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# The response of soil microorganisms and roots to elevated $CO_2$ and temperature in a terrestrial model ecosystem

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**Abstract**

We investigate the response of soil microorganisms to atmospheric $CO_2$ 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 $CO_2$ 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 $CO_2$ did not show consistent treatment effects. Elevation of air temperature at ambient $CO_2$ induced an increase in $C_{mic}$ of the 0–10 cm layer, while at elevated $CO_2$ total phospholipid fatty acids (PLFA) increased after the third generation. The metabolic quotient $qCO_2$ decreased at elevated temperature in the ambient $CO_2$ run. Xylanase and trehalase showed no changes in both runs. Root biomass and C:N ratio were not influenced by elevated temperature in ambient $CO_2$. In elevated $CO_2$, 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 unpredicatable.

## 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 ($CO_2$) on plant physiology and development (Bazzaz, 1990; Rogers et al., 1994), plant litter quality (Kemp et al., 1994), and soil microorganisms (Sadowsky and Schortemeyer, 1997; Schortemeyer et al., 1996). Different studies show that there is variation in the response of root development and below-ground

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processes to climate change between different plant communities. Since the concentration of $CO_2$ in soil is much higher (10–50 times) than in the atmosphere, increasing levels of atmospheric $CO_2$ 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 $CO_2$, such as faster root growth and increased rhizodeposition. Indeed, Zak et al. (1993) suggested the existence of a positive feedback between atmospheric $CO_2$ 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 $CO_2$ 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 $CO_2$ (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 $CO_2$ 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 $CO_2$, 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 $CO_2$ and temperature on root biomass and root N depend on the N content of the ecosystem (Soussana et al., 1996). Simulating seasons in a $CO_2$-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 $CO_2$ 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 $CO_2$. 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 $CO_2$ 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 $\mu$m 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 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 (Jäggi, 1976). $C_{mic}$ ($\mu$g $C_{mic}$ $g^{-1}$ soil) at 25 °C was calculated as [$\mu$L $CO_2$ $g^{-1}$ soil $h^{-1}$] × 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 $qCO_2$ (metabolic quotient) was calculated from the results of respiration and $C_{mic}$ and were expressed as mg $CO_2$–C $g^{-1}$ $C_{mic}$ $h^{-1}$.

$N_{mic}$ 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). $N_{mic}$ ($\mu$g $N_{mic}$ $g^{-1}$ soil) was calculated as [mg ninhydrin-reactive N $g^{-1}$ soil] × 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$\omega$7 and cy19:0 were chosen to represent bacterial PLFAs (bactPLFAs) (Federle, 1986; Frostegård et al., 1993a; Tumid et al., 1989) and 18:2$\omega$6 was used as an indicator of fungal biomass (Federle, 1986). The ratio of 18:2$\omega$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 m*M* 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) for 24 h at 50 °C. The released sugars were analysed colorimetrically (von Mersi and Schinner, 1996).

