Global Change Biology (2003) **9,** 585–600

Six years of *in situ* CO_2 enrichment evoke changes in soil structure and soil biota of nutrient-poor grassland

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Abstract

Nutrient-poor grassland on a silty clay loam overlying calcareous debris was exposed to elevated CO_2 for six growing seasons. The CO_2 exchange and productivity were persistently increased throughout the experiment, suggesting increases in soil C inputs. At the same time, elevated CO_2 lead to increased soil moisture due to reduced evapotransporation. Measurements related to soil microflora did not indicate increased soil C fluxes under elevated CO_2. Microbial biomass, soil basal respiration, and the metabolic quotient for CO_2 (qCO_2) were not altered significantly. PLFA analysis indicated no significant shift in the ratio of fungi to bacteria. 0.5 M KCl extractable organic C and N, indicators of changed DOC and DON concentrations, also remained unaltered. Microbial grazer populations (protozoa, bacterivorous and fungivorous nematodes, acari and collembola) and root feeding nematodes were not affected by elevated CO_2. However, total nematode numbers averaged slightly lower under elevated CO_2 (−16%, ns) and nematode mass was significantly reduced (−43%, $P = 0.06$). This reduction reflected a reduction in large-diameter nematodes classified as omnivorous and predacious. Elevated CO_2 resulted in a shift towards smaller aggregate sizes at both micro- and macro-aggregate scales; this was caused by higher soil moisture under elevated CO_2. Reduced aggregate sizes result in reduced pore neck diameters. Locomotion of large-diameter nematodes depends on the presence of large enough pores; the reduction in aggregate sizes under elevated CO_2 may therefore account for the decrease in large nematodes. These animals are relatively high up the soil food web; this decline could therefore trigger top-down effects on the soil food web. The CO_2 enrichment also affected the nitrogen cycle. The N stocks in living plants and surface litter increased at elevated CO_2, but N in soil organic matter and microbes remained unaltered. Nitrogen mineralization increased markedly, but microbial N did not differ between CO_2 treatments, indicating that net N immobilization rates were unaltered. In summary, this study did not provide evidence that soils and soil microbial communities are affected by increased soil C inputs under elevated CO_2. On the contrary, available data (^{13}C tracer data, minirhizotron observations, root ingrowth cores) suggests that soil C inputs did not increase substantially. However, we provide first evidence that elevated CO_2 can reduce soil aggregation at the scale from μm to mm scale, and that this can affect soil microfaunal populations.

Keywords: carbon and nitrogen dynamics, protozoa, soil aggregates, soil bacteria and fungi, soil food web, soil microfauna

Received 18 September 2002; revised version received and accepted 16 October 2002

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Introduction

Atmospheric CO_2 concentration increased from 280 to 360 $\mu L L^{-1}$ since preindustrial times (Amthor, 1995) and is expected to double before the end of the 21st century (IPCC, 1995). Experimental exposure of plants to elevated CO_2 concentrations has shown that photosynthesis and plant productivity generally increase under these conditions, although large variation among different species and ecosystems was found (Körner, 1996; Poorter *et al*., 1996). In many grasslands, growth stimulation seems in part to reflect soil moisture savings (cf. Jackson *et al*., 1998; Niklaus *et al*., 1998b; Owensby *et al*., 1999; Volk *et al*., 2000). If increases in grassland net primary productivity (NPP) persist over time, soil C inputs will ultimately increase via enhanced plant biomass turnover and/or root carbon (C) losses. Organic C supply limits or colimits soil microbial activity in most grasslands (Smith & Paul, 1990; van de Geijn & van Veen, 1993), and profound changes in soil C cycling and energetics can therefore be expected.

Natural or seminatural grassland cover a significant fraction of the world's terrestrial surface (Houghton, 1995; Parton *et al*., 1995); however, only few studies have investigated C fluxes under elevated CO_2 in such systems (Ross *et al*., 1995; Hungate *et al*., 1997; Niklaus *et al*., 2001a). This is in part due to methodological difficulties with assessment of soil C balances and plant-soil C fluxes (Hilbert *et al*., 1987; Darrah, 1996; Hungate *et al*., 1996; Lund *et al*., 1999; Niklaus *et al*., 2000). Exudation is a major component of soil C inputs, but there is virtually no data available on flux rates and chemical composition of exudates under field conditions. In hydroponic culture, large increases in exudation rates have been detected at elevated CO_2. These effects, however, are much smaller or absent in the field due to soil matrix-effects and resorption of organic compounds exuded by plants (cf. Darrah, 1996; Jones & Darrah, 1996). Experimental evidence on fine root turnover, another major source of soil C inputs, also is controversial (Fitter *et al*., 1996; Arnone *et al*., 2000; Pregitzer *et al*., 2000; Niklaus *et al*., 2001a); several studies even indicate a decrease in fine root turnover at elevated CO_2, sometimes associated with an increase in root longevity. Even less is known on the chemical quality of soil C inputs. The C : N ratio of green leaves generally increases at elevated CO_2, and the content of phenolic compounds and secondary metabolites sometimes also increases (Penuelas & Estiarte, 1998), which could slow down decomposition. However, tissue quality-effects often vanish during leaf senescence and are not any longer found in surface litter. Accordingly, rates of decomposition of naturally senesced litter often are not affected (grassland leaf litter: Hirschel *et al*., 1997; wheat root litter: Van Vuuren *et al*., 2000; but see Cotrufo & Ineson, 1996; Ball & Drake, 1997). Virtually nothing is known of the *in situ* decomposition of root litter or of effects of elevated CO_2 on the chemistry of root exudates. Thus, soil C inputs are likely to increase under elevated CO_2 (because plant biomass increases), but the extent of this increase and the pathways by which extra C enters the soil are currently unknown. Even if we knew the increase in soil C input under elevated CO_2, predictions of the response of the soil biota to this change would be complicated by a number of indirect effects and feedback mechanisms:

1. The C and N cycle are intimately coupled in natural ecosystems, and N availability colimits microbial biomass in many natural ecosystems (Merckx *et al*., 1987; Liljeroth *et al*., 1990; van de Geijn & van Veen, 1993). Microbial responses to elevated CO_2-induced extra C fluxes could therefore be limited. On the other hand, increased microbial growth under elevated CO_2 could lead to increased immobilization of available N, in turn decreasing plant growth (Diaz *et al*., 1993). Extra C supply could also stimulate microbial decomposition of native soil organic matter, thus improving plant N availability (Zak *et al*., 1993). These mechanisms are not exclusive (Hungate, 1999), and large changes in N availability may primarily occur in systems in which nutrient cycles are far from equilibrium (Niklaus & Körner, 1996; Hu *et al*., 1999).
2. The soil biomass (microflora plus soil fauna) is in itself a complex, highly dynamic subsystem, which consist of a wide variety of organisms organized at several trophic levels. Most studies of soil microbial responses to elevated CO_2 have been restricted to measurements of total microbial biomass, which is in essence a test for changes in the mass of the microflora (Hu *et al*., 1999 for a review). However, organisms at higher trophic levels such as protozoa, nematodes, and microarthropods exert important controls on population size and turnover rates of the microflora. Assessment of total microbial biomass alone may therefore miss ecologically important changes in C dynamics within the soil food web (cf. Kampichler *et al*., 1998). For example, extra C supplies at elevated CO_2 might stimulate growth of the microflora, but no extra biomass may accumulate, because it is grazed by soil microfauna, resulting in increased C turnover (and increased metabolic quotient of CO_2). Grazer populations may increase due to the extra food available.

   Despite the variety of organisms involved in and the complexity of the soil food web, the fluxes of C and energy are compartmentalized (Moore *et al*., 1993). Many soil species can be classified as grazing

primarily on bacterial biomass (e.g. protozoa and certain groups of nematodes), whereas others graze predominantly on fungi (other nematodes and microarthropods). Species within the same compartment share similar properties and are thus likely to respond as a unit to environmental changes. For example, species in the bacterial food chain in general have higher turnover rates and are more susceptible to desiccation than species depending on fungi.

