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15 Effects of different levels of atmospheric CO_2 and N fertilization on soil microbial biomass: Kinetics of microbial growth of bulk soil and rhizosphere microorganisms

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Abstract

Kinetics of substrate induced respiration were investigated in bulk and rhizosphere soils from a field experiment with atmospheric CO_2 enrichment (FACE). Only some of the cases studied showed an increase of total microbial biomass (maximum 23 %) in CO_2-fumigated plots. The size of the total microbial biomass was mainly dependent on the proximity of the organisms to the roots (rhizosphere effect) and to a lesser extent on the CO_2 level. However, the specific growth rates (μ_{max}) of soil and rhizosphere microorganisms were higher for plots with elevated CO_2 concentrations in most of the cases. A three-way ANOVA showed that among the three independent factors studied, the CO_2 level had the greatest impact on μ_{max}. Effect of the rate of N application on μ_{max} was inconsistent for soils under sugar beets and winter wheat. The observed increases in microbial specific growth rates are probably connected to the higher input of root exudates from plants grown under elevated CO_2. Microbial communities adapted to the higher level of carbonaceous substrates and species with faster growth rates (r-strategists) have an advantage under these conditions.

Zusammenfassung

Auswirkungen unterschiedlicher atmosphärischer CO_2-Konzentrationen und N Gaben auf die mikrobielle Biomasse: Kinetik des mikrobiellen Wachstums von Boden- und Rhizosphären-Mikroorganismen

Es wurden Wachstumskinetiken mittels der Substrat-induzierten Respiration (SIR) von Boden- und Rhizosphären-Mikroorganismen aus einem Feldexperiment unter atmosphärischer CO_2-Anreicherung (FACE) verglichen. Nur einige der untersuchten Fälle zeigten Zuwächse in der mikrobiellen Biomasse (bis zu 23 %) unter erhöhter CO_2-Begasung. Der Zuwachs der Gesamtbiomasse war hauptsächlich von der Nähe der Mikroorganismen zu den Wurzeln (Rhizosphäreneffekt) und weniger von der CO_2 Konzentration abhängig. Die spezifische Wachstumsrate (Zuwachs pro Biomasseeinheit und Zeit, μ_{max}) war für Boden- und Rhizosphärenorganismen unter erhöhter CO_2 Konzentration in den meisten Fällen schneller. Eine 3-faktorielle ANOVA zeigte, dass unter den untersuchten drei unabhängigen Variablen die erhöhte CO_2 Konzentration den größten Einfluss auf μ_{max} ausübte, während die Höhe der N Applikation oder die Nähe zur Rhizosphäre keinen konstanten Einfluss auf μ_{max} hatten. Die beobachtete Zunahme in der Wachstumsrate von Mikroorganismen wird mit der Zunahme von Wurzelexudaten von Pflanzen unter erhöhter CO_2-Begasung erklärt. Mikrobielle Gemeinschaften, die sich an hohe Kohlenstoff (C)-haltige Substrate adaptiert haben und Organismen mit schnellerem Wachstum (r-Strategen) sind unter diesen Bedingungen im Vorteil.

15.1 Introduction

It is a well-established fact that plant productivity will increase under elevated CO_2. Subsequent increases in the carbon flow entering the soil and changes in root-exudate composition, together with other organic debris available to microbial degradation, may affect the mineralization activity of the soil microbial com-

