



The response of soil microorganisms and roots to elevated CO₂ and temperature in a terrestrial model ecosystem

E. Kandeler^{1,6}, D. Tschirko¹, R.D. Bardgett², P.J. Hobbs³, C. Kampichler⁴ and T.H. Jones⁵

¹Federal Agency and Research Centre for Agriculture, Spargelfeldstr. 191, A-1226 Vienna, Austria* ²School of Biological Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, United Kingdom, ³Institute of Grassland and Environmental Research, North Wyke Research Station, Okehampton, Devon EX20 25B, United Kingdom, ⁴GSF National Research Centre for Environment and Health, Institute of Soil Ecology, Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany and ⁵NERC Centre for Population Biology, Imperial College at Silwood Park, Ascot Berkshire SL5 7PY, United Kingdom. ⁶Present address: Institute of Soil Science, Department of Soil Biology, University of Hohenheim, Emil-Wolff-Straße 27, D-705 99 Stuttgart, Germany

Received 12 November 1997. Accepted in revised form 14 May 1998

Key words: climate change, Ecotron, microbial biomass, microbial community structure, soil enzymes, temperature

Abstract

We investigate the response of soil microorganisms to atmospheric CO₂ and temperature change within model terrestrial ecosystems in the Ecotron. The model communities consisted of four plant species (*Cardamine hirsuta*, *Poa annua*, *Senecio vulgaris*, *Spergula arvensis*), four herbivorous insect species (two aphids, a leaf-miner, and a whitefly) and their parasitoids, snails, earthworms, woodlice, soil-dwelling Collembola (springtails), nematodes and soil microorganisms (bacteria, fungi, mycorrhizae and Protista). In two successive experiments, the effects of elevated temperature (ambient plus 2 °C) at both ambient and elevated CO₂ conditions (ambient plus 200 ppm) were investigated. A 40:60 sand:Surrey loam mixture with relatively low nutrient levels was used. Each experiment ran for 9 months and soil microbial biomass (C_{mic} and N_{mic}), soil microbial community (fungal and bacterial phospholipid fatty acids), basal respiration, and enzymes involved in the carbon cycling (xylanase, trehalase) were measured at depths of 0–2, 0–10 and 10–20 cm. In addition, root biomass and tissue C:N ratio were determined to provide information on the amount and quality of substrates for microbial growth.

Elevated temperature under both ambient and elevated CO₂ did not show consistent treatment effects. Elevation of air temperature at ambient CO₂ induced an increase in C_{mic} of the 0–10 cm layer, while at elevated CO₂ total phospholipid fatty acids (PLFA) increased after the third generation. The metabolic quotient *q*CO₂ decreased at elevated temperature in the ambient CO₂ run. Xylanase and trehalase showed no changes in both runs. Root biomass and C:N ratio were not influenced by elevated temperature in ambient CO₂. In elevated CO₂, however, elevated temperature reduced root biomass in the 0–10 cm and 30–40 cm layers and increased N content of roots in the deeper layers. The different response of root biomass and C:N ratio to elevated temperature may be caused by differences in the dynamics of root decomposition and/or in allocation patterns to coarse or fine roots (i.e. storage vs. resource capture functions). Overall, our data suggests that in soils of low nutrient availability, the effects of climate change on the soil microbial community and processes are likely to be minimal and largely unpredictable.

Introduction

Considerable effort has been put into investigating the effects of climate change on both above- and below-ground processes. In general, studies have mainly

focused on the effects of elevated atmospheric carbon dioxide (CO₂) on plant physiology and development (Bazzaz, 1990; Rogers et al., 1994), plant litter quality (Kemp et al., 1994), and soil microorganisms (Sadovskiy and Schortemeyer, 1997; Schortemeyer et al., 1996). Different studies show that there is variation in the response of root development and below-ground

* FAX No: 00497114593117. E-mail: kandeler@uni-hohenheim.de

processes to climate change between different plant communities. Since the concentration of CO₂ in soil is much higher (10–50 times) than in the atmosphere, increasing levels of atmospheric CO₂ may not directly influence below-ground processes (van de Geijn and van Veen, 1993). Most below-ground responses are likely to be the result of indirect effects of CO₂, such as faster root growth and increased rhizodeposition. Indeed, Zak et al. (1993) suggested the existence of a positive feedback between atmospheric CO₂ enrichment and an increase in root growth, microbial activity, and nitrogen (N) availability in the rhizosphere. However, there is as yet no general agreement (Kampichler et al., 1998; Rogers et al., 1994), and there are too few reported experimental observations to enable the prediction of whether soils will provide a net sink or source for carbon (C) under conditions of CO₂ enrichment (Tate and Ross, 1997). The possible consequences of elevated temperature on below-ground processes can also be attributed to indirect effects. The impact of the average 2 °C increase that is predicted for 2060 (Houghton et al., 1996) is likely to be mediated or even buffered by soil microorganisms; the temperature optimum for soil microorganisms is broad and an increase in soil temperature of 1–2 °C may have very little effect on the soil microbial community (Scharpenseel et al., 1990). Both model- and data-based studies do however predict that even small temperature changes (0.02 °C – 0.5 °C) could result in soils becoming significant sources or sinks of atmospheric CO₂ (Jenkinson et al., 1991; Trumbore et al., 1996). These latter authors calculated that a temperature increase of 0.5 °C would decrease turnover time of the fast-cycling C in temperate regions from 34.9 to 32.6 yr (Trumbore et al., 1996). Elevated temperature may increase plant production, this in turn, can change the nature of the C compounds present in the soil and possibly influence the partitioning of photo-assimilate within the soil/plant system (O'Neill, 1994). As a consequence, increased root respiration and higher growth of soil microorganisms could occur (O'Neill, 1994).

Few experiments have studied the effects of the interaction of rising CO₂ concentrations, and associated temperature increase, on ecosystems (Clark et al., 1995; Monz et al., 1994; Ross et al., 1995, 1996; Soussana et al., 1996). The interactions between CO₂, temperature, photosynthesis, soil moisture and plant-available nutrients can determine not only the structure and composition of plant and microbial communities, but also the size and turnover of the soil C reservoir

(Tate and Ross, 1997). There is experimental evidence that the effects of CO₂ and temperature on root biomass and root N depend on the N content of the ecosystem (Soussana et al., 1996). Simulating seasons in a CO₂-enrichment incubation experiment, Ross et al. (1996) found no consistent changes in total and microbial C and N, or in invertase activity in continuously moist or 'summer'-dried soil. In general, the increasing annual temperature should increase the rate of loss of soil C (Jenkinson et al., 1991; Lal et al., 1995), although this may possibly be counteracted by CO₂ enrichment enhancing plant primary production (Coleman and Crossley, 1996).

The aim of the present study was to determine the response of roots and soil microorganisms in multi-species terrestrial ecosystems to conditions of elevated temperature at both ambient and elevated CO₂. For this purpose the Ecotron controlled environment facility at Silwood Park (Lawton, 1996; Lawton et al., 1993), in which small but complex replicate model ecosystems are maintained, was used. The investigations focused on the response of root growth, root C:N ratio, soil microbial biomass and community structure, soil respiration and the activity of enzymes involved in C-cycling (xylanase and trehalase) to changing environmental conditions. To understand fully the role of soil biota in ecosystem response to elevated temperature and CO₂ enrichment, we linked two approaches: the characterization of pool sizes (root biomass, microbial biomass, fungal and bacterial biomass ratios) and the estimation of process rates (respiration, enzyme activities). Destructive sampling methods of the soil profile could only be performed at the end of each experimental run, microbial biomass and respiration in the top soil on the other hand were followed throughout.

