r*- vs. *K*-strategists in microbial communities associated with soil macro- and microaggregates. Two main questions were addressed. (1) Does elevated CO_2, as mediated by increased C rhizodeposition of plants (van Veen *et al.*, 1991; Cheng, 1999; Phillips *et al.*, 2006), select for *r*- vs. *K*-strategists? (2) Do soil aggregates of different sizes affect the selection for microorganisms with *r*- or *K*-strategy?

Materials and methods

Study site

Soil samples were taken from the FACE facility in Stuttgart-Hohenheim, Baden-Wuerttemberg, Germany (48°43′N latitude, 9°13′E longitude). The FACE experiment started in 2002 and included plots with an elevated level of atmospheric CO_2 ($0.54\,mL\,L^{-1}$, with enclosures), an ambient level of CO_2 ($0.38\,mL\,L^{-1}$, with enclosures), and control plots (ambient CO_2 level, no enclosures) (Erbs & Fangmeier, 2006). Each treatment was replicated five times. Spring wheat (*Triticum aestivum* cv. Triso) was planted annually in the plots from 2002 to 2006. In 2007, oilseed rape (*Brassica napus*) was grown in the plots for the first time. Soil was tilled in spring before crop sowing. Beginning in 2003, inorganic NPK fertilizers were applied ($140\,kg\,ha^{-1}$ N, $60\,kg\,ha^{-1}$ K, and $30\,kg\,ha^{-1}$ P) to each plot under ambient and elevated CO_2; no organic fertilizers were applied. The soil is a Gleyic Cambisol (FAO, 1998) without $CaCO_3$. Soil properties under ambient and elevated CO_2 were identical: 9% sand, 69% silt, 22% clay; pH 6.8; bulk density (0–10 cm) $1.1\,g\,cm^{-3}$; C_{org} 1.59%; N_{tot} 0.17%; C : N ratio 9 : 1.

Aggregate size fractionation at optimal soil moisture

The top 10 cm of soil was sampled from each ambient and elevated CO_2 plot using soil corers (inner diameter: 5 cm) in September 2007, 3 weeks after rape harvest. Soil samples were stored at 7 °C for 1 week before aggregate size fractionation. Aggregates were isolated according to Kristiansen *et al.* (2006). Soil samples were placed in a ventilation box (room temperature 22 °C) and spread out in a thin layer. The soil was dried to a moisture level such that limited mechanical stress would induce maximum brittle failure of aggregates along natural planes of weakness. When individual soil clods had reached the desired condition, they were gently crumbled by hand to < 8 mm. The resulting aggregates were size separated by a brief sieving procedure. Portions (300 g) were transferred to a nest of sieves (2.00- and 0.25-mm pore diameters) and shaken three times for 2 min. All visible roots and stones were removed and aggregates > 2 mm (large macroaggregates) were collected. The same procedure was performed for the material retained on the 0.25-mm sieve to obtain the aggregate class of 2.00–0.25 mm (small macroaggregates). The material that passed through the 0.25-mm sieve was identified as aggregate class < 0.25 mm (microaggregates). Preliminary tests showed that the sieving duration was sufficient to separate the various aggregate size classes quantitatively while minimizing aggregate abrasion. Because of the low sand content (9%) no correction for sand was necessary.

Microbial biomass and the kinetics of substrate-induced respiration

Soil microbial biomass (C_{mic}) and the kinetic parameters of microbial growth response were measured in subsamples of bulk soil and of the three aggregate classes; the soil water content was 20% (1 g water per 100 g dry soil) for bulk soil and large and small macroaggregates and 25% for microaggregates, which constituted about 60% of the water-holding capacity of the soil. This value corresponded to an optimal level for microbial respiration activity as determined in preliminary experiments. Soil samples were preincubated for 24 h at room temperature before measurements.

