Folia Microbiol. 39 (3), 225–230 (1994)

Soil Microorganisms at Different CO_2 and O_2 Tensions

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Received November 10, 1993
Revised version January 6, 1994

ABSTRACT. Microbial biomass and activity were determined in cambisol incubated under ambient and increased (up to 2.23 mmol/L) CO_2 concentrations. An immediate negative response of the soil microbial community to [CO_2] increase was observed during the first day with respect to microbial biomass, soil respiration and specific respiration activity (both expressed as CO_2 evolution). In contrast, O_2 consumption was not affected but anabolic utilization of available substrate increased. These phenomena were observed under conditions of increased CO_2 tension but without any change in O_2 concentration.

The composition of soil air is usually different from that of atmospheric air because of the biological activity of roots and microorganisms and the hindered exchange of air in soil. While the average oxygen concentration is (except at anoxic microsites) close to the atmospheric level (9.1 mmol O_2/L soil air), the average carbon dioxide concentration is 10 times higher (140 μmol CO_2/L soil air) than in the atmosphere (9.4 mmol O_2/L, *i.e.* 21 %, *V/V*, and 14 μmol CO_2/L, *i.e.* 310 ppm, *V/V*, respectively; Richter 1987). The composition of the soil air fluctuates widely under field conditions. Values of 0.05–2.23 mmol CO_2/L air are typical but 4.3 mmol CO_2/L air and higher have been recorded (Glinski and Stepniewski 1985; Nobel and Palta 1989).

Changes of CO_2 concentration in soil air disturb the conditions to which soil microorganisms are adapted. Such a perturbation is accompanied by an immediate microbial response so that a new "equilibrium" characterized by a new aeration status is established. This results in changes in metabolic activity and in the composition of the microbial community. Data showing the effect of high CO_2 concentration on soil microorganisms are frequent in literature. For example, it was shown that a high concentration of CO_2 during O_2 limitation has an inhibitory effect on microbial colony formation (Stotzky and Goos 1965). Inhibition of some yeasts and bacteria by CO_2 dissolved in the surrounding medium at concentrations ranging from 1.4 to 28 mmol CO_2/L air has been reported by Janda and Kotyk (1985). A decrease in soil respiration at carbon dioxide concentration in soil above 0.77 mmol CO_2/L air has been found by MacFadyen (1973). Griffin (1972) concluded that changes in the partial pressure of CO_2, rather than pO_2, appear to be the determining factor in microfungal respiration in soil. However little is known regarding the effect of the exposure of soil microorganisms to a high concentration of CO_2 on their biomass and specific respiration rate under conditions when the O_2 concentration is not the limiting factor. There is also a lack of information concerning the immediate response of the soil microbial community, adapted to a low CO_2 concentration, to an increase of CO_2 concentration in the soil atmosphere. It may be surmised that susceptible groups of microorganisms will die or develop inactive resting stages and that this will facilitate the development of more tolerant groups of microorganisms. We report here the immediate response of microbial assemblies to an increase of CO_2 in soil air with respect to the whole microbial biomass and its activity.

MATERIALS AND METHODS

Experimental design. The soil used was a cambisol (7.0 % clay, 41 % sand), characterized by $pH_{(H2O)}$ 5.3, C_{org} 2.2 %, N_{org} 0.24 % and C/N 9.2, with a content of carbonates ($MgCO_3$, $CaCO_3$) not exceeding 0.3 %. The soil was sampled in November 1991, sieved and the 2–5 mm fraction was stored at 15 °C in oxic conditions (about 14 μmol CO_2/L and 9.4 mmol O_2/L) for 1–2 in two sets of experiments: (*i*) stored soil was exposed to increased CO_2 (1.12 and 2.23 mmol CO_2/L) and atmospheric O_2 concentration without any additional treatment, (*ii*) stored soil was preincubated under anoxic conditions (2.7 mmol CO_2/L air and 0.17 mmol O_2/L air) at 25 °C for 6 d. After preincubation, the soil was aerated for 0.5 h and then treated as in (*i*). Fifty-g samples of wet soil (55 % WHC) were incubated at 25 °C for 1 d in hermetically sealed 250 mL glass vials containing either atmospheric air (14 μmol CO_2/L air and 9.4 mmol O_2/L air) or air supplemented with additional CO_2 to provide the final concentrations. In some experiments microorganisms were activated by addition of glucose solu-

tion dropwise to the soil surface either before preincubation (3.1 mg C/g) or after preincubation just at the beginning of the experiment (180 μg C/g).