munity [MONTEALEGRE *et al.*, 2002]. In turn, this activity, which governs the resultant CO_2 flux from soil into the atmosphere, is dependent on the supply of nitrogen and other nutrients to the soil. Nitrogen availability as well, controls the increase in phytomass caused by elevation of the CO_2 concentration in the atmosphere. The increase of root mass under elevated CO_2 was presented only in variants with high N-content in soil [PREGITZER *et al.*, 2000]. That is, mineralization activity of microorganisms under elevated CO_2 in the atmosphere is directly dependent on both interactions: the plant community and the nitrogen supply of soils. Opposite reactions of these factors apparently cause the discrepancies in results observed in the relevant literature and difficulties in investigating the effect of elevated CO_2 on microbial activity and mineralization processes in soil. It was shown that an increase of CO_2 evolution rates from soil under elevated carbon dioxide content in the atmosphere is substantially due to an increase of root productivity [LUO *et al.*, 1996; ROUHIER *et al.*, 1996; HUNGATE *et al.*, 1997; PREGITZER *et al.*, 2000]. It is still unclear what part the soil microorganisms play under CO_2 enhancement. In root-free soil, however, an increase of microbial respiration was found under elevated CO_2 [ROSS *et al.*, 1995; INSAM *et al.*, 1999]. A build-up of CO_2 concentration in the atmosphere caused a rise as well a decrease of soil microbial biomass [HUNGATE *et al.*, 1996; KANDELER *et al.*, 1998]. It is unclear, however, what factors were responsible for these contradictory reactions. Also, it remains unknown in what manner the composition and the functions of soil microbial communities are changed in response to an increased quantity and availability of organic substrates formed under elevated [ZAK *et al.*, 2000]. Results of investigations on the effect of elevated CO_2 on the mineralization of soil organic matter are also contradictory (HODGE *et al.*, 1998; BALL *et al.*, 2000; VAN GINKEL *et al.*, 2000; SOWERBY *et al.*, 2000). It was found that roots, when formed under elevated CO_2, decomposed more slowly compared to fully grown roots under normal conditions (GORISSEN *et al.*, 1995; VAN GINKEL *et al.*, 2000). This phenomenon, however, has been found in greenhouse experiments and was absent in experiments under open-air CO_2 enrichment (NORBY *et al.*, 2001). Results obtained in field experiments will therefore have a greater significance.

Our goal was to investigate the long-term effects of an elevated concentration of CO_2 in the atmosphere on microbial biomass and specific growth rates of microbial communities of soil and rhizosphere in a field experiment with different levels of nitrogen fertilization. This presentation is an extract of a paper presented at EUROSOIL: Freiburg / Breisgau in September 2004 (BLAGODATSKY *et al.*, 2004)

15.2 Materials and methods

Investigations were carried out on plots under ambient (350 ppm) and elevated (550 ppm) atmospheric CO_2 concentrations in a field experiment at the Institute of Agroecology (FAL, Braunschweig). An automated Free Air Carbon Dioxide Enrichment (FACE) system was used both on control and CO_2 amended plots [Weigel & Dämmgen, 2000]. The system preserves the relevant atmospheric exchange conditions and is a suitable means for simulating future CO_2 study scenarios. Soil samples for analysis were taken in September 2001 and August 2002 when sugar beets and winter wheat were grown in crop rotation, i.e., after 3-4 years since the beginning of the fumigation experiments with elevated CO_2. Two N treatments were included:

a) N100 = conventional N amendments according to local practices
b) N50 = N supply reduced to 50 % of a).

The soil type is a Cambisol / loamy sand of pH (KCl) 6.7, with ~ 1.1 % of organic C and 0.9 % of organic N.

Mean soil samples were taken from the layer 0-10 cm during harvesting time (sugar beets) or immediately after the cropping of the winter wheat. Rhizosphere soil was taken from locations that adhered to the plant roots, whereas bulk (non-rhizosphere) soil was taken from the space between rows of crops. Soil was stored field-fresh in aerated polyethylene bags for a maximum of 8 weeks at 4 °C. Prior to analysis, samples were sieved (<2 mm), soil was separated from fine roots and other plant debris and pre-incubated for 24 h at 22 °C. Soil moisture was adjusted to 60 % WHC.

Specific growth rates of soil microorganisms were determined using the substrate induced growth response expressed as increase of respiration rates. The dynamics of the CO_2 emission rate was recorded after soil amendment with glucose. Samples of 10 g (dry weight) soil were amended with a powder-mixture 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} $MgSO_4 \bullet 7H_2O$ – 3.8 mg g^{-1}. Optimal concentrations of glucose were found in preliminary experiments, so that soil amendments were sufficient to provide an unlimited exponential growth of soil microorganisms during the initial period of time.