The dynamics of the CO_2 emission from soil amended with glucose and nutrients (Blagodatsky *et al.*, 2000) was used for estimation of C_{mic} and the kinetic parameters of microbial growth. A soil sample (12.0–12.5 g wet weight, equivalent to 10 g dry soil) was amended with a powder mixture containing glucose (10 mg g^{-1}), talcum (20 mg g^{-1}), 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}. Substrate concentrations sufficient for unlimited exponential growth of microorganisms were estimated in preliminary experiments in which different amounts of glucose were added. The amount of mineral salts was selected so that the substrate changed the pH of soil to < 0.1 (Blagodatskaya *et al.*, 2007). After addition of the glucose–talcum mixture and mixing with a common, handheld kitchen blender, the soil samples were immediately placed into 24 plastic, 50-mL tubes and the rate of CO_2 production was measured. Each sample was continuously aerated (100 mL min^{-1}) at 22 °C and the evolved CO_2 was measured every 1.5 h using an infrared detector (Gas Exchange Measurement System 2250, ADC, UK) connected to the gas handling unit with a flowmeter (ADC).

Calculation of microbial biomass and growth parameters

Soil respiration was used to calculate C_{mic} and the following kinetic parameters: the specific growth rate of microorganisms (μ), the microbial biomass capable for immediate growth on glucose (x_0'), physiological state index of microbial biomass before substrate addition (r_0), the total microbial biomass (x_0) responding by respiration to glucose addition, and the lag period (t_{lag}).

C_{mic} was calculated according to the equation of Anderson & Domsch (1978) using initial substrate-induced respiration (SIR) rate (vCO_2) and the conversion factor of 30 suggested by Kaiser *et al.* (1992):

$$C_{mic}\ (\mu g\, g^{-1}\ soil) = 30 vCO_2\ (\mu L\, g^{-1}\ soil\, h^{-1}) \quad (1)$$

The change in CO_2 production (p) with time (t) in soil amended with glucose and nutrients was calculated as follows:

$$\frac{dp}{dt} = r\,(Q - Q')x + Q'x \quad (2)$$

where x and r are the current values for microbial biomass and activity status, respectively. The first term on the right-hand side represents respiration coupled with growth and the second term is respiration uncoupled from growth. According to Panikov (1995), the specific respiration activity measured under excess substrate is denoted as Q, and the cyanide-resistant fraction as Q'; the productive fraction of the total respiration is then equal to $Q - Q'$. The dynamics of biomass is described by:

$$\frac{dx}{dt} = \mu\, rx \quad (3)$$

and the dynamics of the r-variable is:

$$\frac{dr}{dt} = \mu r\,(1 - r) \quad (4)$$

Equations 3 and 4 were integrated for initial conditions, i.e. $x = x_0$ and $r = r_0$ at $t = 0$ (Panikov & Sizova, 1996; Blagodatsky *et al.*, 2000), and the CO_2 production rate was derived as an explicit function of time after substitution of x and r into Eqn. (2):

$$CO_2(t) = A + B\exp(\mu t) \quad (5)$$

where A (uncoupled respiration) $= (1 - r_0)Q'x_0$, B (coupled respiration) $= r_0 Q x_0$, and $\mu = Y_{CO_2}(Q - Q')$. Y_{CO_2} is yield of biomass C per unit of respired C-CO_2, assumed to be constant during the experiment and equal to 1.1. The parameters of Eqn. (5) were fitted by minimizing the least-square sum using MODEL MAKER-3 software (SB Technology Ltd). Fitting was restricted to the part of the curve that corresponded to unlimited exponential growth, as indicated by maximum values of r, F, and Q statistic criteria (20–22 h).

r_0 was calculated from the ratio of $A : B$ (Panikov & Sizova, 1996):

$$r_0 = \frac{B(1 - \lambda)}{A + B(1 - \lambda)} \quad (6)$$

where $\lambda = (Q - Q')/Q$ and may be accepted as a basic stoichiometric constant = 0.9 (Panikov & Sizova, 1996). The total glucose-metabolizing microbial biomass (sustaining + growing; x_0) was calculated as following:

$$x_0 = \frac{B\lambda Y_{CO_2}}{r_0 \mu} \quad (7)$$

The growing microbial biomass (GMB, x_0') was calculated using the equation:

$$x_0' = x_0 r_0 \quad (8)$$

The lag period (t_{lag}) was determined as the time interval from substrate amendment to the moment when the increasing rate of growth-related respiration [$B\exp(\mu t)$] became as high as the rate of respiration uncoupled from the growth of microorganisms [A] [Eqn. (5)]. t_{lag} was calculated using the parameters of the approximated respiration curve by the following equation:

$$t_{lag} = \frac{\ln(A/B)}{\mu} \quad (9)$$

C_{mic} is expressed as the amount of C on an oven-dry soil weight basis (105 °C) per gram of bulk soil or isolated aggregates (C_{mic}, mg C g^{-1}). The abundance of C_{mic} in an aggregate fraction is shown as a percentage of the total C_{mic} in bulk soil. When compared with C_{mic} from bulk soil, the sum of C_{mic} calculated as the weight distributions of aggregate size classes indicated the recovery or extent of any losses in C_{mic} due to fractionation of aggregates (Fig. 1). The study was conducted with samples of eight plots (eight independent replicates corresponded to four ambient CO_2 plots and four elevated CO_2 plots).