Gas chromatographic analyses of CO_2 and O_2. CO_2 and O_2 concentrations were quantified in a gas chromatograph equipped with TCD and two parallel glass columns with Porapak Q and/or MS 5A operated at 45 °C. The initial CO_2 concentrations (t = 0) were measured 20–30 min after closing the glass vials. CO_2 measurements were corrected for CO_2 dilution in soil solution using Henry's law (Glinski and Stepniewski 1985) for a given CO_2 concentration and at 25 °C. The reaction of CO_2 with Ca^{2+} and Mg^{2+} ions was not taken into account as their concentration in the studied soil was low.

Determination of microbial biomass, residual glucose and hot-water-extracted saccharides. Microbial biomass was measured using the CFE method (Vance *et al.* 1987). Residual glucose in soil was determined in wet soil immediately after sampling. A 10 g portion of the soil was shaken with 15 mL 0.1 % benzoic acid for 15 min on an end-over-end shaker. The suspension was centrifuged (15 min, 135 Hz) and the supernatant used for glucose determination. Glucose concentration was measured enzymically using Oxo-chrom glucose (Bio-La test, Czech Republic). Subsamples for hot-water-extracted saccharide (HWEC) determination were quickly dried at 40 °C to arrest microbial activity. A 2 g portion of dry, crushed and sieved (<0.25 mm) soil was added to 6 mL distilled water and kept at 100 °C in a water bath for 1 h. After centrifugation and filtration, HWEC were determined by the phenol–sulfuric acid method (Šafařík and Šantrůčková 1992).

Estimation of death quotient. Specific respiration activities were expressed as CO_2 production and/or O_2 consumption per unit microbial biomass and unit time. Loss of microbial biomass was expressed as a death quotient (qD) by applying the equation

$$q\mathrm{D} = (100/C)(\Delta C/\Delta t)\ [\%]$$

where C is biomass at the beginning of the experiment (μg/g), t duration of the experiment (here t = 24 h) and $\Delta C/\Delta t$ the loss of biomass per unit time.

Estimation of glucose mineralization rate. The mineralization rate of glucose (Y) was estimated from the increase of CO_2 production after glucose addition and from glucose depletion (Δs, μg/g) during the given time period:

$$Y = 100(y_2 - y_1)/\Delta s\ [\%]$$

where y_1 and y_2 are CO_2 production as μg (CO_2-C)/g from untreated soil and soil treated with glucose, respectively.

Statistical treatment. Data were statistically analyzed using the confidence limits of means (significant level p = 0.05).

RESULTS AND DISCUSSION

The effect of high [CO_2] on microbial biomass and activity in soil maintained under oxic conditions

After 1 d, CO_2 production in soil decreased significantly with increasing concentration of CO_2 in the soil air while no decrease of O_2 consumption was detected (Table I). The loss of microbial biomass, characterized by the death quotient qD, was almost 0.1 % at atmospheric CO_2 concentration but increased at higher CO_2 concentrations. While at ambient CO_2 the specific respiration activity expressed by CO_2 production reached 2.6 μmol CO_2 per mg microbial carbon per day, it fell to approximate by 1.6 μmol CO_2 per mg microbial carbon per day at the concentration of 2.23 mmol CO_2/L air. The reduced CO_2 production, together with a constant O_2 consumption suggested changes in substrate consumption and utilization. To test this hypothesis, glucose-treated soil (3.1 mg C/g) was incubated at initially ambient [CO_2] (14 μmol CO_2/L air) and at high CO_2 concentration (2.24 mmol CO_2/L air) for 1 d. Both glucose depletion and soil respiration characteristics were measured (Table II). CO_2 production was again inhibited by high CO_2 exposure but O_2 consumption was not. Although glucose consumption was similar at both concentrations of CO_2, the mineralization rate of glucose was halved at high CO_2 exposure. These findings indicate a higher anabolic metabolism attended by enhanced glucose consumption for growth in the first hours after an increase of [CO_2] in soil air.