Material and methods

The model communities were grown in the Ecotron, a controlled environmental facility based at the NERC Centre of Population Biology, Imperial College at Silwood Park (Lawton et al., 1993; Naeem et al., 1995). Temperature, humidity, light and water were controlled by a computer and a 24 h diurnal pattern without seasonality was maintained (for details see Jones et al., 1998; Naeem et al., 1996). Each community was grown in a separate chamber. Rainfall (reverse osmosis water) was delivered from an irrigation lance situated above the soil surface. Irrigation

occurred three times in each 24 h period delivering water at a rate of 4.2 L min^{-1} , each lasting 1 min.

The soil was sterilized by methyl bromylation and consisted of 0.1 m^3 gravel topped with 0.3 m^3 of 40:60 sand:Surry loam mix (carbonate free, 6.4 pH, 41.61 ppm N, 23.50 ppm P, 53.00 ppm K) placed in 0.4 m^3 containers. These contents corresponded to a 10–15 cm layer of gravel covered by a 25–30 cm layer of sand:Surry loam mix. Mean organic-C concentrations ranged from 0.95 to 1.37% (w/w) and total N from 0.09 to 0.13% (w/w) during the course of the experiment. C:N ratios ranged from 9.9 to 10.3 and did not differ significantly between treatments. Once filled with soil each container received 120 mL of a microbial inoculum prepared from a Whatman no. 4, 20–25 μm pore filtrate of Silwood Park clay:loam soil. Described in detail by Naeem et al. (1995) this treatment ensured that a standard microbial inoculum was added to the soil of each chamber (unpublished data; Jones et al., 1998). Characterisation of the soil biota indicated that the inoculum also introduced a relatively species-poor, but standardised protozoa and nematode community.

The model communities consisted of species from four trophic levels. For each trophic group, species that were capable of growing and reproducing under Ecotron conditions were chosen; briefly, the model communities consisted of four plant species which naturally cooccur in weedy fields and disturbed ground throughout Southern England (*Cardamine hirsuta*, *Poa annua*, *Senecio vulgaris*, *Spergula arvensis*), four herbivorous insect species (two aphids, a leaf-miner, and a whitefly) and their parasitoids, snails, earthworms, woodlice, soil-dwelling Collembola (springtails), nematodes and soil microorganisms (bacteria, fungi, mycorrhizae and Protista).

In the first experimental run CO_2 levels were maintained at ambient throughout (ambient CO_2 run) while in the second run CO_2 levels were enhanced by 200 ppm CO_2 (elevated CO_2 run). Ambient conditions varied with diurnal fluctuations with elevated conditions being enhanced appropriately. In both experiments two temperature regimes were maintained; ambient and elevated (ambient + 2 °C). Ambient conditions followed a 24 h cycle with a minimum temperature of 12 °C and a maximum of 20 °C.

For both runs six chambers were used for each treatment and both runs lasted for nine months, the time taken by three generations of plant growth. Above-ground plant material was cut after the first, second and third plant generation and after the first

and second generation returned as small pieces of vegetation on the soil surface.

Root analysis

At the end of both runs, eight soil cores (5 cm dia.) were taken from the 0–10, 10–20 and 20–30 cm layer from each chamber. The samples were stored in plastic bags at –20 °C. The gravel layer (30–40 cm) was also sampled in each chamber by taking eight random cores (10.3 dia., 15 cm length). The plant roots present in the gravel layer were separated by floatation immediately after sampling. After thawing for 24 hs, the roots present in each soil layer (0–10, 10–20 and 20–30 cm) were separated using a washing machine, as described by Smucker et al. (1982). The weight of root dry matter was estimated after drying at 60 °C for two days. The data were expressed as mg cm^{-3} . Finely-ground root material (about 200 mg) was used to determine C and N content of roots in a LECO CNS elemental analyser.

Soil microbial analysis

Eight soil samples (5 cm dia.) were taken from the soil at 0–2, 0–10 and 10–20 cm at the end of both runs. The single samples from any one layer in a particular chamber were bulked and stored in plastic bags at –20 °C for a maximum period of two months. During the elevated CO_2 run additional soil samples (0–5 cm) were taken after the first, second and third generation of plants to follow the temporal change of microbial biomass and respiration. To minimize disturbance only the top soil (0–5 cm) layer was sampled during the course of the experiment. When needed samples were allowed to thaw at 4 °C for about three days before being sieved (< 5 mm) and stored in plastic bags at 4 °C. Within 2 weeks soil microbial analysis was initiated.

Indices for microbial biomass

For the substrate induced respiration (C_{mic}) measurement, substrate saturation and maximum initial respiration responses were obtained with an amendment rate of $4.0 \text{ mg glucose g}^{-1}$ of dry matter. The CO_2 evolved was trapped in 0.05 M NaOH for a 4-h incubation at 25 °C and measured by titration (Jaggi, 1976). C_{mic} ($\mu\text{g C}_{\text{mic}} \text{ g}^{-1}$ soil) at 25 °C was calculated as $[\mu\text{L CO}_2 \text{ g}^{-1} \text{ soil h}^{-1}] \times 25.3$. The factor given by Kaiser et al. (1992) for 22 °C was corrected according to the rate-modifying equation for temperature effects given

by Jenkinson et al. (1987): $y = 47.9/(1+e^{106/(x+18.3)})$. Microbial respiration was obtained by the same titration procedure after a 24 h incubation at 25 °C. The $q\text{CO}_2$ (metabolic quotient) was calculated from the results of respiration and C_{mic} and were expressed as $\text{mg CO}_2\text{-C g}^{-1} C_{\text{mic}} \text{ h}^{-1}$.

N_{mic} was estimated by fumigating soil samples with chloroform for 24 h. Ninhydrin reactive nitrogen was determined after extraction with 2 M potassium-chloride using a colorimetric procedure (Amato and Ladd, 1988, modified by Joergensen and Brookes, 1990). N_{mic} ($\mu\text{g N}_{\text{mic}} \text{ g}^{-1} \text{ soil}$) was calculated as $[\text{mg ninhydrin-reactive N g}^{-1} \text{ soil}] \times 3.1$ according to Amato and Ladd (1988).

Soil microbial community structure: Phospholipid fatty acid (PLFA) analysis

Lipids were extracted from soil, fractionated and quantified using the procedure described by Bardgett et al. (1996) (based on Bligh and Dyer (1959) as modified by White et al. (1979)). Separated fatty acid methyl-esters were identified by chromatographic retention time and mass spectral comparison using standard qualitative bacterial acid methyl ester mix (Supelco) that ranged from C11 to C20. For each sample, the abundance of individual fatty acid methyl-esters was expressed on a dry weight basis per unit area (m^{-2}). Fatty acid nomenclature was used as described by Frostegård et al. (1993a,b). The fatty acids i15:0, a15:0, 15:0, i16:0, 17:0, i17:0, cy17:0, 18:1 ω 7 and cy19:0 were chosen to represent bacterial PLFAs (bactPLFAs) (Federle, 1986; Frostegård et al., 1993a; Tumid et al., 1989) and 18:2 ω 6 was used as an indicator of fungal biomass (Federle, 1986). The ratio of 18:2 ω 6:bactPLFAs was taken to represent the ratio of fungal:bacterial biomass in soil (Bardgett et al., 1996; Yeates et al., 1997).