The effects of aggregate size and CO_2 level were assessed by two-way ANOVAs with size of aggregates and CO_2 treatment as independent factors. The Fischer LSD *post hoc* test was used to evaluate the significance of treatments at $P < 0.05$.

Results

Size distribution and C content of aggregate fractions

The sieving procedure fractionated the bulk soil into aggregate size classes as follows: large macroaggregates (> 2.00 mm), small macroaggregates (0.25–2.00 mm), and microaggregates (< 0.25 mm) representing 31–34%, 58–59%, and 8–10%, respectively, of the bulk soil mass (Fig. 1). Atmospheric CO_2 enrichment did not affect the size distribution of aggregates (Fig. 1). The C_{org} content in each aggregate class was similar to the C_{org} content of the bulk soil. The only difference in C_{org} content was in the aggregate fraction > 2.00 mm under elevated CO_2, which contained less C_{org} than the bulk soil or the 0.25–2.00-mm fraction under elevated CO_2 (Fig. 2, top).

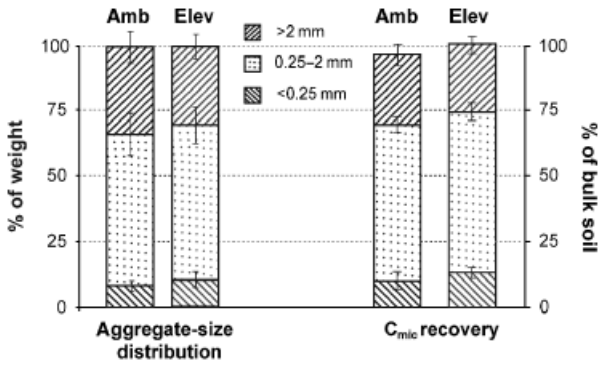

Fig. 1. Aggregate-size distribution (left Y axis) and microbial biomass (C_{mic}; right Y axis) in three aggregate size classes expressed as a percentage of total weight of bulk soil and the total C_{mic} under ambient and elevated CO_2.

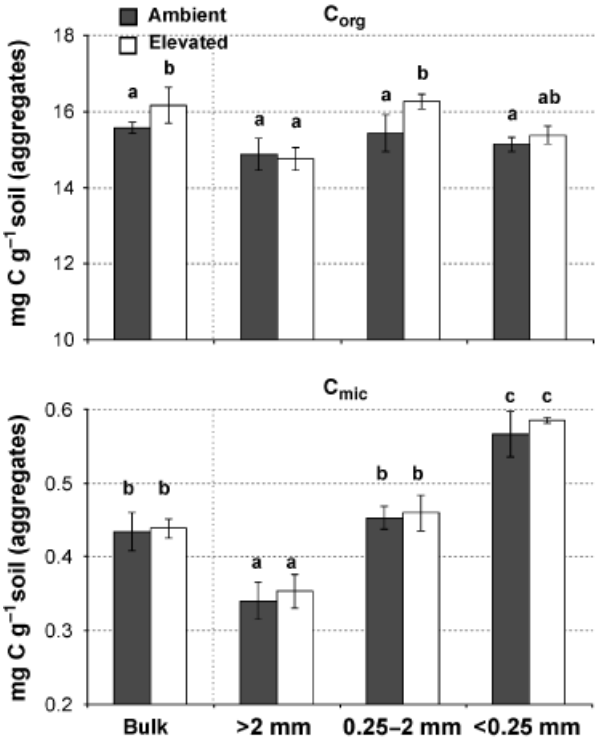

Fig. 2. Organic carbon content (C_{org}), and microbial biomass (C_{mic}) in bulk soil and three aggregate-size classes under ambient and elevated CO_2. Values are averages (± SE) of four replicates. Treatments followed by the same letters are not significantly different between aggregate size classes at $P \leq 0.05$. There were no significant differences between elevated and ambient CO_2 treatments.