3. Elevated CO_2 can affect the soil biota indirectly by altering soil physical conditions. In many experiments, elevated CO_2 increased soil moisture (Jackson *et al*., 1994; Owensby *et al*., 1997; Niklaus *et al*., 1998b), the effect being driven by reduced leaf conductance (Morison, 1985). These water savings can be compensated by increased leaf area, especially in communities with low ground cover. Large-scale atmospheric feedback could reduce these water savings (Amthor, 1999), but general circulation models (GCMs) predict more humid conditions for temperate latitudes so that (on average) future plant growth conditions will include increased CO_2 concentrations and moister soils (Coughenour & Chen, 1997; Neilson & Drapek, 1998).
4. Soil structure is an important determinant of plant productivity and microbial dynamics, primarily, because it affects water and nutrient fluxes and the niches of specific soil microbes. Consequently, indirect effects of elevated CO_2 on soil structure could strongly modify the responses of plants and soil microbes to elevated CO_2. Association of mineral particles with organic matter forms aggregates of different sizes and stability (Tisdall & Oades, 1982), the different size fractions harbouring distinct groups of soil microorganisms and organic matter transformations (Sessitsch *et al*., 2001). Domains of individual, parallel-orientated clay particles ($< 1\,\mu m$ thick) are held together by physical (electrostatic) forces, organic molecules, and cations. Several such domains, held together by polysaccharides and other organic compounds, plant, fungal and bacterial debris, form mechanically relatively stable microaggregates. Microaggregates in turn bind to larger and less stable macroaggregates, which are stabilized primarily by roots and hyphae. Elevated CO_2 has been shown to alter soil moisture and plant productivity. Thus, the amount of soil solution and the concentration of binding agents in soil solution may change, thereby affecting the processes controlling aggregation. Indeed, such effects have been reported in two-field (Rillig *et al*., 1999b; Niklaus *et al*., 2001c) and one greenhouse study (Niklaus *et al*., 2001a).

In this paper, we investigate effects of six years of CO_2 enrichment on soil aggregate structure, nitrogen dynamics, and composition of the soil biota of nutrient-poor calcareous grassland. The CO_2 exchange (Stocker *et al*., 1997) and productivity (Leadley *et al*., 1999) increased at elevated CO_2, suggesting increased soil C inputs. Soil moisture levels were persistently increased under elevated CO_2 (Niklaus *et al*., 1998b) due to reduced stomatal conductance (Lauber & Körner, 1997) and evapotranspiration (Stocker *et al*., 1997).

Specifically, we tested whether (1) soil aggregate size distribution was affected by elevated CO_2; (2) whether elevated CO_2 induced an increase in microbial immobilization and/or N mineralization, thus decreasing and/or increasing plant N availability, respectively; (3) microbial biomass or microbial grazer populations increased as consequence of the presumed extra C supply, and (4) this response, if there was any, occurred within the fungal or bacterial compartment of the soil food web. Results of these analyses are discussed in relation to a range of previously published data from the same trial.

Materials and methods

Study site

The field site is located in a nutrient-poor, south-facing species-rich calcareous grassland in northwestern Switzerland (47°33′N 7°34′E, 520 m asl, 20° slope). This pasture has been used for extensive grazing for at least a hundred years (Schläpfer *et al*., 1998). The plant community, characterized as *Mesobromion* community, contains over 90 plant species and is dominated by *Bromus erectus* Huds. which accounts for *ca.* half of the above-ground community biomass. A detailed description of the vegetation is given in Huovinen-Hufschmid & Körner (1998). These temperate grasslands are largely created by man, and are sustained by biomass removal through livestock grazing or mowing, which prevents dominance of rank grass and scrub. In our study, cattle grazing was replaced by extensive mowing, which is frequent practice. These grasslands once were common throughout central Europe, but today are restricted to remnants, primarily due to land use intensification and abandonment of unproductive sites. Plant communities are natural in the sense that, except for biomass removal, they evolved naturally; soils are not disturbed. The Rendzina-type soil, which is typical for these habitats, consists of a 10–15 cm neutral to slightly basic ($pH \approx 7,8$) silty clay loam top-soil and is underlain with calcareous debris. In the top 10 cm, the horizon, where most of the fine roots occur, organic C and N contents are *ca.* 3.9% and 0.33%, respectively (Niklaus, 1998).

Experimental design

A total of 24 plots (1.27 m^2 each) containing natural undisturbed vegetation and soil were selected. The plots were organized in a randomized complete block design with four pairs of blocks orientated perpendicular to the slope. Treatments, which were assigned randomly within each block, were unscreened ambient control plots ('C', 360 μL CO_2 L^{-1}, no screen), screened ambient CO_2 plots ('A', 360 μL L^{-1}, screened) and screened elevated CO_2 plots ('E', 600 μL L^{-1}, screened). The CO_2 enrichment was applied using a novel CO_2 enrichment technique utilizing small windscreens reaching a height of 50 cm above-ground and leaving a 7-cm gap at the bottom to allow free convection of air and movement of animals. This *screen aided CO_2 control (SACC)* allowed accurate control of CO_2 enrichment while still minimizing microclimatic impacts and permitting high spatial replication (Leadley *et al*., 1997). The CO_2 enrichment began in March 1994 and operated around the clock except during mid-winter (December–February) when it was shut down. In order to maintain the high species diversity common to these plant communities, each year plots were clipped at a height of 5 cm in June and October

Soil sampling

When the experiment was destructively harvested (June 22–August 1, 1999), soil samples (0–10 cm) were taken concurrently with removal of the plant material These samples were used for microbial biomass determination, extraction of microarthropods and nematodes, analysis of enzyme activities, extraction of soluble organic C, and soil physical fractionation. Plots were harvested blockwise, and samples were packed on ice until transport back to the lab. Soil samples have been taken on April 20 for enzyme analysis and on April 26 for microbial biomass determination by substrate-induced respiration (SIR).

Soil moisture

Soil moisture was determined gravimetrically in soil cores probing 0–10 cm, and the result converted to volumetric soil moisture using independent bulk density measurements (Niklaus *et al*., 1998b).

Soil microbial biomass and activity

Soil microbial biomass C and N was determined by chloroform fumigation–extraction (Brookes *et al*., 1985; Vance *et al*., 1987) and by substrate induced respiration (SIR; Anderson & Domsch, 1978). Soil basal respiration, a measure of respiratory activity of the microbe/soil system in the absence of exogenous C sources, was determined by O_2 consumption. The metabolic quotient for CO_2 (qCO_2) was calculated by dividing basal respiration by microbial biomass measurements by SIR.

For chloroform fumigation–extraction soils were sieved (2 mm mesh size), homogenized, and remaining roots carefully removed with tweezers. One of two field-moist subsamples (water content of 40–50% water capacity, weight corresponding to 20 g dry weight) was immediately extracted for 30 min with 100 mL 0.5 M K_2SO_4 (table shaker at 200 rpm). The second sample was fumigated with ethanol-free $CHCl_3$ for 24 h and $CHCl_3$ removed by repeated evacuation. The evacuation time was kept to a minimum in order to minimize losses of volatile nitrogenous compounds (i.e. ammonia and organic amines). After removal of the chloroform, fumigated samples were extracted in the same way as the unfumigated soils. All extracts were centrifuged (10 min at 1000 g), filtered (No. 589 3½, Schleicher und Schüll, Dassel, Germany) and kept frozen until further analysis. Organic C and N in the extracts were determined using an automated TOC/TON analyser (DIMA TOC-100 with TN_b extension, Dimatec, Essen, Germany) and microbial biomass C (C_{mic}) calculated as $C_{mic} = (C_{fumigated} - C_{unfumigated})/k_{EC}$, where k_{EC} is the extraction efficiency for microbial C ($k_{EC} = 0.29$; obtained by calibration against SIR measurements) and $C_{fumigated}$ and $C_{unfumigated}$ are the organic C contents of the extracts of fumigated and unfumigated soils, respectively. Microbial biomass N was similarly calculated using $k_{EN} = 0.54$ (Brookes *et al*., 1985).