Soil enzyme activities

For the determination of trehalase activity, 5 g of fresh soil were incubated with 7.5 mL of 40 mM trehalose solution and 7.5 mL of 2 M acetate buffer (pH 5.5) for 3 h in a shaking water bath at 50 °C (Kiem and Kandeler, 1997). The estimation of the released reducing sugars in the soil extracts was carried out by the ferric-ferrocyanide reaction (von Mersi and Schinner, 1996). For the determination of xylanase activity, 5 g of fresh soil were incubated with 15 mL of 1.2% xylan solutions and 15 mL of 2 M acetate buffer (pH 5.5)

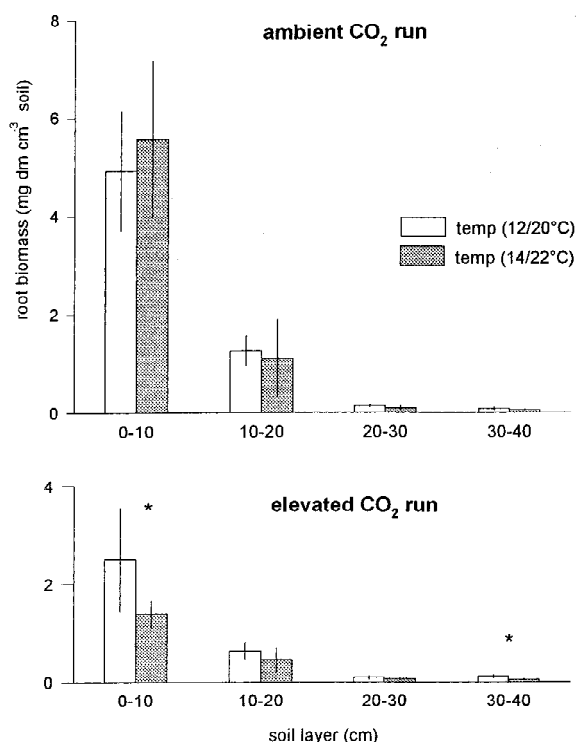


Figure 1. The effect of elevated temperature and elevated CO₂ on root biomass in different soil layers of the Ecotron. (In the elevated CO₂ run CO₂ levels were enhanced by 200 ppm CO₂. Minimum/maximum 24 h cycle temperatures are given in parentheses). Significant temperature effect are marked by an asterisk.

for 24 h at 50 °C. The released sugars were analysed colorimetrically (von Mersi and Schinner, 1996).

Analytical methods and statistical procedure

All analytical results were calculated on the basis of oven-dry (105 °C) weight of soil. Microbial biomass and soil microbial processes were determined in duplicate. Variables were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Levene's test). Mean differences between treatments within each run were tested with an independent *t*-test. Multifactorial ANOVA (generation and treatment) were carried out to determine the source of variation. Stepwise discriminant function analysis using Wilks' Lambda as the selection rule was carried out to separate treatments by given measurements on microbiological variables. Significance was accepted at the $p < 0.05$ level.

Table 1. Temperature response of root carbon content, nitrogen content and C/N ratio under ambient and elevated CO₂. Results are given as means and standard deviation (in parentheses) and *p* of a two-tailed *t*-test (*n*=6 for ambient CO₂ run; *n* = 5 for elevated CO₂ run)

Parameter	Soil layer	Depth (cm)	Ambient CO ₂ run			Elevated CO ₂ run		
			Ambient temp	Elevated temp	<i>p</i>	Ambient temp	Elevated temp	<i>p</i>
C%	top	0–10	43.08 (3.87)	40.38 (3.65)	n.s.	44.45 (0.89)	44.11 (1.48)	n.s.
	bottom	10–20	40.77 (1.57)	39.54 (2.56)	n.s.	44.27 (1.74)	45.92 (1.88)	n.s.
	gravel 1	20–30	40.52 (0.74)	40.40 (0.96)	n.s.	43.10 (3.91)	46.70 (1.45)	n.s.
	gravel 2	30–40	31.30 (2.96)	29.43 (5.52)	n.s.	29.66 (5.57)	37.58 (1.57)	0.014
N%	top	0–10	0.78 (0.09)	0.83 (0.17)	n.s.	0.81 (0.06)	0.82 (0.09)	n.s.
	bottom	10–20	0.89 (0.05)	0.83 (0.12)	n.s.	0.88 (0.10)	0.95 (0.10)	n.s.
	gravel 1	20–30	0.97 (0.04)	0.96 (0.04)	n.s.	0.85 (0.08)	1.04 (0.08)	0.007
	gravel 2	30–40	0.76 (0.05)	0.77 (0.14)	n.s.	0.70 (0.17)	0.93 (0.07)	0.019
C/N	top	0–10	55.5 (5.2)	50.2 (8.5)	n.s.	54.9 (4.1)	54.6 (7.6)	n.s.
	bottom	10–20	46.0 (2.5)	48.0 (6.0)	n.s.	51.0 (5.2)	48.7 (4.9)	n.s.
	gravel 1	20–30	41.8 (1.4)	42.0 (1.7)	n.s.	51.2 (5.9)	45.1 (3.8)	n.s.
	gravel 2	30–40	40.9 (1.9)	38.3 (4.2)	n.s.	43.2 (7.3)	40.6 (3.7)	n.s.

Results

Root biomass and C:N ratio of roots

In the ambient CO₂ run, elevated temperature did not significantly affect the weight and distribution of root biomass within the soil profile (Figure 1). The distribution of roots showed a depth gradient with 96.3% (ambient temperature) and 97.7% (elevated temperature) of root biomass found in the upper 20 cm. This result concurs with the finding that in uniformly wet and well-drained soils (as in the Ecotron) the highest root length density occurs in the top 20 cm of the soil profile (Wellbank et al., 1974). There were no significant effects on root quality (Table 1).

In the elevated CO₂ run, elevated temperature decreased root biomass in both the 0–10 cm and the 30–40 cm layer (Figure 1). Root C and N contents, as well as the C:N ratio, did not differ between treatments in the 0–10 and 10–20 soil layers (Table 1). In the gravel layer, however, N content of roots was significantly higher in elevated temperature, and significant differences of total C content of roots could be detected in the 30–40 cm layer (Table 1). The C:N ratio of roots decreased markedly down the soil profile from about 50 to 40 (Table 1).

Microbial biomass, respiration and metabolic quotient

In general, no significant treatment effects on microbial biomass (C_{mic} and N_{mic}) and respiration could be detected in the different soil layers (0–2, 0–10, 10–20 cm) at the end of both runs (Table 2). There were, however, two exceptions. In the ambient CO₂ run, elevated temperature induced an increase in C_{mic} of the 0–10 cm layer (Table 2) and a decrease in the metabolic quotient (qCO_2 , respiration-to-biomass ratio). Neither were affected in the elevated CO₂ run (Table 2).