Microbial biomass distribution

Elevated CO_2 tended to increase C_{mic} in bulk soil and in the three aggregate size classes, but this increase was not significant (Fig. 2, bottom). The content of C_{mic} significantly increased as aggregate size decreased. Thus, the smallest values of C_{mic} (mg C g^{-1}) were in large macroaggregates (0.340 ± 0.025 under ambient and 0.353 ± 0.023 under elevated CO_2), and the largest C_{mic} values were in microaggregates (0.566 ± 0.031 under ambient and 0.585 ± 0.004 under elevated CO_2) (Fig. 2, bottom).

The total C_{mic} in bulk soil was similar under ambient and elevated CO_2: 0.44 mg C g^{-1} (Fig 2, bottom). C_{mic} in

aggregate fractions was distributed similarly to the aggregate weight distribution, i.e. the largest value was in small macroaggregates and the lowest was in microaggregates (Fig. 1). The quality or efficiency of the fractionation procedure was estimated by the cumulative recovery of C_{mic} in the fractions. Thus, cumulative C_{mic} in aggregate fractions, expressed as a percentage of the C_{mic} in the bulk soil was 97.8% under ambient CO_2 and 100.2% under elevated CO_2 (Fig. 1).

Microbial growth kinetics

Respiration rate (RR)

The glucose and nutrient amendment increased microbial RR exponentially in bulk soil and in the three aggregate size classes under both ambient and elevated atmospheric CO_2, indicating microbial growth after the lag phase (Fig. 3). For the bulk soil, the increase in RR immediately after glucose amendment (due to increase in initial coupled respiration – *B*) was larger under elevated vs. ambient CO_2, but the difference was not significant (Table 1). The initial RR coupled with growth was higher for small macro- and microaggregates under ambient vs. elevated atmospheric CO_2 (Table 1). However, the steepness of the RR curve for aggregates was larger (leading to higher μ) under elevated CO_2 than under ambient CO_2. For soil aggregate size classes under both CO_2 treatments, the initial RR (both *A* and *B*) increased from macro- to microaggregates: large macroaggregates < small macroaggregates < microaggregates (Fig. 3, Table 1).

Specific growth rate

The specific growth rate (μ) of soil microorganisms was substantially affected by atmospheric CO_2 enrichment: μ values were significantly higher under elevated vs. ambient CO_2 for bulk soil, small macroaggregates and microaggregates. The difference in μ between elevated and ambient CO_2 was insignificant only in large macroaggregates (Fig. 4, top). According to the steepness of the respiration curves (Fig. 3), μ values increased more in the fractionated soil than in the bulk soil: bulk soil < large macroaggregates < small

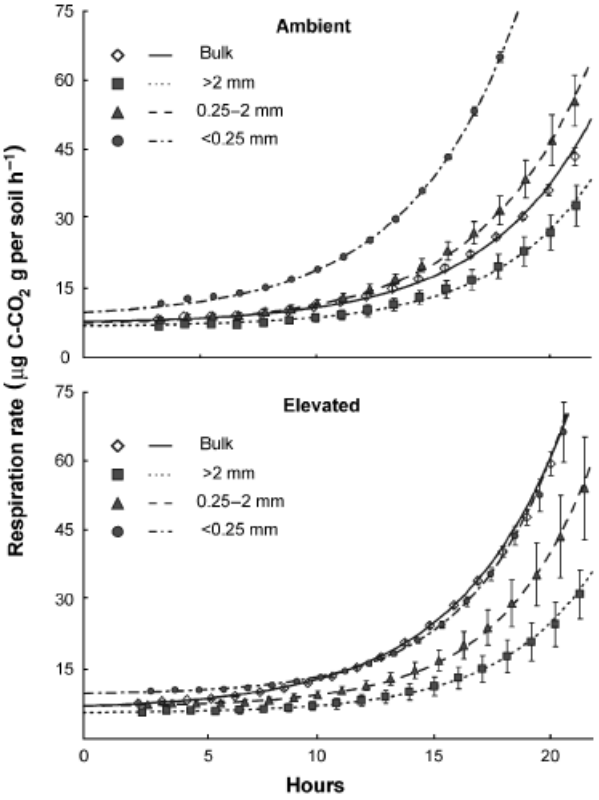

Fig. 3. Measured respiration rates (symbols) and values fitted according to Eq.5 (lines) after glucose and nutrients amendment of the bulk soil and three aggregate size classes under ambient (top) and elevated (bottom) CO_2. Values are averages (± SE) of four replicates.