For determination of microbial biomass by SIR, sieved fresh soil (2 mm mesh size) was adjusted to a water content of 50% of its water holding capacity and preincubated for 3 days at 22 °C. Thereafter samples were amended with a glucose/talcum mixture, substrate-induced respiration measured for 6 h in an automated, custom-built multichannel device equipped with an infrared gas analyser, and SIR converted to the plot's microbial biomass C following the procedure outlined in Anderson & Domsch (1978). Basal respiration and qCO_2 were determined similarly using a slightly different system. Soil samples were preincubated as described above, but for 5 days at 20 °C. Then, respiration of samples was measured in an automated system measuring O_2 consumption by electrolytic O_2 microcompensation (thus accounting only for aerobic activity, see Scheu, 1992 for a detailed description). Basal respiration was determined as average O_2 consumption between 15 and 20 h, and the metabolic quotient for CO_2 (qCO_2, h^{-1}) was calculated by dividing basal respiration by the microbial biomass of the same samples (which was measured by SIR in the same apparatus).

Microarthropods

Soil microarthropods were extracted from soil using a Macfayden canister extractor (Edwards, 1991). Briefly, soil samples were inserted upside down into the sample holder, and animals extracted by heating the soil samples from the top thus driving soil animals downwards were they were collected in a trap containing saturated aqueous picric acid. Temperatures applied for extraction were 3 days at 30 °C, followed by (in this order) 2 days at 35 °C, 1 day at 40 °C, 1 day at 45 °C and finally three days at 50 °C. Thereafter, the animals were transferred to a 75% aqueous ethanol in which they remained stored until counting.

Nematodes

Soil samples were sieved (4 mm) and nematodes extracted using a modified O'Connor wet extraction apparatus. Forty hours after the beginning of the extraction, temperature was increased from 20 °C to 45 °C over 6 h, while trapping vessels were cooled to 13–15 °C (Alphei, 1995). Subsequently, nematodes were killed by heat and fixed in 4% formaldehyde solution. Total numbers of nematodes were counted under a dissection microscope. Out of each sample, 100 specimens were picked at random and mounted on slides for identification and biomass determination. Nematodes were sorted into six trophic groups (bacterial feeders, fungal feeders, root hair feeders, omnivores, plant parasites, predators) according to Yeates *et al*. (1993). Individual fresh weights were calculated using the formula of Andrássy (1956). Dry weights were calculated as 22.5% of fresh weights (cf. Buecher & Hansen, 1971; Sohlenius, 1979).

PLFA

Phospholipid fatty acids (PLFA) were analysed following the procedure used by Frostegård *et al*. (1993). Briefly, 1 g of moist soil was incubated for 2 h in a single-phase chloroform–methanol–citrate buffer mixture. Then, the solution was split into two phases by further addition of chloroform and citrate buffer and the samples centrifuged. The organic phase containing the microbial lipids was dried at 40 °C under a nitrogen stream. The lipids isolated were then fractionated into neutral, glyco- and polar-lipids including phospholipids using Si-columns (Varian, Harbor City, USA). Then, the phospholipid fraction was dried under a stream of N_2 and methylnonadecanoate added as internal standard to the samples. The phospholipids were converted to fatty acid methyl esters by mild alkaline methanolysis and analysed by capillary gas chromatography (Autosystem XL, Perkin-Elmer, Norwalk, CT, USA, fitted with a 50 m capillary column [HP-5, Agilent, Palo Alto, CA, USA] and a flame ionisation detector).

Following Frostegård & Bååth (1996), the ratio of the fungal 18 : 2**ω**6–10 bacterial PLFA was used as indicator of fungal to bacterial biomass ratios (the bacterial PLFA i15 : 0, a15 : 0, 15 : 0, i16 : 0, i17 : 0, a17 : 0, 17 : 0, cy17 : 0, 18 : 1**ω**7, cy19 : 0 were used in the calculation; Frostegård and Bååth also included 16 : 1**ω**5 and 16 : 1**ω**9, but these could not be determined with our equipment). A ratio of 20 : 4**ω**5 to total PLFA was used as indicator of protozoan biomass fraction (Paul & Clark, 1996).

Soluble organic C

Fresh soil samples corresponding to 25 g dry matter were extracted for 20 min in 80 mL 0.5 M KCl. Suspensions were filtered (No. 589 3½, Schleicher und Schüll, Dassel, Germany), acidified with HCl to remove inorganic carbonate, and analysed for total organic C and N using an automated TOC/TON analyser (as above).

Soil aggregate size distribution

Soil physical structure was determined using two methods: (1) mild chemical dispersion followed by wet-sieving, yielding macroaggregates, and (2) determination of the microaggregate size distribution after soil disintegration using soil organic matter oxidation and chemical dispersion:

Fresh soil samples corresponding to 150 g dry matter were suspended in 170 mL of 0.5% sodium hexametaphosphate and gently shaken for 2 h. Hexametaphosphate improves dispersion and binds Ca^{2+} ions, which otherwise would precipitate with the density separation agent (polytungstate). The suspension was washed through a stack of sieves (2000, 1000, 500, 250 and 125 μm) with 3 L of water. A few macroaggregates, held together by plant root systems, were larger than 2000 μm and remained on the top sieve. These aggregates were disintegrated by forcing them through the top-most sieve. The fraction < 125 μm was centrifuged for 10 min at 2000 g and the supernatant discarded. The obtained size fractions were stirred into 100 mL aqueous sodium polytungstate (SOMETU, Falkenried, Germany) solution with a density of 1.7 g cm^{-3}. The suspension was allowed to settle for 10 min. Then, the low-density macroorganic supernatant was removed and the high-density organomineral fraction ($\rho > 1.7$ g cm^{-3}) was vacuum-filtered through a glass fibre filter (GF9, Schleicher und Schüll, Dassel, Germany), washed with distilled water and dried at 80 °C for 48 h.

The distribution of microaggregates was determined using samples corresponding to 10 g dry weight. Organic matter was destroyed by amending moist soils with 60 mL H_2O_2 (15% volume) and heating to 90 °C. Further

H_2O_2 was added until gas development ceased. Then, soils were dispersed by adding 200 mL 0.0125 M $Na_4P_2O_7$ and shaking end-over-end for 16 h. The sand size fraction (> 63 μm) was separated from the resulting suspension by wet sieving. The remaining fractions were further separated into silt and clay fractions by sedimentation. All samples were dried overnight at 105 °C, weighed, and the result corrected for the mass of $Na_4P_2O_7$ contained in the samples. This procedure does *not* yield the fundamental mineral particles – there is still some level of aggregation, which is what we were interested in (see Results for details).

Statistical analysis

All data were log-transformed prior to analysis of variance (ANOVA). According to the experimental design, the ANOVA model included the factors Block and SACC/CO_2 treatment. To test for effects of SACC and CO_2 alone, *a priori* linear contrasts were used to compare the corresponding treatment within the SACC/CO_2 factor. For analysis of subplot data (e.g. different aggregate size-fractions within each plot), a hierarchical design containing the following additional terms was used: Plot (CO_2), Size, CO_2·Size. The main effects of CO_2 were tested against Plot (CO_2). Size- and CO_2·Size-effects were tested against the residual. All model terms were fitted sequentially as in Type I ANOVA models. The CO_2 effects within size classes were tested using linear contrasts (LMATRIX subcommand of the UNIANOVA procedure in SPSS 9.0; SPSS, Chicago, IL). Repeated measures analysis was done with the multivariate repeated measures ANOVA procedure of SPSS. Effects with $P \leq 0.05$ are considered as significant, effects with $P \leq 0.1$ as marginally significant. Error estimates given in the text and error bars in figures are standard errors of the means. In figures (*) indicates $P \leq 0.1$, *$P \leq 0.05$ and **$P \leq 0.01$, ***$P \leq 0.001$.