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 glucose according to the equation by ANDERSON & DOMSCH (1978):

$$C_{mic}\ (\mu g \bullet g^{-1}\ soil) = (\mu l\ CO_2 \bullet\ g^{-1}\ soil \bullet h^{-1}) \times 40.04 \quad (1)$$

Specific maximal growth rate (μ_m) was determined by best-fitting the experimental data on CO_2 evolution rate (ν) to the equation:

$$\nu(t) = A + B * \exp(\mu_m * t) \quad (2)$$

where A = initial rate of uncoupled (non-growth) respiration,
B = initial rate of coupled (growth) respiration,
t = time (BLAGODATSKY *et al.*, 2000; PANIKOV & SIZOVA 1996)

The parameters of Equation 2 were determined by least-square fitting of the experimental data using the Model Maker software (SB Technology Ltd.). The approximation was restricted to the part of the curve corresponding to an unlimited exponential growth which was indicated by maximal values of Q and r statistic criteria.

Three way completely randomized ANOVA were applied in order to characterize the effect of following factors: ambient versus elevated CO_2 (CO_2); rhizosphere versus non-rhizosphere soil (Rhi) and different N amendments (N). When significant effects were found a multiple comparison using Student-Newman-Keuls test ($P < 0.05$) or Duncan's Multiple Range test (significance level 5 %) were performed. All variables treated passed normality and equal variance tests.

15.3 Results and discussion

Mean values of SIR-determined microbial biomass were higher under elevated CO_2 for soil under sugar beets in 2001 (**Figure 1**). This increase was significant, however, only in two cases and comprised 23 % of the control. This result was confirmed only as a trend in soil under winter wheat in 2002: in all cases differences between microbial biomass under elevated and ambient CO_2 were not significant. The effect of elevated CO_2 in the atmosphere on biomass and the activity of soil microorganisms is noteworthy even if it is only a tendency, since in most of the investigations, the increase of biomass and/or activity of microorganisms has been noted under elevated CO_2, but the extent of such effects varies to a great extent [ZAK *et al.*, 2000].