Table 1. Model parameters optimized for respiration curves (Fig. 3) of bulk soil and three aggregate size classes under ambient (amb) and elevated (elev) CO_2 treatments

	Goodness of fit (r^2)		*F* values		Parameter value ± SE					
					Uncoupled respiration (*A*)* (μg C g^{-1} soil h^{-1})		Coupled respiration (*B*)* (μg C g^{-1} soil h^{-1})		Lag period (h)	
Aggregate-size classes/soil	Amb	Elev	Amb	Elev	Amb	Elev	Amb	Elev	Amb	Elev
> 2 mm	0.9979	0.9990	12330	7586	5.97 ± 0.77^{Aa}	5.71 ± 0.59^{Aa}	0.36 ± 0.12^{Aa}	0.20 ± 0.06^{Aa}	14.3 ± 2.9^{Ab}	15.8 ± 0.9^{Ab}
0.25–2 mm	0.9992	0.9994	8111	21483	6.92 ± 0.69^{Aa}	7.31 ± 0.44^{Ab}	0.61 ± 0.25^{Aa}	0.24 ± 0.01^{Ba}	12.0 ± 2.6^{Aab}	14.8 ± 0.6^{Ab}
< 0.25 mm	0.9998	0.9997	64738	25448	8.78 ± 0.85^{Ab}	10.02 ± 0.07^{Bc}	1.21 ± 0.30^{Ab}	0.30 ± 0.01^{Bb}	9.3 ± 1.3^{Aa}	14.5 ± 0.2^{Bb}
Bulk	0.9996	0.9994	29509	18347	7.04 ± 0.88^{Aab}	7.05 ± 0.22^{Ab}	0.61 ± 0.11^{Aa}	0.69 ± 0.01^{Ac}	12.7 ± 0.6^{Bb}	11.2 ± 0.1^{Aa}

Values are averages (± SE) of four replicates. Treatments followed by the same letters are not significantly different between elevated and ambient CO_2 (uppercase letters) and aggregate size classes (lowercase letters) at $P \leq 0.05$.

*Parameter values *A* and *B* are used in the Eqn. (5).

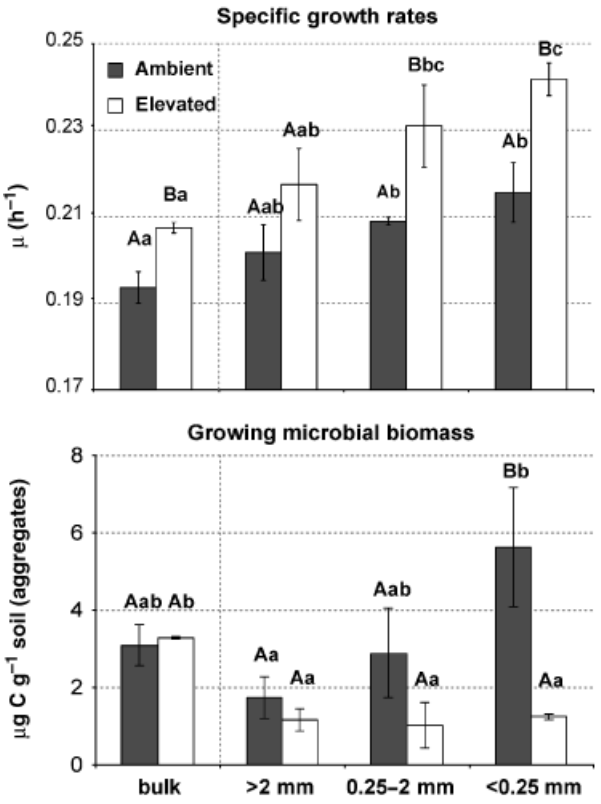

Fig. 4. Specific growth rates (top) and amount of growing microbial biomass (bottom) in bulk soil and three aggregate size classes under ambient and elevated CO_2. Values are averages ($\pm$ SE) of four replicates. Treatments followed by the same letters are not significantly different between elevated and ambient CO_2 (uppercase letters) and aggregate size classes (lowercase letters) at $P \leq 0.05$.

macroaggregates < microaggregates (Fig. 4, top). However, this increase in μ values from macro- to microaggregates was only significant between large macro- and microaggregates under elevated CO_2; the increase was not significant under ambient CO_2 (Fig. 4, top).