To compare the immediate response of soil microorganisms with their adaptation to high CO_2, the incubation of soil samples in an atmosphere containing 2.23 mmol CO_2 was prolonged for 3 d. At

Table I. The effect of elevated CO_2 concentration on soil respiration (CO_2 production, O_2 consumption), death quotient (qD) and specific respiration activities after 1 d of incubation[a,b]

Initial [CO_2] mmol CO_2/L	Soil respiration μmol g^{-1} d^{-1}		qD %	Specific respiration activity μmol (mg C_{mic})$^{-1}$ d^{-1}	
	CO_2	O_2		CO_2	O_2
0.014[c]	1.69 ± 0.15	6.02 ± 2.62	0.09	2.6	9.3
1.12	1.07* ± 0.25	6.06 ± 0.81	0.61	1.9	9.0
2.23	0.82* ± 0.31	4.85 ± 1.32	0.90	1.6	9.3

[a]Mean values and standard deviations (n = 5) are given.
[b]Values followed by an asterisk are significantly different (confidence limits, p = 0.05) compared to those of ambient [CO_2].
[c]Ambient.

Table II. Respiration and glucose depletion characteristics in glucose-treated soil (3.1 mg C/g) incubated for 1 d at initially ambient (14 μmol/L air) and high (2.23 mmol/L air) CO_2 concentrations[a]

Measured variables	Initial CO_2 concentration mmol/L	
	0.014[b]	2.23
CO_2 production, μmol g^{-1} d^{-1}	24.71 ± 0.71	13.99* ± 6.88
O_2 consumption, μmol g^{-1} d^{-1}	23.78 ± 2.46	22.37 ± 1.77
Respiratory quotient	1.04	0.63
Residual glucose in soil, mg C/g	1.478 ± 0.074	1.398 ± 0.084
Mineralization rate of glucose, %/d	17	9

[a]For other details see the footnote to Table I.
[b]Ambient.

Table III. The influence of high CO_2 concentration on the respiration of soil preincubated at low oxygen and high carbon dioxide status and then aerated, measured after 1-d incubation[a]

Treatment with glucose	Initial [CO_2] mmol CO_2/L	Soil respiration, μmol g^{-1} d^{-1}		RQ
		CO_2	O_2	
No	0.014	4.76 ± 0.35	10.10 ± 0.56	0.47
	2.23	2.62* ± 0.16	9.81 ± 0.38	0.27
Before pre-incubation	0.014	13.29 ± 0.62	15.15 ± 1.84	0.88
	2.23	4.18* ± 1.22	14.28 ± 2.99	0.29
After pre-incubation	0.014	9.03 ± 0.26	7.54 ± 1.01	1.19
	2.23	6.08* ± 0.38	9.76 ± 1.07	0.62

[a]For other details see the footnote to Table I.

the beginning of the incubation, the microbial biomass was 663 µg C/g. After 1 d it had decreased to 519 µg C/g which corresponds with the death quotient shown in Table I, but after further 2 d it increased again and reached a value of 760 µg C/g. Similarly, the respiration rate initially decreased (Table I) and subsequently increased to 1.66 µmol CO_2 per g per day. These results show that differ-

ences occur between the initial and long-term responses of soil microorganisms exposed to high CO_2 concentrations.

The effect of high [CO_2] on microbial biomass and metabolic activity in soil transferred from anoxic to oxic conditions

The results indicate that an immediate loss of microbial biomass (see qD, Table I) and a depression of respiration activity result from an increase in CO_2 concentration under conditions of oxygen excess in soil stored in oxic conditions. These same features were studied in soil preincubated in anoxic conditions (Table III). One day after the change of anoxic to oxic conditions, CO_2 production was significantly decreased by high CO_2 exposure. A further decrease was observed when microorganisms were stimulated by the addition of glucose. The consumption of O_2 was not significantly influenced, but the respiratory quotient (RQ), characterizing the mineralization rate of consumed substrate, decreased.