Results

Soil moisture

Soil moisture generally increased under elevated CO_2 ($P < 0.001$; Fig. 1). For a detailed description of CO_2-effects on soil water relations see Niklaus *et al.* (1998b), where seasonal and diurnal dynamics of soil moisture are presented and the interplay between leaf area index (LAI), stomatal conductance, and canopy temperature is reported. Screened ambient CO_2 plots were generally drier than unscreened control plots, primarily because of increased air movement due to the blowers used for CO_2 disposal. Soil moisture under elevated CO_2 was approximately equal to that in unscreened control plots, i.e., CO_2 enrichment and screening of plots had opposite effects on soil water of approximately equal magnitude. It should be noted, however, that the differences in soil moisture between CO_2 treatments, though systematic, were only a small fraction of the changes in soil moisture occurring during regular soil drying cycles and that inter-annual variability also is considerable.

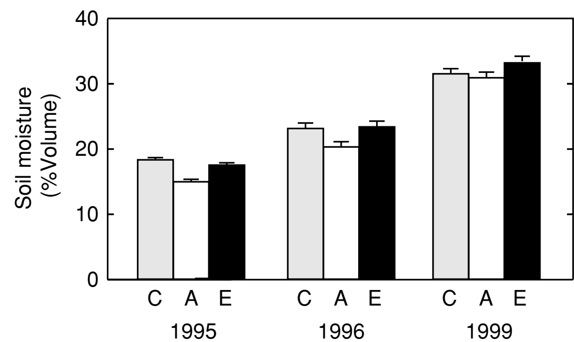

Fig. 1 Peak biomass (June) water content in top 10 cm of soil. The effects of elevated CO_2 and plot screening shown are typical for soil moisture conditions throughout the experiment and significant at $P < 0.01$ (repeated measures analysis; C = unscreened control plots, A = ambient CO_2 plots, E = elevated CO_2 plots).

Soil physical structure

The CO_2 enrichment caused a shift in soil organomineral aggregate distribution towards smaller sizes ($P = 0.004$; Fig. 2a). There was a lower mass of macroaggregates > 125 μm in diameter ($P = 0.03$), and consequently a greater mass < 125 μm. Screening of plots caused a similar effect, but opposite in direction.

Microaggregate size distribution also revealed a shift towards smaller sizes at elevated CO_2 (Fig. 2b; $P = 0.01$ for the CO_2-size interaction). Again, an opposite effect of plot screening was detected ($P = 0.02$ for the SACC-size interaction).

X-ray diffraction measurements, which were run test-wise on a limited number of samples, did not reveal differences in clay mineral composition between CO_2 treatments. The dominant clay minerals present were illite, kaolinite, and chlorite. Quartz and calcite were not present in the samples.

Extractable organic C and N

Increased plant root exudation or fine root turnover would increase microbial C availability and could be reflected in increased dissolved or extractable organic C levels. However, no such increase was found (Fig. 3). Total extractable N, comprising both dissolved and extractable organic N as well as inorganic N also did not change under elevated CO_2.

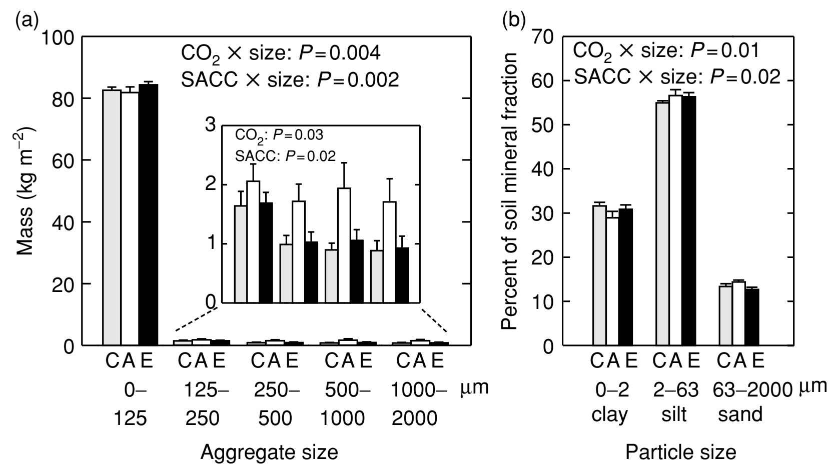

Fig. 2 Effects of elevated CO_2 on soil structure. (a) Organomineral aggregate sizes and (b) microaggregate size distribution after oxidation of soil organic matter with hydrogen peroxide and dispersion with pyrophosphate. (Note that dispersion is still incomplete. After further dispersion and organic matter oxidation, 7% of the minerals were recovered in the sand size fraction, 52% as silt, and 41% as clay; C = unscreened control plots, A = ambient CO_2 plots, E = elevated CO_2 plots).

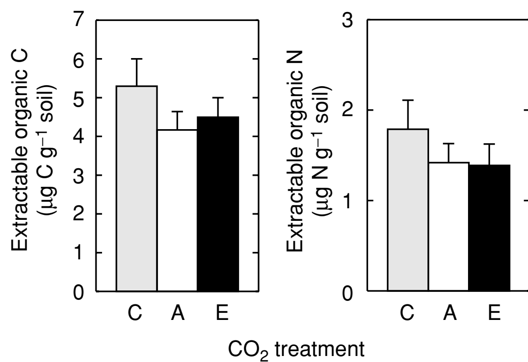

Fig. 3 Soil organic C and total N extractable with 0.5 M KCl (C = unscreened control plots, A = screened ambient CO_2 plots, E = screened elevated CO_2 plots).

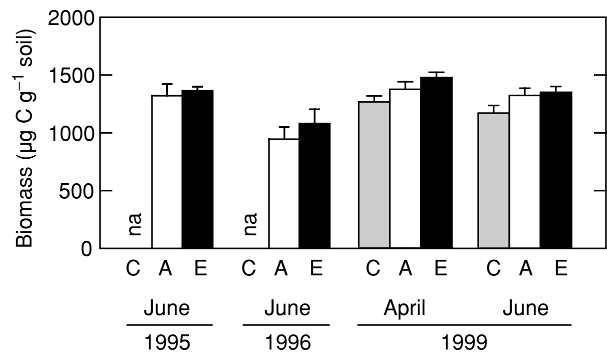

Fig. 4 Biomass of soil microflora as determined by substrate-induced respiration (SIR). Differences between ambient and elevated CO_2 are not significant. Peak season data from pervious years (June 1995 and 1996; Niklaus *et al*., 1998b) is also included in the figure. Due to the limited number of samples that could be handled parallel and the absence of windscreen effect in previous harvests (cf. Niklaus *et al*., 1998b), SIR of unscreened controls was not assessed in June 1995 and 1996. (C = unscreened control plots, A = ambient CO_2 plots, E = elevated CO_2 plots; na = not assessed).

Microbial biomass

Soil microbial biomass C measurements made by chloroform fumigation–extraction, and by SIR were significantly correlated ($r^2 = 0.45$, $P < 0.001$). Elevated CO_2 did not alter microbial C throughout the six years of CO_2 treatment, regardless of the method used (−1%, ns, when measured by fumigation–extraction; +6%, ns, when measured by SIR/O_2 consumption; +2%, ns, when measured by SIR/CO_2 release, see Fig. 4 and Table 1). Also, microbial N and C/N were not significantly altered by elevated CO_2 (Table 1). Repeated measures analysis covering all measurements made during the experiment also did not reveal any effect of elevated CO_2 (measurements in Fig. 4 plus data from the first three years of treatment previously published in Niklaus, 1998, i.e., additional measurements in October 1994, March 1995, October 1995, March 1996 and October 1996).

Screening of plots caused no effect on microbial biomass as measured by $CHCl_3$ fumigation–extraction (+6%, ns). Plot screening caused a marginally significantly decrease in SIR when measured by CO_2 release (−11%, $P = 0.07$), but no change when measured by O_2 consumption (+7%, ns), possibly indicating an increase in mass or activity of aerobe relative to anaerobe organisms.

Basal respiration and qCO_2

Elevated CO_2 altered neither basal respiration (+10%, ns) nor qCO_2 [+3.8%, ns $(3.0 \pm 0.3)\cdot 10^{-3}\,h^{-1}$ and $(3.1 \pm 0.3)\cdot 10^{-3}\,h^{-1}$ at ambient and elevated CO_2, respectively]. Screening of plots reduced basal respiration (−22%, $P = 0.02$) and consequently marginally significantly reduced qCO_2 [−18%, $P = 0.07$ $(3.7 \pm 0.3)\cdot 10^{-3}\,h^{-1}$ in the unscreened control plots].