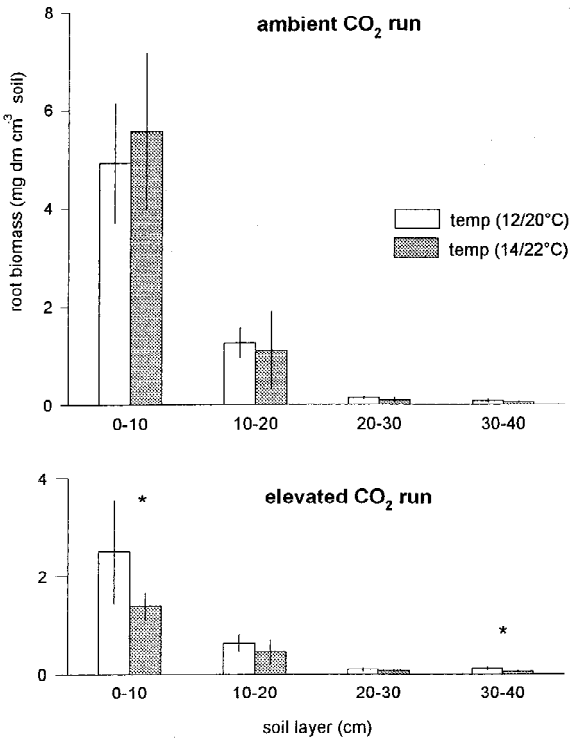


*Figure 1.* The effect of elevated temperature and elevated $CO_2$ on root biomass in different soil layers of the Ecotron. (In the elevated $CO_2$ run $CO_2$ levels were enhanced by 200 ppm $CO_2$. Minimum/maximum 24 h cycle temperatures are given in parentheses). Significant temperature effect are marked by an asterisk.

*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 $CO_2$. Results are given as means and standard deviation (in parantheses) and *p* of a two-tailed *t*-test ($n$=6 for ambient $CO_2$ run; $n$ = 5 for elevated $CO_2$ run)

| Parameter | Soil layer | Depth (cm) | Ambient $CO_2$ run | | | Elevated $CO_2$ run | | |
|---|---|---|---|---|---|---|---|---|
| | | | Ambient temp | Elevated temp | *p* | Ambient temp | Elevated temp | *p* |
| 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 $CO_2$ 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 $CO_2$ 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 $CO_2$ 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 $CO_2$ 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 $CO_2$ 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 $CO_2$ 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)

| | | Ambient $CO_2$ run | | | Elevated $CO_2$ run | | |
|---|---|---|---|---|---|---|---|
| | Soil layer (cm) | Ambient temp | Elevated temp | *P* | Ambient temp | Elevated temp | *P* |
| $C_{mic}$ | 0–2 | 384.98 (68.40) | 493.35 (149.27) | n.s. | 319.00 (96.90) | 413.04 (116.58) | n.s. |
| ($\mu$g C $g^{-1}$ dm) | 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. |
| $N_{mic}$ | 0–2 | 2.63 (1.06) | 2.11 (1.20) | n.s. | 1.38 (0.58) | 2.04 (0.85) | n.s. |
| ($\mu$g N $g^{-1}$ dm) | 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 | 0–2 | 9.77 (1.52) | 9.65 (3.02) | n.s. | 7.62 (1.44) | 7.97 (0.92) | n.s. |
| ($\mu$g $CO_2$ $g^{-2}$ dm $h^{-1}$) | 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. |
| $qCO_2$ | 0–2 | 7.10 (1.57) | 5.32 (0.36) | 0.022 | 6.83 (1.61) | 5.50 (1.09) | n.s. |
| (mg $CO_2$-C $g^{-1}$ $C_{mic}$ $h^{-1}$) | 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 | 0–2 | 24.61 (3.83) | 31.72 (8.84) | n.s. | 32.86 (8.84) | 42.21 (9.28) | n.s. |
| (mg $C_{mic}$ $g^{-1}$ $C_{org}$) | 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 exeption, 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 enzmye activities on the basis of $\mu$g 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 occured 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 occured during the course of the experimental run. Temperature elevation associated with $CO_2$-enrichment reduced root