GMB

Microbial biomass capable of immediate growth on glucose [x_0', Eqn. (8) – GMB] in bulk soil (μg C g^{-1}) was not affected by atmospheric CO_2 enrichment (Fig. 4, bottom). In isolated soil aggregates, GMB was always lower under elevated vs. ambient CO_2. However, this pattern was significant only in microaggregates, where GMB content was 3.4-fold lower under elevated vs. ambient CO_2 (Fig. 4, bottom). GMB increased from 1.94 ± 0.27 to 5.63 ± 1.55 μg C g^{-1} as the aggregate size decreased under ambient CO_2. In turn, GMB content under elevated CO_2 was similar in all three aggregate size classes (Fig. 4, bottom).

Lag period (t_{lag})

According to the calculated t_{lag} (Eq. 9), microorganisms in bulk soil required significantly less time to start exponential growth after glucose amendment under elevated (11.0 h) than ambient (12.5 h) CO_2 (Table 1). Fractionation of the bulk soil into aggregate classes substantially increased t_{lag} to 14.5–15.0 h under elevated CO_2 and to 9–14 h under ambient CO_2 (Table 1). Values of t_{lag} in soil aggregates had different trends under ambient vs. elevated CO_2. While t_{lag} was similar in all aggregate size classes under elevated CO_2 (average of 14.7 h), t_{lag} under ambient CO_2 treatment became shorter (from 14 to 9 h) with decreasing aggregate size. However, the difference in t_{lag} between macro- and microaggregates under ambient CO_2 was not significant (Table 1).

Discussion

Distribution of microorganisms in soil aggregates

Some studies have reported that microbial biomass is greater in macro- vs. microaggregates (Chotte *et al.*, 1998; Guggenberger *et al.*, 1999) but other investigations have stated the opposite (Šantrůčková *et al.*, 1993; Schutter & Dick, 2002; Askin & Kizilkaya, 2006). These contrasting results could reflect the differences in aggregate separation by wet and dry sieving (discussed in details in Dorodnikov *et al.*, 2009). Like Kristiansen *et al.* (2006), we fragmented the soil into constituent aggregates when the water content of individual clods and peds was near the lower plastic limit, corresponding to the optimum water content of medium textured soils at which soil friability is maximal (Dexter & Bird, 2001). We thereby maximized brittle fracturing along natural planes of weakness while minimizing changes in aggregate size distribution (Kristiansen *et al.*, 2006). The procedure used is considerably different from conventional wet- and dry-sieving procedures, and we therefore propose that the procedure be termed *optimal moist sieving*. Because the procedure is gentle (Kristiansen *et al.*, 2006), it minimizes destruction of microorganisms in aggregate separates. Thus, fractionation of bulk soil by optimal moist sieving in our study maintained the microbial biomass: cumulative recovery of C_{mic} in aggregates vs. bulk soil was 97.8% under ambient CO_2 and 100.2% under elevated CO_2 (Fig. 1). Such a high recovery of microbial biomass in separated soil aggregates is one of the good indicators of the appropriateness of the method for soil microbiological studies. It is also important to emphasize that microbial biomass content presented in Fig. 2 (bottom) was estimated by SIR. It follows that the fractionation procedure did not change the cumulative substrate-induced respiration of microbial biomass in

aggregate separates as compared with the bulk soil. This was a prerequisite for application of the SIR approach to evaluate the activity parameters of microbial biomass. Further studies are required, however, to compare traditional aggregate fractionation methods with optimal moist sieving separation and to link results with response of soil functions other than SIGR.

Higher microbial biomass in micro- than in macroaggregates (Fig. 2, bottom) in our study could be explained by the contrasting environments of micro- vs. macroaggregates. The location of microorganisms within the soil matrix is a key factor affecting their survival (Chenu *et al.*, 2001). Small pores (micropores, $< 10\,\mu m$) dominate in microaggregates, whereas both small and large pores (macropores, $> 10\,\mu m$) occur in macroaggregates (Jastrow *et al.*, 2007; Paul, 2007). Micropores, in turn, enhance survival by protecting microorganisms from predation by protozoa and soil animals (Vargas & Hattori, 1986; Postma & van Veen, 1990; Wright *et al.*, 1993) or from desiccation (Nishiyama *et al.*, 1992).