Microbial biomass (C_{mic} and N_{mic}) and respiration were estimated after the first, second and third plant generations in the elevated CO₂ run (Figure 2). Although N_{mic} showed a trend for higher values under elevated temperature, none of the variables showed significant differences (Figure 2). The temporal increase of C_{mic} and respiration under both ambient and elevated temperature indicated increasing maturation of the ecosystem (Figure 2). Calculating qCO_2 showed no influence of the elevated temperature on the system, but an obvious temporal generation effect (Figure 2).

Microbial community structure

Using the temporal results from the elevated CO₂ run, total PLFA, and hence total 'active' microbial biomass, was higher under elevated temperature than

Table 2. Temperature response of biomass C (C_{mic}), biomass N (N_{mic}), respiration, metabolic quotient and C_{mic}/C_{org} ratio in the Ecotron soil under ambient and elevated CO_2 . Results are given as means and standard deviation (in parenthesis) and p of a two-tailed t-test (ambient CO_2 run: $n = 6$ for samples of the 0–2 cm and 0–10 cm layers, $n = 2$ for the 10–20 cm layer; elevated CO_2 run: $n = 6$ for ambient temperature treatment, $n = 5$ for elevated temperature treatment)

	Soil layer (cm)	Ambient CO_2 run			Elevated CO_2 run		
		Ambient temp	Elevated temp	P	Ambient temp	Elevated temp	P
C_{mic} ($\mu g C g^{-1} dm$)	0–2	384.98 (68.40)	493.35 (149.27)	n.s.	319.00 (96.90)	413.04 (116.58)	n.s.
	0–10	180.05 (57.30)	279.14 (86.62)	0.042	295.47 (64.96)	223.35 (54.29)	n.s.
	10–20	145.48	191.02		223.44 (51.96)	268.17 (40.99)	n.s.
N_{mic} ($\mu g N g^{-1} dm$)	0–2	2.63 (1.06)	2.11 (1.20)	n.s.	1.38 (0.58)	2.04 (0.85)	n.s.
	0–10	1.37 (1.16)	1.47 (0.60)	n.s.	0.95 (0.56)	1.41 (0.67)	n.s.
	10–20	1.52	1.34		0.66 (0.39)	0.81 (0.35)	n.s.
Microbial respiration ($\mu g CO_2 g^{-2} dm h^{-1}$)	0–2	9.77 (1.52)	9.65 (3.02)	n.s.	7.62 (1.44)	7.97 (0.92)	n.s.
	0–10	3.41 (0.66)	2.88 (1.00)	n.s.	5.90 (0.97)	5.33 (1.15)	n.s.
	10–20	1.88	1.53		3.95 (0.65)	4.51 (1.08)	n.s.
qCO_2 ($mg CO_2-C g^{-1} C_{mic} h^{-1}$)	0–2	7.10 (1.57)	5.32 (0.36)	0.022	6.83 (1.61)	5.50 (1.09)	n.s.
	0–10	5.47 (1.58)	3.10 (1.45)	0.022	5.52 (0.55)	6.94 (2.78)	n.s.
	10–20	3.93	2.16		5.01 (1.40)	4.66 (1.16)	n.s.
C_{mic}/C_{org} ratio ($mg C_{mic} g^{-1} C_{org}$)	0–2	24.61 (3.83)	31.72 (8.84)	n.s.	32.86 (8.84)	42.21 (9.28)	n.s.
	0–10	13.60 (4.12)	20.93 (5.86)	0.044	32.06 (6.29)	24.52 (7.70)	n.s.
	10–20	10.93	15.46 (2.58)		25.66 (9.37)	29.06 (11.52)	n.s.

under ambient after the third plant generation (Table 3). Likewise, BactPLFA, a measure of bacterial biomass, was also enhanced by elevated temperature treatment but again only in the last generation. On the other hand, fungal PLFA was significantly higher in elevated temperature than under ambient temperature after the first plant generation. Although not significant, fungal PLFA remained consistently higher under conditions of elevated temperature in the following two generations. Only at the end of the first plant generation did temperature have any effect on the fungal:bacterial PLFA ratio (Table 3).

Xylanase and trehalase activity

With one exception, xylanase and trehalase activity in the 0–2, 0–10 and 10–20 cm layers did not differ between temperature treatments in either run (Figure 3). Trehalase activity in the 0–10 cm layer did decrease in conditions of both elevated temperature and CO_2 increased. The response of trehalase activity was highly dependent on soil depth. In all treatments, trehalase activity decreased significantly within the soil profile, while xylanase activity showed only a trend for lower values in deeper soil layers. Calculating both of the

enzyme activities on the basis of μg glucose $g^{-1} h^{-1}$, the decay of trehalose was faster than the decay of xylan in the 0–2 cm layer, but the reverse effect occurred in the 10–20 cm layer (data not shown). This result may be due to the lower production of trehalase by microorganisms in the 10–20 cm layer. Discriminant analysis yielded no significant function that distinguished soil from chambers with ambient or elevated temperature in either the ambient or elevated CO_2 run.

Discussion

Root biomass and C:N ratio of roots

In both runs, Ecotron plant communities changed from a *S. vulgaris* dominated community (measured as biomass) after the first generation to a *P. annua* dominated system after the third generation (unpublished data). While root biomass and C:N ratios were not influenced by elevated temperature in the ambient CO_2 run, this single measurement at the end of the experiment does not show possible changes that occurred during the course of the experimental run. Temperature elevation associated with CO_2 -enrichment reduced root