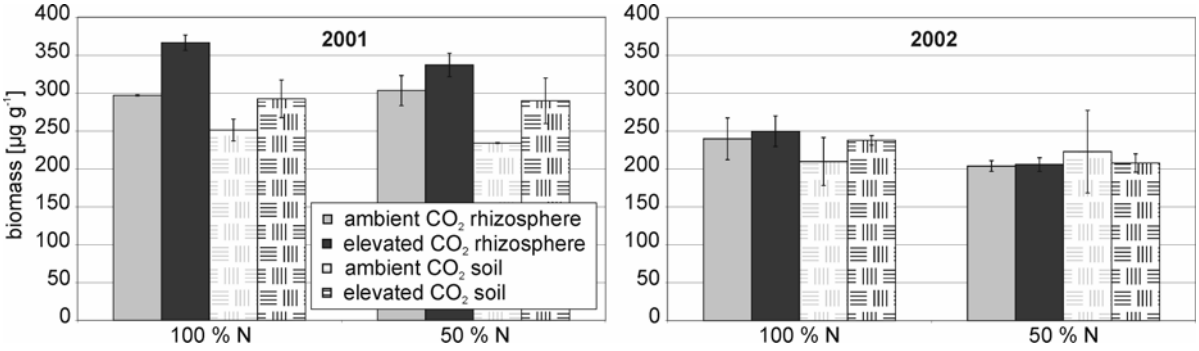


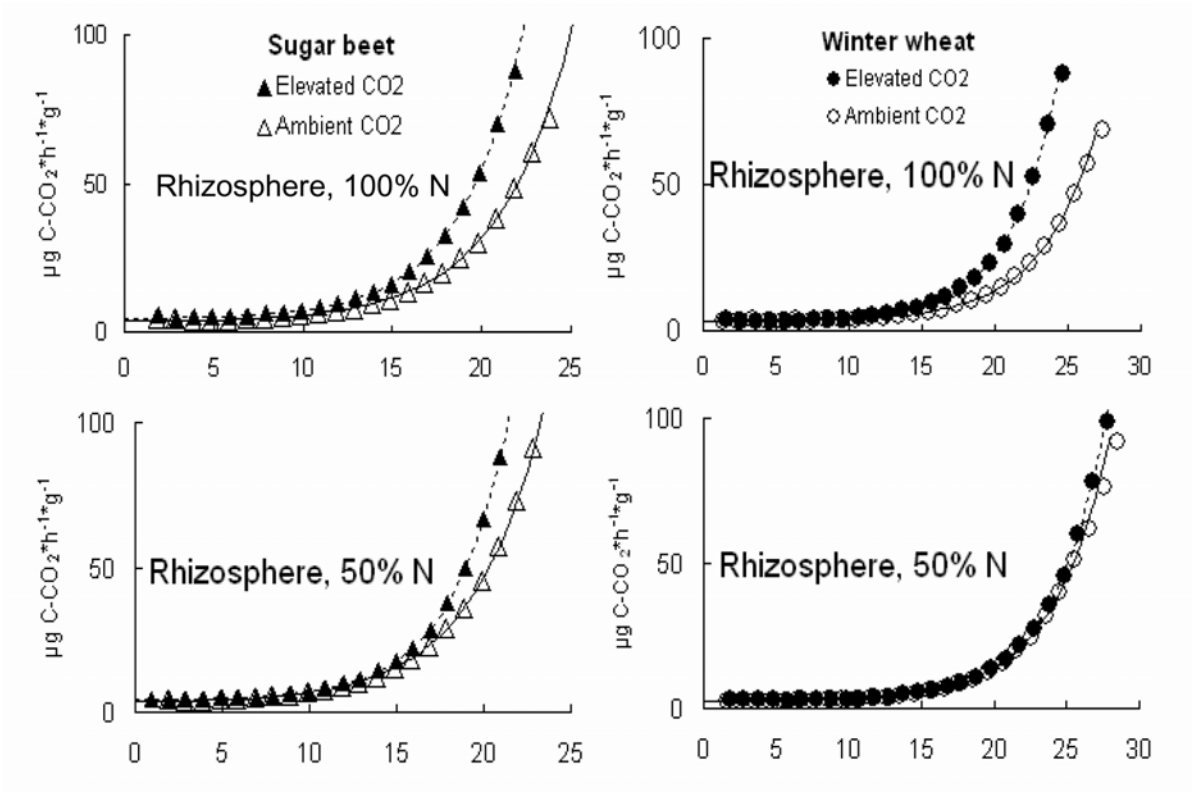
Figure 1: Microbial biomass measured by SIR-technique in rhizosphere and bulk soil as dependent on atmospheric CO_2 concentration and level of N fertilization. In 2001, sugar beets were cropped, and in 2002, winter wheat.

Table 1: Specific growth rates of microorganisms from bulk or rhizosphere soils at two levels of atmospheric CO_2 concentrations, and N fertilization grown under sugar beets and winter wheat.

crop	**soil source**	**N recomm. rate [%]**	**atmosph. CO_2 [ppm]**	**specific growth rate on glucose [μ_{max} h^{-1}]**
sugar beet	bulk soil	100	350	0.625 ± 0.032
			550	0.656 ± 0.027
		50	350	0.655 ± 0.029
			550	0.665 ± 0.023
	rhizosphere soil	100	350	0.608 ± 0.012
			550	0.637 ± 0.024
		50	350	0.638 ± 0.018
			550	0.684 ± 0.023
winter wheat	bulk soil	100	350	0.578 ± 0.010
			550	0.622 ± 0.014
		50	350	0.585 ± 0.004
			550	0.626 ± 0.010
	rhizosphere soil	100	350	0.588 ± 0.008
			550	0.640 ± 0.008
		50	350	0.599 ± 0.007
			550	0.630 ± 0.003