Protozoa, bacteria and fungi

PLFA data did not reveal significant shifts in the ratio of protozoa, bacteria, and fungi.

Soil nematodes

Total nematode numbers averaged lower under elevated CO_2 (−16%, ns; Fig. 5a) and total nematode weight was significant reduced (−43%; $P = 0.06$). This reduction was primarily driven by a decline of large nematodes under elevated CO_2 (omnivores and predators), whereas mass and numbers of other nematodes trophic groups did not change significantly (Fig. 6). Screening of plots generally had opposite effects, so that nematode numbers and weights in elevated CO_2 were equal to numbers in unscreened control plots.

Soil microarthropods

Not one of acari (−1.5%, ns), collembola (+7%, ns) or total microarthropod individual numbers (+0.4%, ns) were altered by elevated CO_2 (Fig. 5b). Screening of plots, however, decreased the numbers of microarthropods (−25% for acari, −40% for collembola, and −30% for total microarthropods, $P < 0.01$ for all).

Nitrogen dynamics and partitioning within the ecosystem

At peak biomass, microbes contained approximately the same amount of N as did plant shoots plus roots (Fig. 7). The N stocks in living plants and surface litter increased, but the amount in soil organic matter and microbes remained unaltered. Nitrogen mineralization, as assessed in soil incubations, increased markedly in April (+30%; $P = 0.05$) but not significantly in June (Table 2).

Discussion

During the six years of treatment, above-ground plant biomass accumulation (> 5 cm layer) increased in response to CO_2 alone between 5 and 32% (average response of 21%). Below-ground biomass showed substantial spatial variability (Leadley *et al*., 1999) and short-term (up to several weeks) turnover of fine root biomass may be a more important component of below-ground NPP than are seasonal growth (spring) and die-back (autumn) of roots. Nevertheless, increased root biomass at the end of the experiment, and increased above-ground growth both suggest increases in soil C inputs under elevated CO_2, although the magnitude of these fluxes remains uncertain.

Table 1 Microbial C and N as determined by chloroform fumigation–extraction (June 1999)

	CO_2 treatment Control	Ambient CO_2	Elevated CO_2	Relative effect of CO_2
Microbial C (μg C (g soil)$^{-1}$)	1238 ± 52	1319 ± 46	1307 ± 31	−1%, ns
Microbial N (μg N (g soil)$^{-1}$)	133 ± 9	154 ± 8	150 ± 7	−2%, ns
Microbial C : N (μg C (μg N)$^{-1}$)	9.5 ± 0.3	8.6 ± 0.2	8.8 ± 0.2	+2%, ns

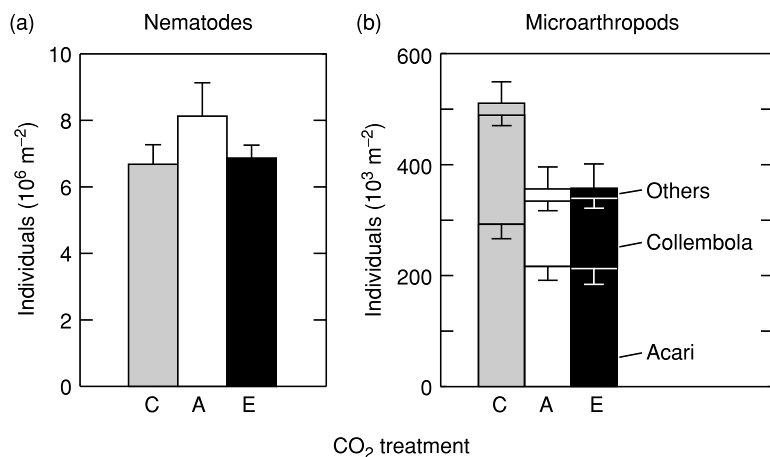

Fig. 5 (a) Number of nematode individuals and (b) number of collembola, acari and total microarthropod individuals in experimental treatments (C = unscreened control plots, A = ambient CO_2 plots, E = elevated CO_2 plots).

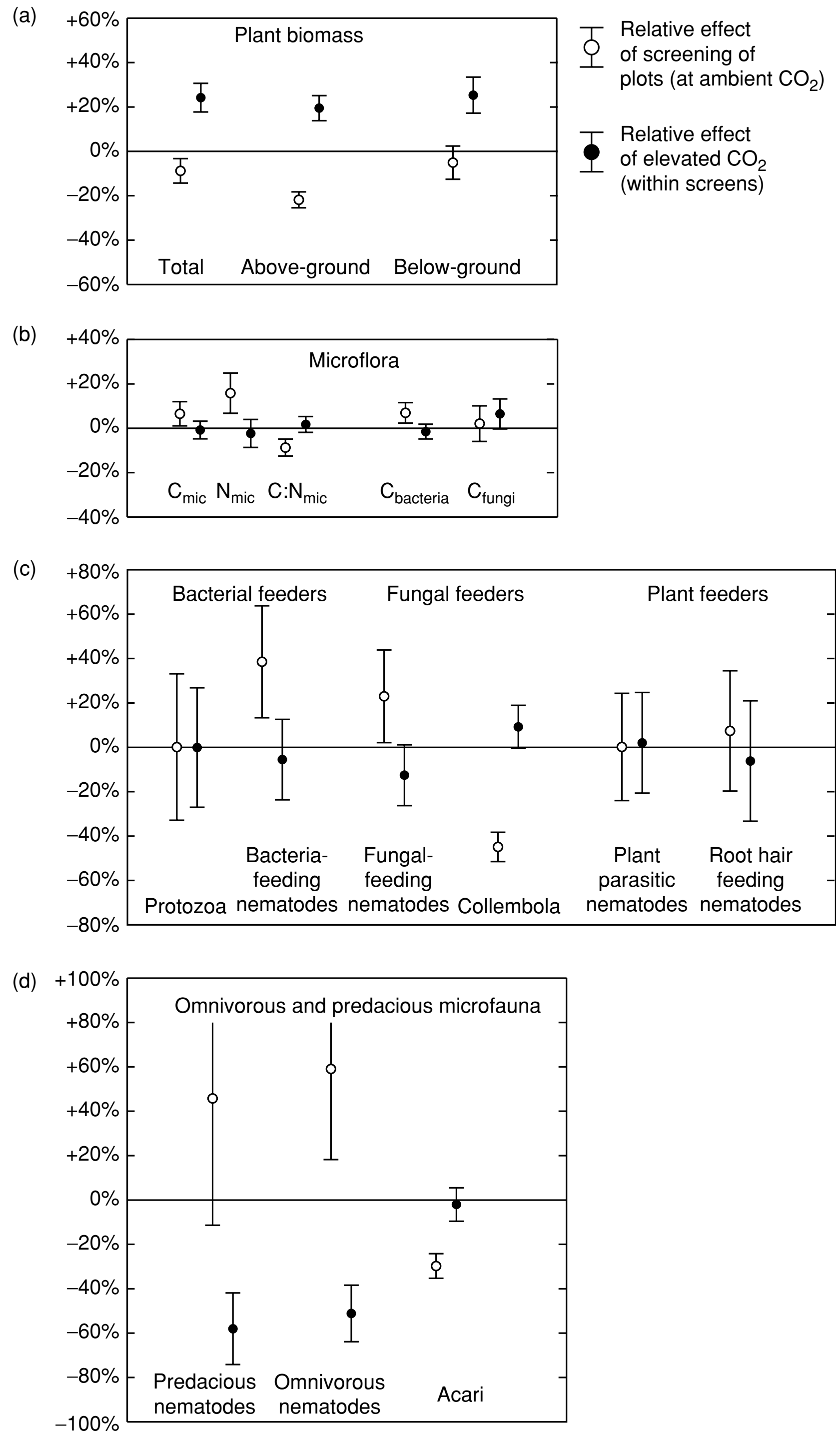

Fig. 6 Relative effects of windscreens (at ambient CO_2) and CO_2 enrichment (within windscreens) on soil biota components. (a) Effects on plant biomass, above- and below-ground, are given for comparison. (b) Effects on total soil microflora and on the bacterial and fungal fraction. The bacteria/fungi-separation is based on characteristic bacterial and fungal PLFA. (c) Effects on soil microfauna, tentatively grouped into bacterial and fungal grazers and organisms feeding predominantly on plants. (d) Effects on omnivorous or predacious organisms, or groups of organisms not sorted to species and containing predacious or omnivorous animals (mites). All data are weight based, with the exception of microarthropods for which no weight data was available and effects are based on the number of individuals.