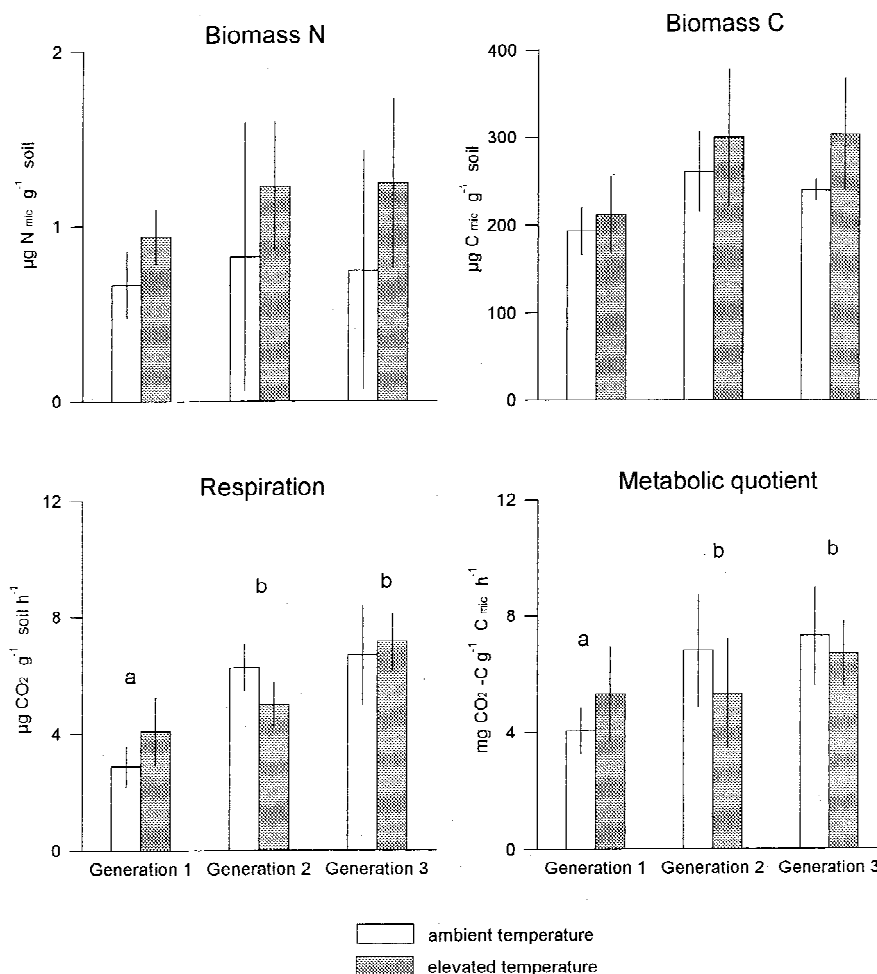


Figure 2. The response of biomass C (C_{mic}), biomass N (N_{mic}), respiration and metabolic quotient ($q\text{CO}_2$) in the 0–5 cm soil depth to elevated temperature in the elevated CO_2 run. Soil samples were taken after the first, second and third plant harvest corresponding to an experimental duration of 3, 6 and 9 months. Results are shown as arithmetic means (\pm sd). Significant time effects are marked by different letters.

Table 3. The response of soil microbial community structure, measured by PLFA to elevated temperature in the elevated CO_2 run during plant succession. Soil samples were taken after the first, second and third plant harvest corresponding to a duration of the experiment of 3, 6 and 9 months. Values are means (SE). NS, *, **, ***, Not significant, $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively. Treatments 1 and 2 refer to ambient and elevated temperature, respectively. For the summary ANOVA, T = treatment, D = date, and $T \times D$ = treatment \times date interaction

	Generation 1		Generation 2		Generation 3		Summary of ANOVA		
	1	2	1	2	1	2	T	D	$T \times D$
Total PLFA ($\text{nmol g}^{-1} \text{soil}$)	7.23 (1.0)	6.23 (0.96)	12.10 (1.55)	10.92 (1.99)	10.92 (0.96)	22.02*	NS	***	**
BactPLFA ($\text{nmol g}^{-1} \text{soil}$)	2.85 (0.4)	2.17 (0.29)	4.26 (0.6)	3.79 (0.59)	3.8 (0.42)	8.37**	*	***	***
Fungal PLEA ($\text{nmol g}^{-1} \text{soil}$)	0.12 (0.02)	0.20 (0.02)	0.26 (0.12)	0.45 (0.23)	0.58 (0.22)	0.91 (0.25)	NS	**	NS
Ratio Fungal:BactPLFA	4.33	9.55	6.19	10.39	14.42	10.49	NS	NS	NS

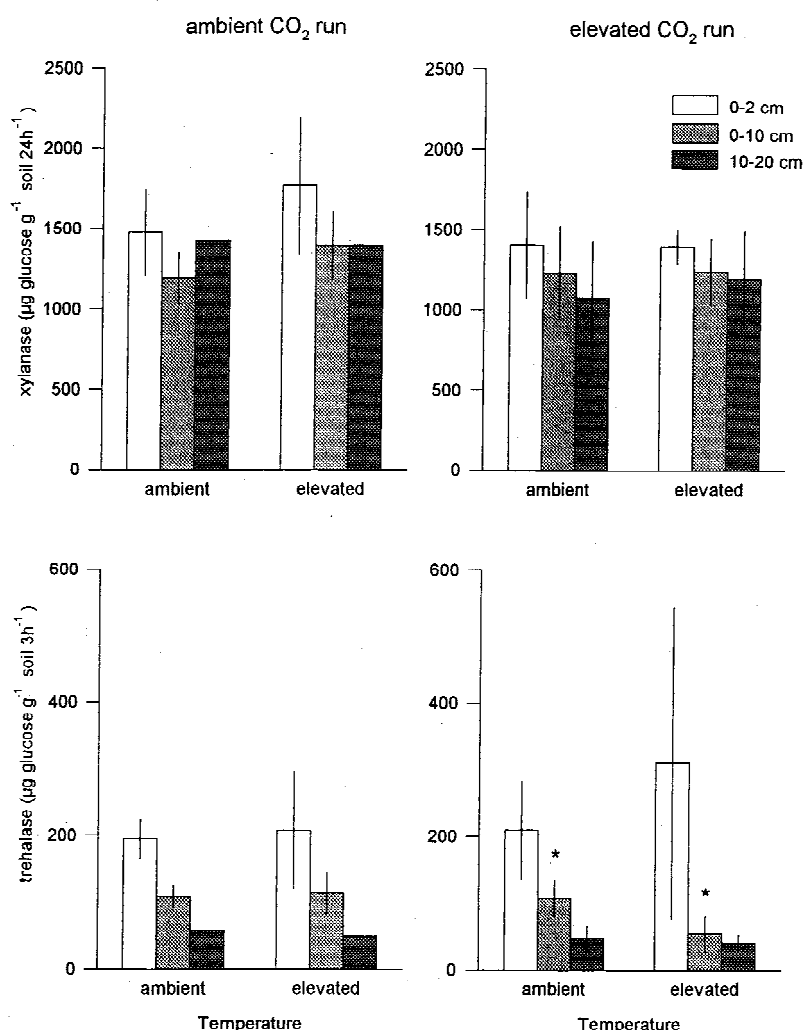


Figure 3. The effect of elevated temperature and elevated CO₂ on xylanase and trehalase activity in different soil layers of the Ecotron. (In the elevated CO₂ run CO₂ level were enhanced by 200 ppm CO₂). Significant temperature effects are marked by an asterisk.

biomass significantly in the 0–10 cm and 30–40 cm layers. Similar results were reported by Soussana et al. (1996) who found a significant reduction in root phytomass of perennial ryegrass (*Lolium perenne*) at suboptimal N-fertilizer levels, under elevated CO₂ and temperature (3 °C increase).

It is not clear from the two experimental runs, why elevated temperature at ambient CO₂ did not change root biomass, whereas elevated temperature and elevated CO₂ decreased root biomass. There are various possible explanations; the die-back already observed for above ground plant biomass in elevated CO₂ and temperature (unpublished data) may have consequences on root biomass, the dynamics of root

decomposition may be different in the two experiments or/and the allocation of C to coarse or fine roots (i.e. storage vs. resource capture functions) may have been changed in conditions of CO₂-enrichment. According to Curtis et al. (1994) while allocation of C-compounds to coarse roots should have little short-term effect on below-ground function, and indeed tends to buffer responses of ecosystems, allocation to fine roots could have an immediate effect on below-ground processes.

The increase in N-content of roots observed in elevated temperature in the 20–30 and 30–40 cm layer of the elevated CO₂ run may be dependent on the species composition of the ecosystem (Table 1). Hunt et al.

(1992) reported a significant increase in root N with elevated temperature in *Pascopyrum smithii* (western wheatgrass, C₃) but not in *Bouteloua gracilis* (blue grama, C₄); a difference related to photosynthetic temperature optima in *P. smithii*, a cool season grass, and *B. gracilis*, a warm season grass.