The character of the respiration curves were markedly different under elevated and ambient CO_2 in all variants of soil or rhizosphere of sugar beets (**Figure 2**). A comparison of the microbial growth parameters best-fitted to experimental data according to Equation 2 (**Table 1**), allows the conclusion that microbial communities existing under elevated CO_2 are markedly distinct in specific growth rate (μ_m) values from microbial communities under control plants growing under ambient CO_2. During the next crop rotation with winter wheat, maximal specific growth rates were also higher under elevated as compared to ambient CO_2 (**Table 1**).

a)



b)

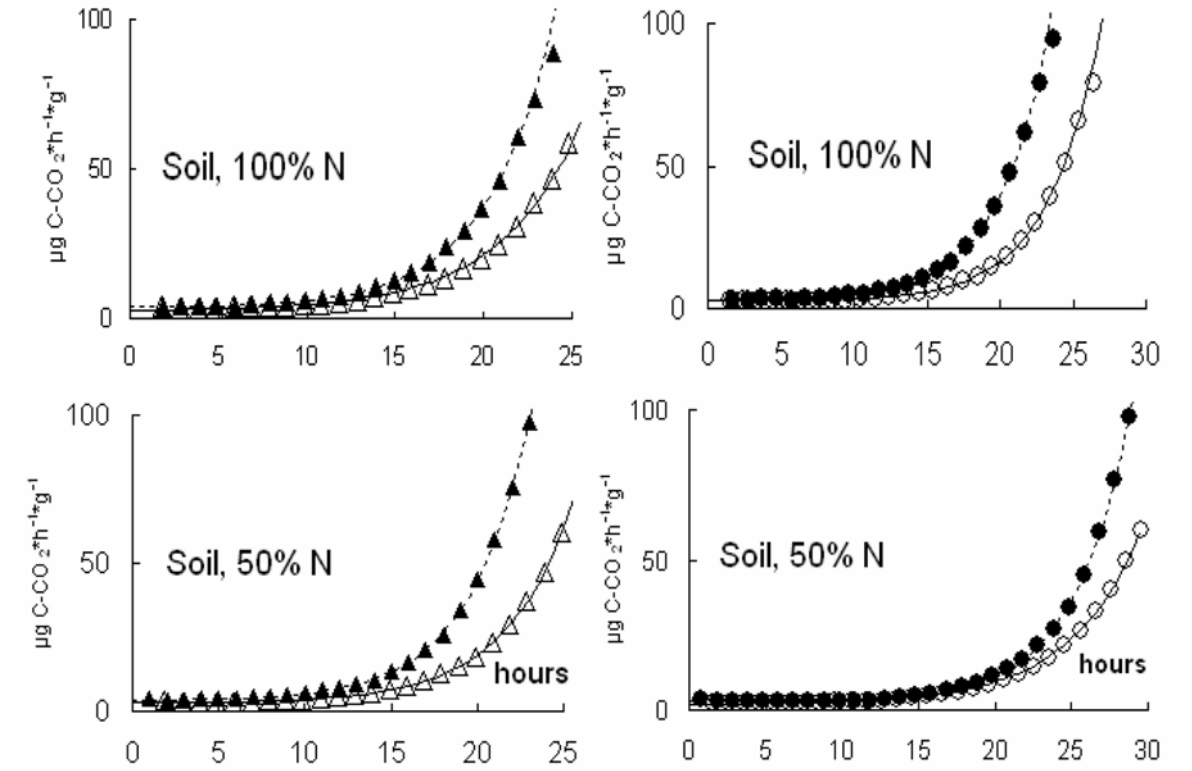


Figure 2: CO_2-evolution rates from rhizosphere soil (a) and from bulk soil (b) under sugar beets (year 2001) and winter wheat (year 2002) after glucose addition.