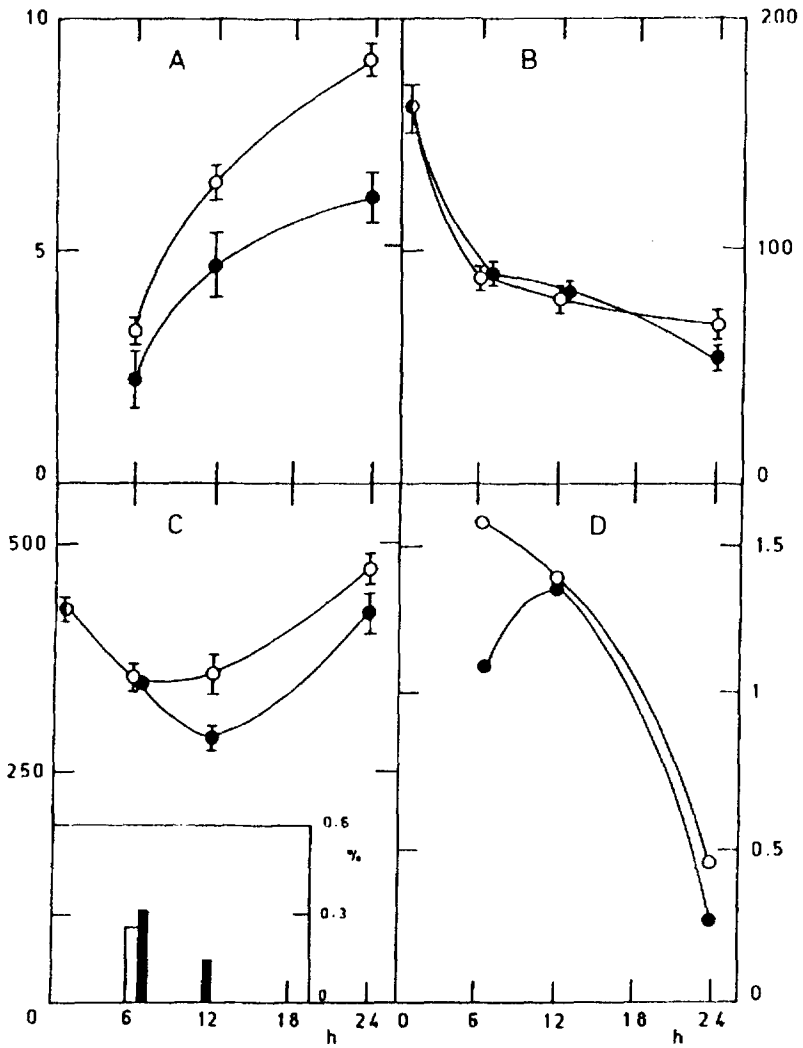

Fig. 1. The effect of low (14 μmol CO_2/L air, *open symbols*) and high (2.23 mmol CO_2/L air, *closed symbols*) CO_2 concentration on the microbial populations of soil preincubated in anoxic conditions, measured during 1-d incubation. Mean values and in A, B, C confidence limits of the means ($n = 5, p = 0.05$) are given; **A** – CO_2 production (μmol/g), **B** – hot-water-extracted saccharides (μg C/g), **C** – microbial biomass (μg C/g, *curves*) and death quotient qD (%, *columns*; under low [CO_2] determined after 6 h only), **D** – specific respiration activity (μmol CO_2 mg C_{mic}^{-1} h^{-1}).

The microbial response to a change of aeration from anoxic to oxic conditions under different initial CO_2 concentrations is shown in Figs 1 and 2. Soil samples were incubated either untreated (Fig. 1) or treated with glucose (180 μg C/g soil; Fig. 2). This addition of glucose is sufficient to permit

respiratory activity of the initial population of microorganisms without affecting their growth and multiplication (Anderson and Domsch 1978). From the beginning of incubation, a high CO_2 concentration in soil air lowered the CO_2 production in both treatments (Figs 1A, 2A). Glucose consumption was not affected and no residual glucose in soil samples was found 6 h after either treatment. At this time approximately 13.3 % of glucose was mineralized to CO_2 at the lower CO_2 exposure and only 7.1 % at the higher CO_2 exposure. The quantity of hot-water-extracted saccharides (HWEC), which represent mainly the microbial metabolites (Redl *et al.* 1990) that were accumulated during the previous anoxic incubation (162 μg C/g), decreased rapidly after the first 6 h by approximately 40 % in both treatments. This decrease of HWEC during the incubation was most rapid with the higher CO_2 concentration treatments and especially with treatments which were not enriched with glucose (Figs 1B, 2B). The loss of accumulated HWEC supports the hypothesis that substrate consumption is not inhibited at a high CO_2 concentration.