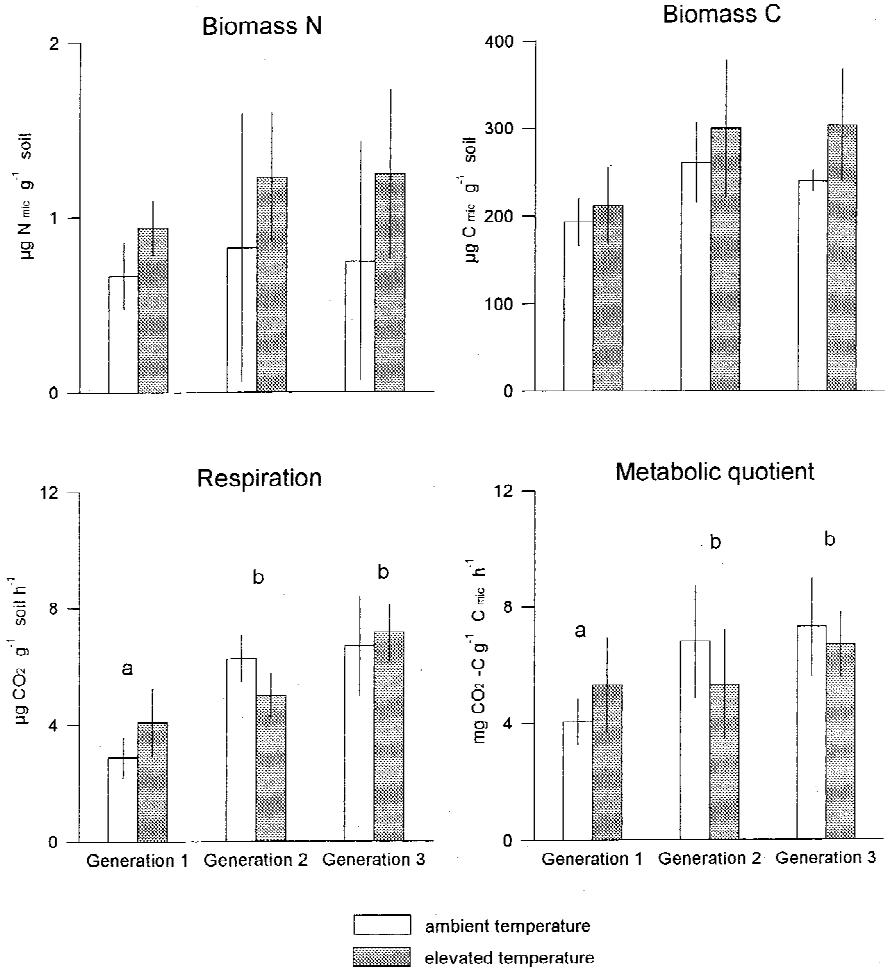


*Figure 2.* The response of biomass C ($C_{mic}$), biomass N ($N_{mic}$), respiration and metabolic quotient ($qCO_2$) in the 0–5 cm soil depth to elevated temperature in the elevated $CO_2$ run. Soil samples were taken after the first, second and third plant harvest corresponding to an experimental duration of 3, 6 and 9 months. Results are shown as arithmetric means (+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 × D = treatment × date interaction

| | Generation 1 | | Generation 2 | | Generation 3 | | Summary of ANOVA | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 1 | 2 | 1 | 2 | T | D | T × D |
| Total PLFA (nmol $g^{-1}$ soil) | 7.23 | 6.23 | 12.10 | 10.92 | 10.92 | 22.02* | NS | *** | ** |
| | (1.0) | (0.96) | (1.55) | (1.99) | (0.96) | (3.74) | | | |
| BactPLFA | 2.85 | 2.17 | 4.26 | 3.79 | 3.8 | 8.37** | * | *** | *** |
| (nmol $g^{-1}$ soil) | (0.4) | (0.29) | (0.6) | (0.59) | (0.42) | (1.19) | | | |
| Fungal PLEA | 0.12 | 0.20 | 0.26 | 0.45 | 0.58 | 0.91 | NS | ** | NS |
| (nmol $g^{-1}$ soil) | (0.02) | (0.02) | (0.12) | (0.23) | (0.22) | (0.25) | | | |
| Ratio Fungal:BactPLFA | 4.33 | 9.55 | 6.19 | 10.39 | 14.42 | 10.49 | NS | NS | NS |