Microbial biomass and community structure

The values obtained for the Ecotron soils are within the range reported for agricultural soils (under crop rotation and conventional tillage) (Kandeler and Murer, 1993; Kandeler and Böhm, 1996). Compared to temporary and permanent grassland however the range of microbial biomass is relatively low (Kandeler and Murer, 1993). Although Ecotron soil is characterized by a relatively low microbial biomass (C_{mic} and N_{mic}) (Table 2, Figure 2), the range of C_{mic}/C_{org} values found in the top soil (11.08 – 25.83 mg $C_{mic} g^{-1} C_{org}$) agrees well with data previously published (Insam, 1990). The low absolute values of C_{mic} are mainly due to either the low content of organic C and other nutrients in the soil, or the sterilization procedure. The C_{mic}/C_{org} ratio decreased with soil depth showing that the most labile pool of organic matter was concentrated in the top soil. The two runs failed to detect a consistent treatment effect on indices of soil microbial biomass (C_{mic} , N_{mic} and total PLFA) (Table 2–3). The increase of C_{mic} in the 0–10 cm layer of the elevated temperature (ambient CO₂ run) were not accompanied by an increase in N_{mic} . Elevated temperature and CO₂ increased the total PLFA after Generation 3 and this increase was associated with an increase in BactPLFA. This suggests that the biomass increase was largely attributed to a response of bacteria rather than fungi. That various approaches showed differing effect of elevated temperature and CO₂ on microbial biomass may be attributed to the fact that the chloroform-fumigation technique measures both active and inactive (i.e. resting and dead) microbial biomass (Anderson and Domsch, 1978; Van de Werf and Verstaete, 1987), whereas PLFA detects the active proportion alone (Tunlid and White, 1992). PLFA is therefore likely to give a more realistic picture of the response of the soil microbial community to climate changes.

Investigating the microbial biomass of the bulk soil may have masked the possible reaction of rhizosphere microorganisms. Schortemeyer et al. (1996) detected some changes of microbial community in the rhizosphere of white clover (*Trifolium repens*) and

perennial ryegrass (*L. perenne*) in a field experiment. The authors concluded that the strongest effect of climate change can be expected in the immediate vicinity of roots, because the rhizosphere will be subject to quantitative and qualitative changes in root exudates. Aside from the spatial variability of microbial biomass within the bulk soil, we hypothesise that microbial biomass in the bulk soil did not change in the elevated temperature due to nutrient limitation. Klironomos et al. (1996) reported an increase of microbial biomass only under high nutrient conditions.

The temporal course of C_{mic} and total PLFA in the elevated CO₂ run showed that microbial biomass increased with ecosystem development in the upper 5 cm of soil (Table 3, Figure 2). As well as an increased growth of microbial population with root development, changes in the structural and functional components of the microbial community occurred. Whereas elevated temperature increased the FungalPLFA:BactPLFA ratio after Generation 1, this effect could not be detected in the later phase of the succession. These early changes in microbial communities appeared to be related to increased nutrients availability during initial stages of ecosystem development (Bardgett et al., 1998)

Respiration

Other model experiments have shown that the rates of CO₂ efflux vary as a function of soil temperature: Q₁₀ values for the response of soil respiration ranges from 1.3–3.3, with a median value of 2.4 (Raich and Schlesinger, 1992). In most soils with a mesophilic microbial community there is an approximate doubling of microbiological activity for each 10 °C rise in temperature between 0 °C and 30 °C (Killham, 1994). Many authors believe that global warming will result in a global increase in the rate of soil respiration (Schlesinger, 1995). We could not detect an increase in respiration at elevated temperature. Whether this arose from either the low levels of mineral N in the Ecotron soil limiting the photosynthetic response of plants or the temperature increase having modified the decomposition kinetics of low molecular weight compounds is unknown. Zogg et al. (1997) showed that temperature changed the amount of available substrates to a greater extent than it influenced the first-order rate constant of decomposition. They further showed that dominant populations at higher temperatures (25 °C) have the ability to metabolize substrates that are not used by members of the microbial community at lower

temperatures (10 °C). The temperature difference of only 2 °C used in our experiments may have been too small to induce such effects. At both elevated temperature and CO₂ there was no change in the moisture content of Ecotron soil; atmospheric CO₂ enrichment may therefore have not increased the water use efficiency of the plant community. Others have shown that the response of microbial biomass and respiration to CO₂ enrichment was only significant under dry soil water conditions (Rice et al., 1994)

Metabolic quotient

The difference in $q\text{CO}_2$ in the ambient CO₂ run may have been caused by the difference in microbial community structure. Under conditions of elevated temperature, simple-substrate decomposers may have been replaced by slower growing strategists. In the elevated CO₂ run, increased temperature did not change the $q\text{CO}_2$ during the development of the soil microbial community, despite a large, but insignificant increase in the ratio of fungal:bacterial PLFA over time. In another study where the effects of elevated CO₂ (700 $\mu\text{L L}^{-1}$), and elevated CO₂ and a temperature enhancement of 6 °C were compared, Ross et al. (1995) found a small decrease of the $q\text{CO}_2$ of a three-year-old ryegrass/white clover pasture. The increase of the $q\text{CO}_2$ from the first to the second generation may be due to increasing root growth and consequently greater substrate supply through decomposing litter, roots and/or rhizodeposition (van Veen et al., 1991).

Xylanase and trehalase activity

Xylanase and trehalase activity of the Ecotron soil were within the range of activity previously reported for agricultural soils (Kandeler and Böhm, 1996; Kiem and Kandeler, 1997). Elevated temperature did not change xylanase and trehalase in either runs. Comparable investigations of CO₂ and temperature responses of soil enzymes involved in C-cycling are scarce. Ross et al. (1995) found that invertase activity was significantly higher in the elevated CO₂ treatment at two of three samplings and that the additional increase of temperature decreased invertase activity to the level of the control. They discuss these results on the basis of the greater input of plant-derived invertase and/or greater synthesis by soil organisms in response to the greater C input under elevated CO₂. Arctic plants responded to elevated CO₂ by higher cellulase activity in ectomycorrhizae, whereas cellulase activities of the soil microflora was inhibited due to

the increase in C-exudation from plant roots (Moorhead and Linkins, 1997). Modeling cellulase activities showed that the observed reduction in cellulase activity could diminish cellulose turnover by 45% in arctic soils within rooting zones. As xylanase is mainly bound to the particulate organic matter (Stemmer et al., 1998), large differences in substrate input of xylan due to the different treatments are unlikely in the bulk soil. Effects which could have been seen in the rhizosphere may have been masked in the bulk soil. The difference of activity profiles of xylanase and trehalase – equal distribution of xylanase within the profile vs. decrease of trehalase activity with soil depth – showed that soil substrate quality and/or microbial community changed within the soil profile. Trehalose, the major storage disaccharide in many fungi and some lichens (Singleton and Sainsbury, 1987) was found mainly in the upper soil layers.