According to the ecological theory of life strategies, microorganisms with an r-strategy are characterized by higher values of μ_m and a lower efficiency of substrate use under non-limiting conditions compared with K-strategists [ANDREWS & Harris, 1986]. Hence, the growing of both sugar beets and winter wheat under an atmosphere of elevated CO_2 caused an increase in the portion of r-strategists in the microbial communities of bulk soil and rhizosphere soil, and therefore led to a change in the dominating ecological strategy of microbial community towards r-direction. Increases in the numbers of cultivated bacteria (usually r-strategists) together with intensive rates of substrate mineralization on BIOLOG-plates were found in samples from the soil microcosm under ryegrass grown under elevated CO_2 as compared to microcosm under ambient CO_2 concentration (HODGE *et al.*, 1998).

The effect of other factors (N or distance from root surface) on microbial biomass and growth parameters was not as evident as the effect of elevated CO_2 on μ_m. In most cases, the response of microbial communities on these factors in soil under sugar beets was opposite to that of winter wheat. This ambiguity of microbial responses at different stages of crop rotation is due to the contrasting plants chosen for the investigations. It is evident that during the harvesting period, microbial metabolism of the root-exudates of sugar beets differs widely from interactions between microorganisms and the root system of cereal plants such as winter wheat. In this regard, the uniquely pronounced effect of elevated CO_2 on μ_m, which was significantly exhibited in all variants of experiments under both plant cultures, is of utmost importance.

A three-way completely randomized ANOVA allowed an assessment of the range of effects of elevated CO_2, rate of N-fertilizers and distance from root surface on maximal specific growth rate of soil microorganisms. Respectively, 96 % and 83 % of the total dispersion of μ_m values was described by the set of these factors for sugar beets and winter wheat. Elevated CO_2 was the most important factor contributing to the dispersion of μ_m in both crop rotations (**Figure 3**). Doses of N-fertilizers was the second most important factor and affected μ_m values under both investigated plant crops.

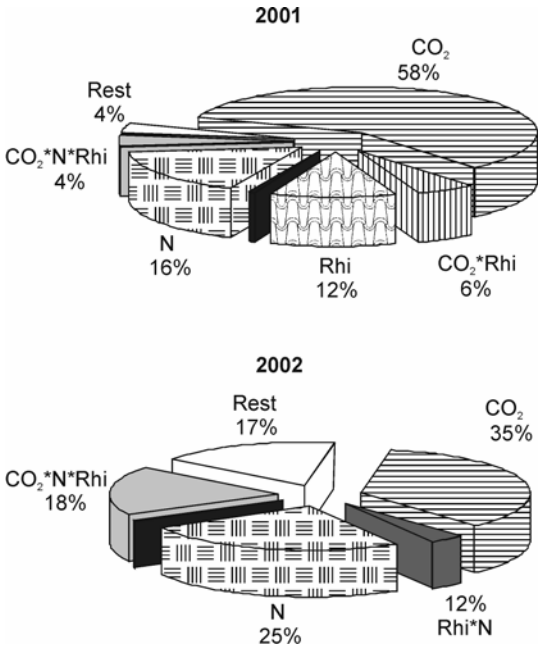


Figure 3: Contribution of independent factors: elevated CO_2, dose of N-fertilizers and distance from root surface on total dispersion of maximal specific growth rate of soil microorganisms as a result of three-way completely randomized ANOVA;
2001 – experimental plots under sugar beets;
2002 – experimental plots under winter wheat.

These results indicate that the maximal specific growth rate (μ_{max}) of a microbial community could be used as a sensitive indicator for showing impacts of environmental change on soil microbes. Our data suggest that the observed change towards a faster growth rate under elevated CO_2- fumigation is due to the higher level of easily available carbonaceaous

substrates from plants grown under these conditions. This promotes a shift in favour of species with faster growth rates (r-strategists) within the total soil microbial community.

15.4 Acknowledgements

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