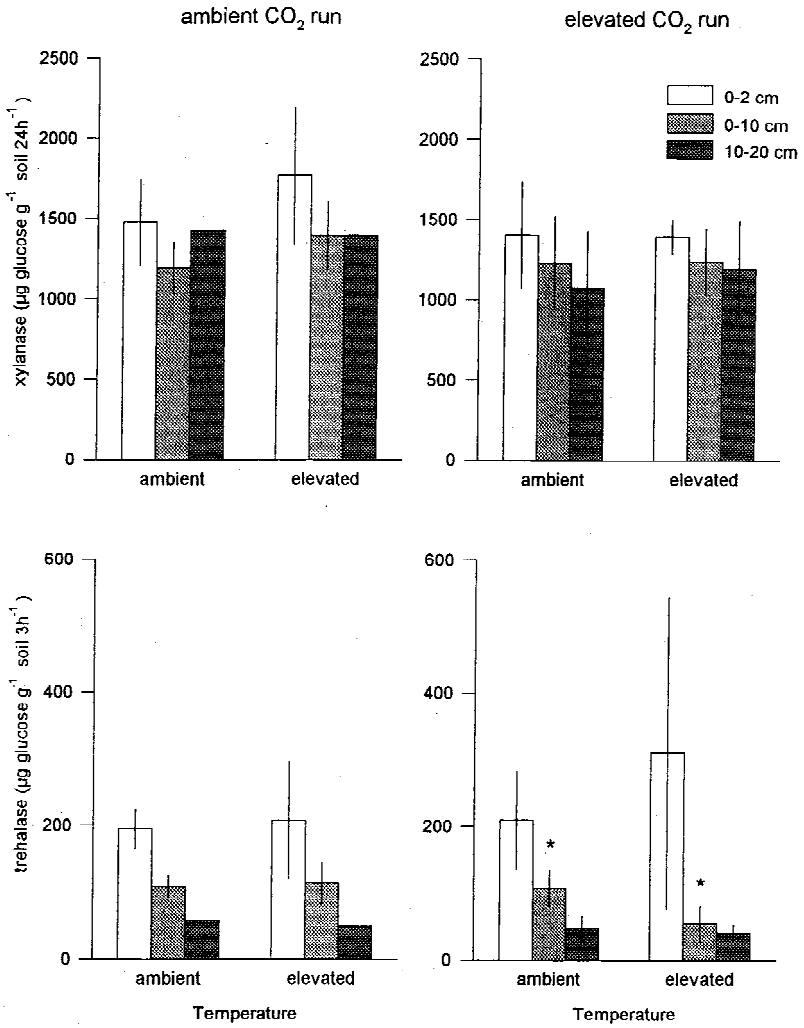


*Figure 3.* The effect of elevated temperature and elevated $CO_2$ on xylanase and trehalase activity in different soil layers of the Ecotron. (In the elevated $CO_2$ run $CO_2$ level were enhanced by 200 ppm $CO_2$). Significant temperature effects are marked by an asterisk.

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 $CO_2$ and temperature (3 °C increase).

It is not clear from the two experimental runs, why elevated temperature at ambient $CO_2$ did not change root biomass, whereas elevated temperature and elevated $CO_2$ decreased root biomass. There are various possible explanations; the die-back already observed for above ground plant biomass in elevated $CO_2$ 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 $CO_2$-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 $CO_2$ run may be depent 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, $C_3$) but not in *Bouteloua gracilis* (blue grama, $C_4$); a difference related to photosynthethic 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 $CO_2$ run) were not accompanied by an increase in $N_{mic}$. Elevated temperature and $CO_2$ increased the total PLFA after Generation 3 and this increase was associated with an increase in BactPFLA. 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 $CO_2$ 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 $CO_2$ 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 occured. 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 $CO_2$ efflux vary as a function of soil temperature: $Q_{10}$ 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 $CO_2$ there was no change in the moisture content of Ecotron soil; atmospheric $CO_2$ 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 $CO_2$ enrichment was only significant under dry soil water conditions (Rice et al., 1994)

*Metabolic quotient*

The difference in $qCO_2$ in the ambient $CO_2$ 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 $CO_2$ run, increased temperature did not change the $qCO_2$ 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 $CO_2$ (700 $\mu$L $L^{-1}$), and elevated $CO_2$ and a temperature enhancement of 6 °C were compared, Ross et al. (1995) found a small decrease of the $qCO_2$ of a three-year-old ryegrass/white clover pasture. The increase of the $qCO_2$ 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 $CO_2$ 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 $CO_2$ 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 $CO_2$. Arctic plants responded to elevated $CO_2$ 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 $CO_2$ 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. $CO_2$ 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 by 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.

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