The diffusion of dissolved glucose goes faster than microbial growth and respiration: under conditions of our experiment we observed 9–16-h lag phase, while dissolving of glucose and its subsequent diffusion takes minutes. We estimated relative concentration gradient for glucose using Eqn. (3) of Darrah (1991) for our experimental conditions. If impedance factor (f_L) for silty soil from Hohenheim with sufficient water content (60% of WHC) is equal to 0.15 (So & Nye, 1989), the half of the applied glucose will diffuse in 1-mm distance in 1 h. That is why only differences in microbial properties affect the respiration parameters rather than diffusion of glucose in soil. Formerly, we compared the modifications of SIGR method with different glucose application techniques: namely as a solution added dropwise to the fine soil layer or in powder. Both modifications led to similar results (Blagodatsky *et al.*, 2000).

Effect of elevated CO_2 on the microbial biomass content

In other studies, elevated CO_2 has either increased soil microbial biomass (e.g. Zak *et al.*, 1993; Mikan *et al.*, 2000; Drissner *et al.*, 2007) or had no effect on soil microbial biomass (e.g. Kampichler *et al.*, 1998; Niklaus *et al.*, 2003). Our results (Fig. 2, bottom) support numerous studies that failed to find a significant response of microbial biomass to elevated CO_2. Moreover, we observed no changes in total microbial biomass under elevated vs. ambient CO_2 in isolated soil aggregates (Fig. 2, bottom; Fig. 1, right). This indicates that the effect of atmospheric CO_2 enrichment on allocation of microorganisms in soil macro- and microaggregates is negligible, at least when intensively managed agroecosystems are considered. We therefore conclude that total microbial biomass content is insensitive to elevated CO_2 and its estimation is not helpful in detecting the effects of elevated CO_2.

Effect of aggregate size on microbial growth strategies

The analysis of microbial respiratory response to the amendment of bulk soil and isolated aggregates with easily available substrate showed the pronounced effect of aggregate size on characteristics of microbial community. Thus, RRs increased with decreasing soil aggregate size (Fig. 3, Table 1). Similar but not statistically significant patterns were found for specific growth rates (Fig. 4, top). These results indicate that fast- vs. slow-growing microorganisms contribute more of the total microbial community in soil microaggregates than in macroaggregates.

A number of studies have indicated that the structure and functions of soil microbial community vary between different aggregate size classes (Gupta & Germida, 1988; Guggenberger *et al.*, 1999; Schutter & Dick, 2002; Väisänen *et al.*, 2005). Thus, Schutter & Dick (2002) reported higher microbial activity in micro- vs. macroaggregates, whereas Väisänen *et al.* (2005) reported the opposite: microbial activity was greater in large (> 2 mm) and small macroaggregates (0.25–2.00 mm) than in microaggregates (0.053–0.250 mm). Such inconsistencies could again be explained by the use of different aggregate fractionation methods, i.e. wet vs. dry sieving. The studies reporting higher microbial activity in macro- than in microaggregates used wet sieving (Elliot, 1986; Franzluebbers & Arshad, 1997; Väisänen *et al.*, 2005), while those reporting higher microbial biomass and activity in microaggregates used dry sieving (Seech & Beauchamp, 1988; Mendes *et al.*, 1999; Schutter & Dick, 2002). Because both dry and wet sieving techniques could change water potential and gas diffusion in soil, which, in turn, affect the microbial activity, the direct comparison of the results mentioned in the studies above and reported here must be conducted with care.

Effect of elevated CO_2 on microbial growth strategies

Although the initial RRs after addition of glucose to aggregates indicated no significant effect of atmospheric CO_2 enrichment (Fig. 3), the kinetic parameters of microbial growth – specific growth rate (Fig. 4, top), GMB (Fig. 4, bottom, bulk soil), and the duration of lag period (Table 1, bulk soil) – revealed the shift in growth strategy of soil microorganisms induced by the indirect effect of elevated CO_2. This shift in growth strategy of the total microbial community to *r*-selected species was most probably mediated by increasing plant rhizodeposition stimulated by elevated CO_2 (van Veen *et al.*, 1991; Paterson *et al.*, 1997; Rogers *et al.*, 1998; Cheng, 1999). In turn, the increasing role

of *r*-strategists in the total soil microbial community under elevated atmospheric CO_2 indicates acceleration of available C mineralization in soil, which may counterbalance the additional C input under elevated CO_2 in terrestrial ecosystems.