Elevated CO_2 did affect soil community structure and nutrient dynamics. The observed effects appear to rely on three mechanisms: (1) increased soil moisture availability under elevated CO_2; (2) reduced soil aggregate sizes, which may be related to effects on soil moisture; (3) plant productivity-related effects of elevated CO_2.

Plant productivity-driven effects of elevated CO_2 can be separated from soil-moisture driven effects on soil communities by factorially combining the CO_2 treatment with a soil moisture/irrigation treatment (cf. Volk *et al.*, 2000). In the present study, this was tentatively possible because (1) the windscreen-effect on soil moisture was

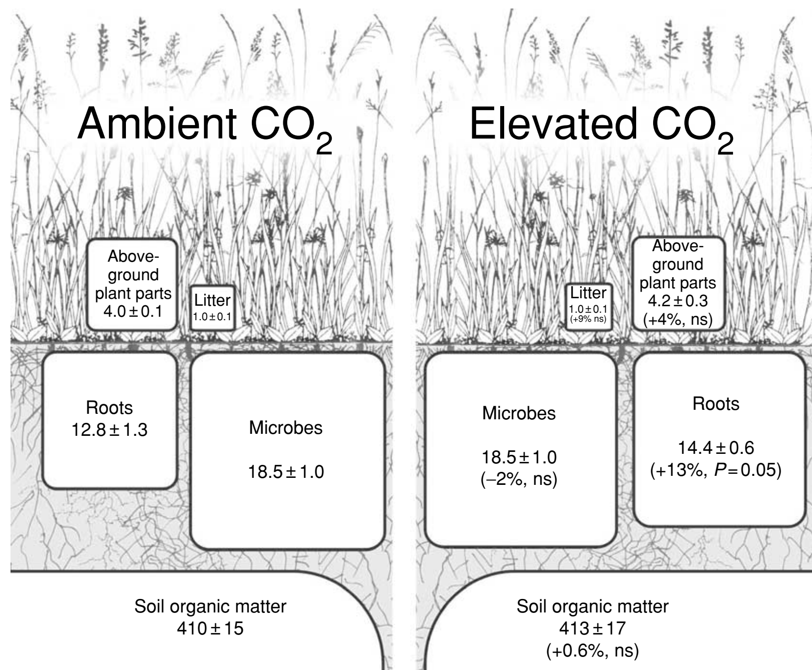

Fig. 7 Peak biomass nitrogen partitioning within the ecosystem. Data refer to 1999 when the experiment was terminated after six growing seasons. All data are in $g\,N\,m^{-2} \pm$ standard error of the mean.

Table 2 Nitrogen mineralization rates determined in lab-incubated soil samples

Date	CO_2 treatment Control	Ambient CO_2 ($\mu g\,N\,g^{-1}$ soil d^{-1})	Elevated CO_2	Relative effect of screening	Relative effect of CO_2
April 1999	14.7 ± 1.0	13.3 ± 1.6	17.3 ± 1.4	−9%, ns	+30%, $P = 0.02$
June 1999	8.7 ± 0.7	8.9 ± 0.8	9.1 ± 0.4	+2%, ns	+3%, ns

almost exactly compensated for by the elevated CO_2-induced reduction in evapotranspiration (cf. also Fig. 1), and (2) by and large plant growth responded to CO_2 enrichment, but not to screening (averaged over all years of treatment: screening effect: −6%, ns; CO_2-effect: +21%, $P < 0.01$). This indicates that at least part of the observed effect of elevated CO_2 on productivity did not depend on soil water savings. Overall, the comparison of unscreened control and screened ambient treatment roughly tests for soil moisture effect alone, whereas the comparison on screened ambient and screened elevated CO_2 plots tests for the effect of elevated CO_2 including the alteration of soil H_2O.

Soil structure

Elevated CO_2 caused a shift towards smaller aggregate sizes. Interestingly, this shift was also found in microaggregates containing only few mineral particles (Lal, 2000). The CO_2-effects observed in these microaggregates suggest that the aggregate size shift occurred over the entire size range of μm to mm.

Treatment effects on aggregate sizes and on soil moisture exhibited similar patterns, suggesting that soil water savings at elevated CO_2 drove the changes in soil physical structure. Soil aggregates are created by shrinkage on drying and stabilization by microbial debris, fungal hyphae, roots and polysaccharides. More pronounced drying–wetting cycles in screened ambient CO_2 plots and increased concentrations of binding agents at lower soil moisture may have improved aggregation (J. M. Oades, pers. comm.; Oades, 1978; 1984).

It is noteworthy that Rillig *et al*. (1999b) observed the opposite effect, i.e., increased aggregate sizes in two Mediterranean grassland ecosystems under elevated CO_2. Root and fungal biomass were increased, which is probably the driver of these changes (Rillig *et al*., 1999a). Rillig *et al*. further speculated that increased mycorrhizal secretion of glomalin in elevated CO_2 plots was a key in increased soil aggregation. Another potential explanation for the discrepancy to our study might be that Rillig *et al*. used a much more gentle soil fractionation technique than we did (immersion of soil in H_2O, whereas in the present study soils were very gently

shaken for several hours in 1% hexametaphosphate). It may therefore well be that both methods simply assess a different hierarchical level of soil aggregation.

The consequences of these particle size shifts are yet largely unclear. Since soil texture is an important determinant of gas diffusivity and redox potential, it appears likely that the release of radiatively active trace gases such as CH_4 and N_2O could increase under elevated CO_2. Indeed, this has been observed in several studies (Megonigal & Schlesinger, 1997; Arnone & Bohlen, 1998; Ineson *et al*., 1998; Phillips *et al*., 2001a, b).

Carbon and nitrogen dynamics

Total soil microbial biomass did not increase at elevated CO_2, even after six years of treatment. It is noteworthy that a disparity between microbial biomass responses and the stimulation of root system size has also been observed in most other studies. This suggests either that soil C inputs do not scale proportionally with root mass (e.g. because the ratio of coarse to fine roots changes, possibly for purely allometric reasons), or that soil microbes respond less than proportionally to increased C inputs. Reasons for the latter may include strongly delayed responses due to long turnover times of related soil pools and nutrient limitations.

We have previously speculated that the lack of effect on total microbial biomass was related to N-limitation of microbial growth (Niklaus, 1998). The basis for this reasoning was that short-term (one week) laboratory incubations with extra C supplied only induced substantial microbial growth when samples were amended with extra mineral N as well. Phosphorus, on the other hand, was readily acquired from soil organic matter. These limitations correspond to the limitations observed on plant growth: overall productivity of this grass-dominated ecosystem is N-limited; legumes fix atmospheric N but comprise only $<5\%$ of peak community biomass, because their growth is *P*-limited (J. Stöcklin, pers. comm.; Niklaus *et al*., 1998a). Another possible explanation for the lack of microbial biomass response to elevated CO_2, however, is that soil C inputs did not increase as much as we initially believed, based on photosynthetic responses. This suspicion is supported by the lack of increase in DOC concentrations; by ^{13}C pulse labelling experiments (Niklaus *et al*., 2001a) which did not show any statistically significant increases in microbial biomass labelling and turnover at elevated CO_2; by soil fractionation experiments which did not reveal any increases in low-density macro-organic matter (Niklaus *et al*., 2001c); and by minirhizotron observations which did not show any increase in fine root turnover under elevated CO_2 (Arnone *et al*., 2000).