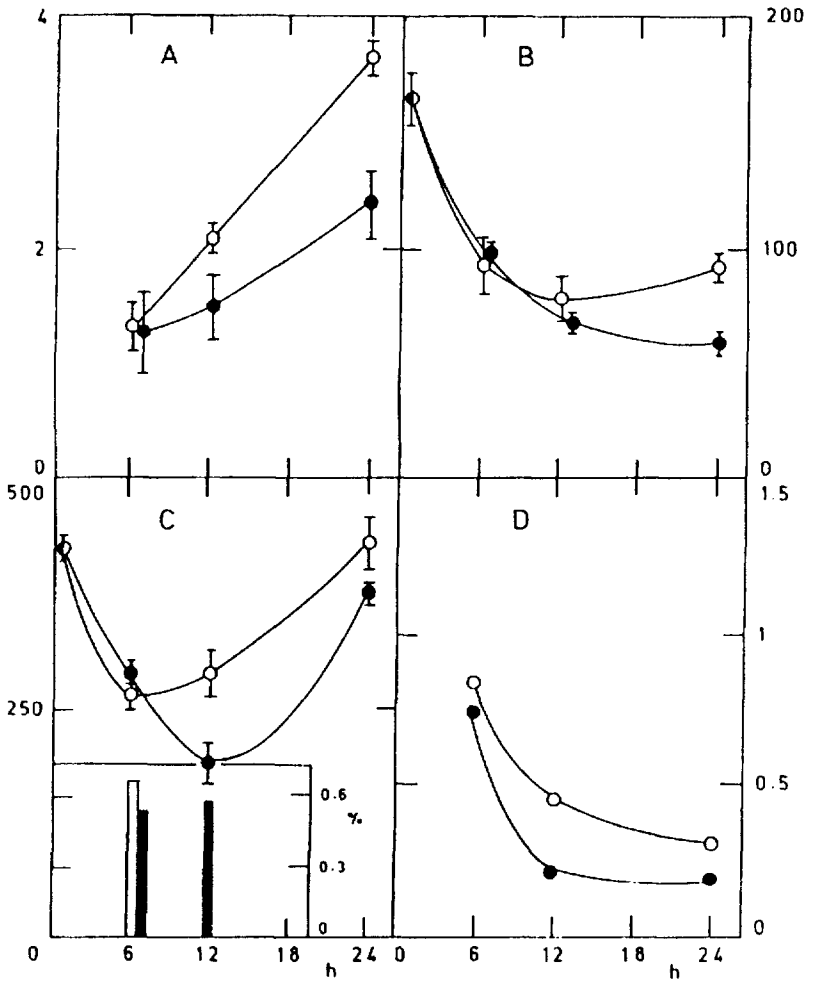

Fig. 2. The effect of low (14 μmol CO_2/L air, *open symbols*) and high (2.23 mmol CO_2/L air, *closed symbols*) CO_2 concentration on soil preincubated in anoxic conditions and then treated with glucose (180 μg C/g soil). For other symbols and details see Fig. 1.

The specific respiration activity of microorganisms, expressed by CO_2 production, decreased with the duration of the incubation in all treatments (except that of the glucose-treated soil) during the first 6 h of incubation at high CO_2 concentration (Figs 1D, 2D). The decrease in specific respiration activity was more pronounced in conditions of high CO_2 exposure. The change of the aeration status caused a decrease in microbial biomass during the first 6 h; a decrease which was enhanced and prolonged by up to 12 h in the higher CO_2 exposure but was partly relieved by glucose (Figs 1C, 2C). The death quotient reached 0.64 and 0.28 % in untreated and treated soil, respectively. It can be concluded

that the microbial response to the change of aeration status from anoxic to oxic conditions under high [CO_2] is similar to that found during continually oxic conditions.

The experiments confirmed the occurrence of an immediate response of the soil microbial community to CO_2 increase with respect to microbial biomass, soil respiration (CO_2 production) and specific respiration activity (CO_2). All these parameters decreased during the first day. During the same period O_2 consumption was not affected but anabolic utilization of available substrate increased. The decrease in microbial biomass and respiratory activity was followed by its reinstatement during the following period up to 2 d. These phenomena were observed using conditions of increased [CO_2] from ambient to 2.23 mmol CO_2/L air, which lies within the range of the typical concentration of CO_2 in soil, and without any marked change in oxygen concentration. In other words, CO_2 can quickly affect soil microorganisms, independently of the O_2 status. Such an immediate response of the microbial community to an increase in CO_2 concentration in soil air should be taken into account in experiments conducted in a closed system in which CO_2 is not absorbed. During several hours or days under closed conditions the internal atmosphere can change and an increased CO_2 concentration may arise and consequently contribute a source of experimental error.

The authors gratefully acknowledge the help of Dr. R.J. Smith and Mr. Paul O'Hara, *Lancaster University* (UK), for their kind comments.

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