The results of this study agree with results from the FACE facility at Braunschweig, where the microbial growth strategies were determined for bulk soils under winter wheat and sugar beet exposed for 3–4 years to elevated CO_2 (Blagodatsky *et al.*, 2006). In this earlier FACE study, elevated CO_2 stimulated the microbial community and increased the contribution of *r*-strategists. These findings also agree with those reported by Hodge *et al.* (1998), who used Biolog MicroPlate assays and found increases numbers of cultivatable bacteria and high rates of substrate mineralization in soil under ryegrass exposed to elevated atmospheric CO_2. Lipson *et al.* (2006) showed that glucose SIGR was essentially the only variable that had a consistent and significant direct effect of elevated atmospheric CO_2 when they measured a number of soil process rates, including salicylate, glutamate, and glycine substrate-induced respiration and proteolysis, as well as microbial community structure based on 16S rRNA gene libraries. Direct comparison of total (DNA-based) and active (RNA-based) bacterial communities (Jossi *et al.*, 2006) showed that increased pCO_2 mainly influenced active and root-associated bacterial components. The measurement of specific growth rates of microorganisms responding to substrate addition can, therefore, serve as a robust and simple method for quantitative estimation of changes in the whole microbial community caused by environmental changes, including increased pCO_2.

The relative fraction of microbial biomass capable of immediate growth on glucose (GMB) estimated in our study as 0.2–1% from total microbial biomass is consistent with the view that only about 1% of soil biomass can be readily cultured/grow on substrates such as glucose. In contrast to the bulk soil, the fractionated soil aggregates contained a smaller quantity of GMB and had a longer lag period, which was especially pronounced under elevated CO_2 (Fig. 4, bottom). We explain such a decrease in GMB and increase in the lag-period by high sensitivity of *r*-strategists to the physical impact of sieving (shaking). However, even with the decrease of GMB in the three aggregate size classes, the relative contribution of *r*- vs. *K*-strategists to GMB remained higher under elevated CO_2 than under ambient CO_2 as demonstrated by the higher specific growth rates.

Lastly, we should acknowledge that observed differences in specific growth rates are just an indirect evidence for the shift in *r*- and *K*-continuum of soil microbial community. Hence, our investigation is not a final point, but rather an interesting perspective for future studies, which could combine kinetic respiration analysis and RNA-based estimations of microbial community structure. By these means, linking of microbial community structure and functions devised as a challenge in microbial ecology (Paul, 2007) will be approached.

Conclusions

Fractionation of soil samples at optimal moisture satisfactorily separated the bulk soil into constituent micro- and macroaggregates. As demonstrated by high recovery of microbial biomass and its respiratory activity, this fractionation technique had minimal impact on soil microorganisms. Sizes of aggregates had a pronounced effect on the abundance and characteristics of the microbial community: (1) the abundance of microbial biomass per soil mass unit increased with decreasing aggregate size and (2) fast growing microorganisms constituted more of the microbial community in microaggregates than in macroaggregates.

Estimation of GMB and kinetics of substrate-induced respiration in bulk soil and aggregate fractions after 5 years of CO_2 enrichment leads to the following conclusions:

(**1**) The kinetics of the microbial respiratory response to amendment of soil with a readily available substrate is an effective tool for describing the shift of soil microbial biomass with environmental changes. In contrast to total microbial biomass content, the kinetic characteristics of soil microorganisms (maximum specific growth rate, GMB, and duration of lag period) were sensitive to changes in CO_2 and are therefore useful for characterizing the changes in soil microbial community induced by elevated CO_2.

(**2**) Elevated atmospheric CO_2 stimulated *r*-selected soil microorganisms presumably because of increased inputs of root-derived, easily decomposable organic substrates. The increased activity of *r*-strategy microorganisms under elevated atmospheric CO_2 was especially pronounced in soil microaggregates (< 0.25 mm).

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