At peak biomass, microbes contained approximately the same amount of N than plant shoots plus roots did (cf. Fig. 7). Almost all vascular plant species in this grassland are perennial. Plants cover part of their N needs during growth from retranslocation from root and rhizomes to the shoot; nevertheless, a large part of the N required is taken up from the soil. Immobilization of extra N in microbial biomass could therefore critically reduce plant N availability and limit growth. This limitation would be most severe when nutrient assimilation by plants and enhanced input of easily available C into the soil coincide, i.e., during periods of vigorous shoot (high N demand) and root growth (high soil C inputs). In our grassland, this corresponds to the mid-spring period (cf. root and shoot biomass data in Leadley *et al*., 1999 and Niklaus *et al*., 2001b). Microbial biomass N, however, did not differ between CO_2 treatments in April 1999, indicating that net N immobilization rates did not differ. However, soil N mineralization was substantially increased under elevated CO_2 (+30%, but no effect of windscreens; cf. Table 2). Taken together, our data supports the feedback mechanism proposed by Zak *et al*. (1993) rather than the one demonstrated by Diaz *et al*. (1993), although these are not mutually exclusive.

Soil food web

Animals inhabiting the litter layer or living very close to the soil surface (collembola and mites) were affected by plot screening; their numbers were reduced, presumably due to the increased air movement. Soil microbial C and plant growth, however, were not affected; and nematode numbers increased in the drier, screened plots (and decreased in elevated CO_2). Nematodes presumably did not suffer from drier soils because they live within an aggregates surface water film, which does not dry out until the soil matrix potential gets very low. Locomotion of nematodes is even more effective in thin water films (Wallace, 1958). Experiments (G. Yeates, pers. comm; Yeates *et al*., 2002 and references therein) demonstrated that nematode activity including reproduction occurs in water films as thin as 1 μm (which is a fraction of the nematodes body diameters of up to ~50 μm). Yeates *et al*. argue that the concentration of soil nutrients would increase in thin water films and that grazing of surface-bound microbes would also become more efficient. This mechanism is compatible with our finding that nematode numbers decreased under elevated CO_2 (higher soil H_2O), despite the anticipated extra food available.

Nematode migration is limited by pore neck sizes (cf. Yeates *et al*., 2002). The reduction in soil aggregate sizes under elevated CO_2 will also have reduced pores sizes, providing an alternative explanation for the reduced nematode numbers. This effect would be most

Table 3 Elevated CO_2 studies reporting microfaunal effects (a) Experiments with planted ecosystems, conducted in pots and microcosms (b) Studies in natural or seminatural ecosystems

Ecosystem	CO_2 treatment ($\mu mol\ CO_2\ mol^{-1}$)	Experimental duration	Plant responses		Soil microbial responses						Reference
			Shoots	Roots	Microflora	Fungi	Bacteria	Protozoa	Nematodes	Microarthropods	
(a) Pot and microcosm studies											
Artemisia tridentata	700, low N	12 weeks	+11%, ns	+41%	+16%, ns	+32%	+6%, ns		+32%	+14%, ns	Klironomos *et al.*, 1996
	700, High N	12 weeks	+23%, ns	+41%	+70%, ns	+100%	+44%		+88%	+47%	
Brassica x euramericana	700, low N	23 weeks			−15%, ns†			+548%†		+38%†	Lussenhop *et al.*, 1998
	700, high N	23 weeks			−34%, ns†			+151%†		−24%†	
Brassica nigra	700	4 weeks	−11%, ns	+2%, ns			−7%, ns	+6%, ns			Treonis & Lussenhop, 1997
Mixtures of *Cardamine hirsuta*, *Poa annua*, *Senecio vulgaris* and	560	4.5 months		−46%*	ns	ns	ns			52%	Kampichler *et al.*, 1998
spergula arvensis	560	9 months		88%*	ns	ns	ns			ns	Jones *et al.*, 1998
(b) Naturally established ecosystems											
Perennial pasture	700	420 days	+10%, ns	77%	ns				+11%		Yeates *et al.*, 1997; Ross *et al.*, 1996
Annual grassland on	700	4 growing seasons				ns	ns	ns	ns		Hungate *et al.*, 2000
sandstone soil	700	6 growing seasons		+23%, ns		+68%	−8%	+8%		+109%	Rillig *et al.*, 1999a
Annual grassland on	700	4 growing seasons				ns	ns	ns	ns		Hungate *et al.*, 2000
sand stone soil	700	6 growing seasons		+49%		+93%	+17%	−15%	ns	+39%	Rillig *et al.*, 1999a
Perennial low-fertility grassland	600	6 growing seasons	+19%	+25%	ns	ns	ns	ns	−43%	ns	This study

*0–10 cm, no significant response in 10–20 cm layer.
†rhizo sphere soil.

pronounced in large animals; indeed, the largest reductions in animal numbers were observed in omnivorous and predator nematodes (Fig. 6), whereas smaller nematodes showed no such effect. These nematodes are at relatively high trophic levels; therefore, the effect of CO_2 enrichment on soil structure could trigger a soil-structure mediated top-down effect on the soil food web.

Microfaunal effects of elevated CO_2 have only been studied in a limited number of studies (Table 3). There are probably too few experiments to draw any generalizations. Nevertheless, we speculate that effects occur primarily when root biomass is stimulated by elevated CO_2 and that the bacterial part of the food web responds more than the fungal part when rapid root growth occurs (which favours exudation and sloughing of root tissue) and when elevated CO_2 increases soil moisture. Indeed, increased protozoan numbers have been observed in rhizosphere soil of *Populus x euramericana* hybrids grown at elevated CO_2 and were associated with a decrease in microbial biomass (Lussenhop *et al*., 1998). In this experiment, root biomass and fine root turnover increased strongly under elevated CO_2. No effects on total protozoa were detected in annual grassland (Hungate *et al*., 2000) or in *Brassica nigra* pot systems (Treonis & Lussenhop, 1997; but plant growth did not increase under elevated CO_2). We did not detect any effects on protozoan biomass in this study; however, estimates were based on characteristic protozoan PLFA and statistical power was relatively low (Ebersberger *et al.* unpublished). A general problem with effects on organisms turning over quickly is that huge population changes can occur after relatively minor disturbances, triggering a series of successive population peaks starting with bacteria, followed by protozoan population growth, in turn stimulating protozoan predators, etc. (Clarholm, 1994). Any CO_2 effects detected could therefore be transitional, and many repeated observations are required to obtain a good measure of average populations.

Effects on the fungal compartment were detected in *Artemisia tridentata* shrub microcosms (Klironomos *et al*., 1996) in which fungi and microarthropods increased markedly. This effect was associated with relatively strong effects on root mass (+41%); effects on bacteria were smaller. Hungate *et al*. (2000) and Rillig *et al*. (1999a) observed strong seasonal dynamics of microbial measurements in the grassland they investigated, most likely because of its annual character. Fungal biomass and microarthropod numbers increased in the harvest in year six of treatment (Rillig *et al*., 1999a) but were not increased in the two harvests in year four (Hungate *et al*., 2000).

Seasonal dynamics of microbial measurements are probably less pronounced in perennial ecosystems (e.g. Newton *et al*., 1996; this experiment). Ross *et al*. (1996) did not find increases in microbial biomass in temperate pasture, despite a 77% increase in root biomass; nematode numbers increased by 11% (Yeates *et al*., 1999).

Conclusions

Elevated CO_2 did not alter total soil microbial biomass, even after six years of increased plant productivity. Our initial hypothesis that either soil microbial biomass or grazer populations would increase due to better substrate availability could not be corroborated. In contrast to other studies reporting a shift from the fungal to the bacterial part of the food web, we failed to find any indication of such a change. However, elevated CO_2 decreased soil aggregate sizes, most likely due to increased soil moisture in elevated CO_2 plots. This change in soil structure may have far-reaching consequences for water and nutrient fluxes and organic matter binding. Trace gas production may also be affected; N_2O production occurs in the anaerobic centre of aggregates, and a reduction in aggregate diameter is likely to reduce the proportion of anaerobic–aerobic sites. This effect may be counteracted by the overall increase in soil moisture found. Reduced aggregate sizes and pore neck diameters are probably responsible for the reduction in larger-diameter soil nematodes we observed. Because these nematodes are relatively high up the soil food web, this could trigger as top-down effect on the soil food web.