Conclusion

Understanding the effects of climate change on the amount and fate of C fluxes below-ground, including soil organic matter dynamics, soil biota mass and turnover, and soil C-efflux is essential if we are predict future consequences of elevated CO₂ and temperature (Curtis et al., 1994). Our studies have focused on the nature of the regulation and how C entering the soil is partitioned into root biomass, microbial biomass and microbial respiration. Under conditions of nutrient limitation and high rainfall below-ground processes may react differently to what has been described for many other ecosystems. Although occasionally, elevated temperature did affect root biomass, C_{mic} , N_{mic} and soil microbial respiration, in general the model ecosystem was able to buffer a 2 °C temperature increase. CO₂ enrichment associated with elevated temperature reduced root biomass in the 0–10 cm and 30–40 cm layers and increased N content of roots in the deeper layers. Soil respiration and soil enzyme activities involved in C-cycling did not change. Most of the results may be attributed to differences in the dynamics of root decomposition and/or in allocation pattern of C to coarse or fine roots (i.e. storage vs. resource capture functions). Further studies are necessary to quantify the release of root exudates and the turnover of roots as substrates for microbial growth under different climate conditions. In addition, estimates of microbial activity in the rhizosphere could

provide information on the extent of climate change effects in the close vicinity of roots.

Acknowledgments

The authors wish to thank Eva Kohlmann, Doris Busch, Hildegard Peisser, Gerhard Schlatter and Michael Stemmer for their technical assistance. We thank Monika Sobotik (Austrian Federal Institute of Agriculture in Alpine Regions, Gumpenstein) for the opportunity to use her laboratory for the root biomass analysis. Thanks also to the Ecotron Research Team and in particular Martijn Bezemer for detailed comments on an earlier version of the manuscript. The Ecotron Climate Change experiments were partially funded by the NERC TIGER Initiative (GST/02/646) and the British Council (VIE/891/11). The contribution of Ellen Kandeler and Dagmar Tschirko was financed by the Austrian Academy of Sciences (IGBP11/95) and by the Federal Ministry of Agriculture and Forestry.

References

- Amato M and Ladd J N 1988 Assay for microbial biomass based on ninhydrin-reactive nitrogen in extracts of fumigated soils. *Soil Biol. Biochem.* 20, 107–114.
- Anderson W E and Domsch K H 1978 A physiological method for quantitative measurement of microbial biomass in soils. *Soil Biol. Biochem.* 10, 215–221.
- Bardgett R D, Kandeler E, Tschirko D, Hobbs P J, Jones T H, Thompson U and Bezemer T M 1998 Below-ground microbial community development in a high temperature world. *Oikos*. (*in press*).
- Bardgett R D, Hobbs P J and Frostegård A 1996 Changes in the structure of soil microbial communities following reductions in the intensity of management of an upland grassland. *Biol. Fertil. Soils* 22, 261–264.
- Bazzaz F A 1990 The response of natural systems to the rising of global CO₂. *Annu. Rev. Ecol. Syst.* 21, 167–196.
- Bligh E G and Dyer W J 1959 A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* 37, 911–917.
- Clark H, Newton P C D, Bell C C and Glasgow E M 1995 The influence of elevated CO₂ and simulated seasonal changes in temperature on tissue turnover in perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) dominated pasture turves. *Appl. Ecol.* 32, 128–136.
- Coleman D C and Crossley Jr D A 1996 *Fundamentals of Soil Ecology*. Academic Press, London.
- Curtis P S, O'Neill E G, Teen J A, Zak D R and Pregitzer K S 1994 Below-ground responses to rising atmospheric CO₂: Implications for plants, soil biota and ecosystem processes. *Plant Soil* 165, 1–6.
- Federle T W 1986 Microbial distribution in soil – new techniques. *In Perspectives in Microbial Ecology*. Eds. F Megusar and M Gantar. pp. 493–498. Slovene Society for Microbiology, Ljubljana.
- Frostegård A, Bååth E and Tumid A 1993a Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biol. Biochem.* 25, 723–730.
- Frostegaard Å, Tunlid A and Bååth E 1993b Phospholipid fatty acid composition, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. *Appl. Environ. Microbiol.* 59, 3605–3617.
- Houghton J T, Meira-Filho L G, Callander B A, Harris N, Kattenberg A and Maskell K 1996 *Climate Change 1995: The Science of Climate Change*. Cambridge University Press, Cambridge.
- Hunt H W, Elliott E T, Detling J K, Monz C A and Reuss D E 1992 Plant size and shoot to root ratio in intact sods of native short-grass prairie exposed to elevated CO₂ for two growing seasons. *Bull. Ecol. Soc. Am.* 73, 254.
- Insam H 1990 Are the soil microbial biomass and basal respiration governed by the climate regime? *Soil Biol. Biochem.* 22, 525–532.
- Jäggi W 1976 Die Bestimmung der CO₂-Bildung als Mass der bodenbiologischen Aktivität. *Schweizer Landwirtschaftliche Forschung* 15, 371–380.
- Jenkinson D S, Hart P B S, Rayner J H and Pary L C 1987 Modelling the turnover of organic matter in long-term experiments at Rothamsted. *INTECOL Bulletin* 15, 1–8.
- Jenkinson D S, Adams D E and Wild A 1991 Model estimates of CO₂ emissions from soil in response to global warming. *Nature* 351, 304–306.
- Joergensen R G and Brookes P C 1990 Ninhydrin-reactive nitrogen measurements of microbial biomass in 0.5 M K₂SO₄ soil extracts. *Soil Biol. Biochem.* 22, 1023–1027.
- Jones T H, Thompson L J, Lawton J H, Bezemer T M, Bardgett R D, Blackburn T M, Bruce K D, Cannon P F, Hall G S, Hartley S E, Howson G, Jones C G, Kampichler C, Kandeler E and Ritchie D A 1998 Impacts of rising atmospheric CO₂ on soil biota and processes in model terrestrial ecosystems. *Science*, vol. 280, 441–443.
- Kaiser E A, Mueller T, Joergensen R G, Insam H and Heinemeyer 1992 Evaluation of methods to estimate the soil microbial biomass estimations and the relationship with soil texture and organic matter. *Soil Biol. Biochem.* 24, 675–683.
- Kampichler C, Kandeler E, Bardgett R D, Jones T H and Thompson L J 1998 Impact of elevated atmospheric CO₂ concentration on soil microbial biomass and activity in a complex, weedy field model ecosystem. *Glob. Change Biol.* 4, 335–346.
- Kandeler E and Murer E 1993 Aggregate stability and soil microbial processes in a soil with different cultivation. *Geoderma* 56, 503–513.
- Kandeler E and Böhm K 1996 Temporal dynamics of microbial biomass, xylanase activity, N-mineralisation and potential nitrification in different tillage systems. *Appl. Soil Ecol.* 4, 181–191.
- Kemp P R, Waldecker D G, Owensby C E, Reynolds J F and Virginia R A 1994 Effects of elevated CO₂ and nitrogen fertilization pretreatments on decomposition on tallgrass prairie leaf litter. *Plant Soil* 165, 115–127.
- Kiem R and Kandeler E 1997 A simple method for the determination of trehalase activity in soils. *Microbiol. Res.* 152, 19–25.
- Killham K 1994 *Soil Ecology*. Cambridge University Press, Cambridge. 242 pp.
- Klironomos J N, Rilling M C and Allen M F 1996 Below-ground microbial and microfaunal responses to *Artemisia tridentata* under elevated atmospheric CO₂. *Funct. Ecol.* 10, 527–534.

- Lal R, Kimble J, Levine E and Stewart B A (Eds.) 1995 Soils and Global Change. CRC Lewis Publishers, Boca Raton, Florida. p 23.
- Lawton J H 1996 The Ecotron facility at Silwood park: the value of 'big bottle' experiments. *Ecology* 77, 665–669.
- Lawton J H, Naeem S, Woodfin R M, Brown V K, Gamge A, Godfray H C J, Heads P A, Lawler S, Magda D, Thomas C D, Thompson L J and Young S 1993 The Ecotron: a controlled environmental facility for the investigation of population and ecosystem processes. *Phil. Trans. Roy. Soc. Lon., Series B* 341, 181–194.
- Monz C A, Hunt H W, Reeves F B and Elliott E T 1994 The response of mycorrhizal colonization to elevated CO₂ and climate change in *Pascopyrum smithii* and *Bouteloua gracilis*. *Plant Soil* 165, 75–80.
- Moorhead D L and Linkins A E 1997 Elevated CO₂ alters below-ground exoenzyme activities in tussock tundra. *Plant Soil* 189, 321–329.
- Naeem S, Thompson L J, Lawler S, Lawton J H and Woodfin R M 1995 Empirical evidence that declining species diversity may alter the performance of terrestrial ecosystems. *Phil. Trans. Roy. Soc. Lon., Series B*, 347, 249–262.
- Naeem S, Thompson L J, Jones T H, Lawler S, Lawton J H and Woodfin R M 1996 Changing community composition and elevated CO₂. In *Carbon Dioxide, Populations and Communities*. Eds. C Körner and F A Bazzaz. pp 93–99. Academic Press, London.
- O'Neill E G 1994 Responses of soil biota to elevated atmospheric carbon dioxide. *Plant Soil* 165, 55–65.
- Raich J W and Schlesinger W H 1992 The global carbon dioxide flux in soil respiration and its relationship to vegetation and climate. *Tellus* 44B, 81–99.
- Rice C W, Garcia F O, Hampton C O and Qwensby C E 1994 Soil microbial response in tallgrass prairie to elevated CO₂. *Plant Soil* 165, 67–74.
- Rogers H H, Runion G B and Krupa S V 1994 Plant responses to atmospheric CO₂ enrichment with emphasis on roots and rhizosphere. *Environ. Poll.* 83, 155–189.
- Ross D J, Tate K R and Newton P C D 1995 Elevated CO₂ and temperature effects on soil carbon and nitrogen cycling in ryegrass/white clover turves of an Endoaquept soil. *Plant Soil* 176, 37–49.
- Ross D J, Saggat S, Tate K R, Feltham C W and Newton P C D 1996 Elevated CO₂ effects on carbon and nitrogen cycling in grass/clover turves of a Psammaquept soil. *Plant Soil* 182, 185–198.
- Sadowsky M J and Schortemeyer M 1997 Soil microbial responses to increased concentration of atmospheric CO₂. *Glob. Change Biol.* 3, 217–224.
- Scharpenseel H W, Schomaker M and Ayoub A, Eds 1990 Soils on a Warmer Earth. Elsevier, Amsterdam.
- Schinner F, Öhlinger R, Kandeler E and Margesin R 1996 *Methods in Soil Biology*. Springer, Berlin.
- Schlesinger W H 1995 Soil respiration and changes in soil carbon stocks. In *Biotic Feedbacks in the Global Climatic Systems*. Eds G M Woodwell and F T Mackenzie. pp 160–168. Oxford University Press, New York, Oxford.
- Schortemeyer M, Hartwig U A, Hendrey G R and Sadowsky M J 1996 Microbial community changes in the rhizospheres of white clover and perennial ryegrass exposed to free air carbon dioxide enrichment (FACE). *Soil Biol. Biochem.* 28, 1717–1724.
- Singleton P and Sainsbury D 1987 *Dictionary of Microbiology and Molecular Biology*. John Wiley & Sons, Chichester, New York.
- Smuckner A J M, McBurney S L and Srivastava A K 1982 Quantitative separation of roots from compacted soil profiles by the Hydropneumatik Elutriation Systems. *Agron. J.* 74, 500–503.
- Soussana J F, Casella E and Loiseau P 1996 Long-term effects of CO₂ enrichment and temperature increase on temperate grass sward. II. Plant nitrogen budgets and root fraction. *Plant Soil* 182, 101–114.
- Stemmer M, Gerzabek M H and Kandeler E 1998 Organic matter and enzyme activity in particle-size fractions of soils obtained after low-energy sonication. *Soil Biol. Biochem.* 30, 9–17.
- Tate K R and Ross D J 1997 Elevated CO₂ and moisture effects on soil carbon storage and cycling in temperate grassland. *Glob. Change Biol.* 3, 225–235.
- Trumbore S E, Chadwick O A, Amundson R 1996 Rapid exchange between soil carbon and atmospheric carbon dioxide driven by temperature change. *Science* 272, 393–396.
- Tunlid A, Hoitink H A J, Low C and White D C 1989 Characterization of bacteria that suppress *Rhizoctonia* damping-off in bark compost media by analysis of fatty acid biomarkers. *Appl. Environ. Microbiol.* 55, 1368–1374.
- Tunlid A and White D C 1992 Biochemical analysis of biomass, community structure, nutritional status, and metabolic activity of microbial communities in soil. In *Soil Biochemistry*, vol 7. Eds. G Stotzky, J M Bollag. pp 229–262. Marcel Dekker, New York.
- van de Geijn S C and van Veen J A 1993 Implications of increased carbon dioxide levels for carbon input and turnover in soils. *Vegetatio* 104/105, 283–292.
- van de Werf H. and Verstraete W. 1987 Estimation of active soil microbial biomass by mathematical analysis of respiration curves: calibration of the test procedure. *Soil Biol. Biochem.* 19, 261–265.
- van Veen J A, Liljeroth E, Lekkerkerk L J A and van de Geijn S C 1991 Carbon fluxes in plantsoil systems at elevated atmospheric CO₂ levels. *Ecol. Applic.* 1, 175–181.
- von Mersi W and Schinner F 1996 Xylanase activity. In *Methods in Soil Biology*. Eds. F Schinner, R Öhlinger, E Kandeler and R Margesin. pp 193–196. Springer, Berlin.
- Wellbank P J, Gibbs M J, Taylor P J and Williams E D 1974 Root growth of cereal crops. Reports of Rothamsted Experimental Station for 1973, Part 2, pp. 26–66.
- White D C, Davis W M, Nickels J S, King J C and Bobbie R J 1979 Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia* 40, 51–62.
- Yeates G W, Bardgett R D, Cook R, Hobbs P J, Bowling P J and Potter J F 1997 Faunal and microbial diversity in three Welsh grassland soils under conventional and organic management regimes. *J Appl. Ecol.* 34, 453–470.
- Zak D R, Pregitzer K S, Curtis P S, Teeri J A, Fogel R and Randlett D L 1993 Elevated atmospheric CO₂ and feedbacks between carbon and nitrogen cycles. *Plant Soil* 151, 105–117.
- Zogg G P, Zak D R, Ringelberg D B, MacDonald N W, Pregitzer K S and White D C 1997 Compositional and functional shifts in microbial communities due to soil warming. *Soil Sci. Soc. Am. J.* 61, 475–481.

Section editor: R Merckx