Increased mineralization of soil organic matter N under elevated CO_2 occurred in spring when plant growth was rapid and soils were moist. This extra N available may have allowed for faster growth under elevated CO_2; however, total N removed in plant mass over the course of the experiment did not differ between CO_2 treatments (unpublished results), suggesting that this effect was only transitory. Microbial N pools also did not change, indicating that elevated CO_2 did not stimulate net microbial immobilization of N which could have imposed a negative feedback on plant growth as reported in other studies.

Acknowledgements

We are grateful to Christian Körner for continuous support of these investigations. Gregor Yeates is acknowledged for helpful discussions on effects of soil structure on nematodes. John Dando helped with complete fractionation for mineral particle size analysis. This work was funded by the Swiss National Science Foundation (grant SPPU 5001-035214 to Ch. Körner) and contributes to the GCTE core research programme.

References

Alphei J (1995) Die freilebenden Nematoden von Buchenwäldern mit unterschiedlicher Humusform: Struktur

der Gemeinschaften und Funktion in der Rhizosphäre der Krautvegetation. *Berichte Des Forschungszentrums Waldökosysteme Göttingen*, **125**, 1–165.

Amthor JS (1995) Terrestrial higher-plant response to increasing atmospheric [CO_2] in relation to the global carbon cycle. *Global Change Biology*, **1**, 243–274.

Amthor JS (1999) Increasing atmospheric CO_2, water use, and water stress: scaling up from the plant to the landscape. In: *Carbon Dioxide and Environmental Stress* (eds Luo Y, Mooney HA), pp. 33–59. Academic Press, San Diego.

Anderson JPE, Domsch KH (1978) A physiological method for the quantitative measurement of microbial biomass in soils. *Soil Biology and Biochemistry*, **10**, 215–221.

Andrássy I (1956) Die Rauminhalts-und Gewichtsbestimmung der Fadenwürmer (Nematoda). *Acta Zoologica Hungarica*, **2**, 1–15.

Arnone JAI, Bohlen PJ (1998) Stimulated N_2O flux from intact grassland monoliths after two growing seasons under elevated CO_2. *Oecologia*, **116**, 331–335.

Arnone JA, Zaller JG, Spehn E *et al.* (2000) Dynamics of roots systems in intact native grasslands: effects of elevated atmospheric CO_2. *New Phytologist*, **147**, 73–85.

Ball AS, Drake BG (1997) Short-term decomposition of litter produced by plants grown in ambient and elevated atmospheric CO_2 concentrations. *Global Change Biology*, **3**, 29–35.

Brookes PC, Landman A, Pruden G *et al.* (1985) Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology and Biochemistry*, **17**, 837–842.

Buecher EJ, Hansen EL (1971) Mass culture of axenic nematodes using continuous aeration. *Journal of Nematology*, **3**, 199–200.

Clarholm M (1994) The microbial loop in soil. In: *Beyond the Biomass: Compositional and Functional Analysis of Soil Microbial Communities* (eds Ritz K, Dighton J, Giller KE), pp. 221–230. John Wiley, Chichester.

Cotrufo MF, Ineson P (1996) Elevated CO_2 reduces field decomposition rates of *Betula pendula* (Roth.) leaf litter. *Oecologia*, **106**, 525–530.

Coughenour MB, Chen DX (1997) Assessment of grassland ecosystem responses to atmospheric change using linked plant-soil process models. *Ecological Applications*, **7**, 802–827.

Darrah PR (1996) Rhizodeposition under ambient and elevated CO_2 levels. *Plant and Soil*, **187**, 265–275.

Diaz S, Grime JP, Harris J *et al.* (1993) Evidence of a feedback mechanism limiting plant response to elevated carbon dioxide. *Nature*, **364**, 616–617.

Edwards CA (1991) The assessment of populations of soil-inhabiting invertebrates. *Agriculture, Ecosystems and Environment*, **34**, 145–176.

Fitter AH, Self GK, Wolfenden J *et al.* (1996) Root production and mortality under elevated atmospheric carbon dioxide. *Plant and Soil*, **187**, 299–306.

Frostegård A, Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils*, **22**, 59–65.

Frostegård A, Bååth E, Tunlid A (1993) Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biology and Biochemistry*, **25**, 723–730.

van de Geijn SC, van Veen JA (1993) Implications of increased carbon dioxide levels for carbon input and turnover in soils. *Vegetatio*, **104/105**, 283–292.

Hilbert DW, Prudhomme TI, Oechel WC (1987) Response of tussock tundra to elevated carbon dioxide regimes: analysis of ecosystem CO_2 flux through nonlinear modelling. *Oecologia*, **72**, 466–472.

Hirschel G, Körner C, Arnone JA (1997) Will rising atmospheric CO_2 affect leaf litter quality and *in situ* decomposition rates in native plant communities. *Oecologia*, **110**, 387–392.

Houghton RA (1995) Land-use change and the carbon cycle. *Global Change Biology*, **1**, 275–287.

Hu S, Firestone MK, Chapin FS (1999) Soil microbial feedbacks to atmospheric CO_2 enrichment. *Trends in Ecology and Evolution*, **14**, 433–437.

Hungate BA (1999) Ecosystem responses to rising atmospheric CO_2: feedbacks through the nitrogen cycle. In: *Carbon Dioxide and Environmental Stress* (eds Luo Y, Mooney HA), pp. 265–285. Academic Press, San Diego.

Hungate BA, Holland EA, Jackson RB *et al.* (1997) The fate of carbon in grasslands under carbon dioxide enrichment. *Nature*, **388**, 576–579.

Hungate BA, Jackson RB, Field CB *et al.* (1996) Detecting changes in soil carbon in CO_2 enrichment experiments. *Plant and Soil*, **187**, 135–145.

Hungate BA, Jaeger CH, Gamara G *et al.* (2000) Soil microbiota in two annual grasslands: responses to elevated atmospheric CO_2. *Oecologia*, **124**, 589–598.

Huovinen-Hufschmid C, Körner C (1998) Microscale patterns of plant species distribution, biomass and leaf tissue quality in calcareous grassland. *Botanica Helvetica*, **108**, 69–83.

Ineson P, Coward PA, Hartwig UA (1998) Soil gas fluxes of N_2O, CH_4 and CO_2 beneath *Lolium perenne* under elevated CO_2: The Swiss free air carbon dioxide enrichment experiment. *Plant and Soil*, **198**, 89–95.

IPCC (1995) *Radiative Forcing of Climate Change and an Evaluation of the IPCC 1992 IS92 Emission Scenarios* (eds Houghton JT, Meira Filho LG, Hoesung Lee BJ, Callander BA, Haites E, Harris N, Maskell K), p. 339. Cambridge University Press, Cambridge.

Jackson RB, Sala OE, Field CB *et al.* (1994) CO_2 alters water use, carbon gain, and yield for the dominant species in a natural grassland. *Oecologia*, **98**, 257–262.

Jackson RB, Sala OE, Paruelo JM *et al.* (1998) Ecosystem water fluxes for two grasslands in elevated CO_2: a modelling analysis. *Oecologia*, **113**, 537–546.

Jones DL, Darrah PR (1996) Resorption of organic compounds by roots of *Zea mays* L. and its consequences in the rhizosphere. *Plant and Soil*, **178**, 153–160.

Jones TH, Thompson LJ, Lawton JH *et al.* (1998) Impacts of rising atmospheric carbon dioxide on model terrestrial ecosystems. *Science*, **280**, 441–443.

Kampichler C, Kandeler E, Bardgett RD *et al.* (1998) Impact of elevated atmospheric CO_2 concentration on soil microbial biomass and activity in a complex, weedy field model ecosystem. *Global Change Biology*, **4**, 335–346.

Klironomos JN, Rillig MC, Aellen MF (1996) Below-ground microbial and microfaunal responses to *Artemisia tridentata* grown under elevated atmospheric CO_2. *Functional Ecology*, **10**, 527–534.

Körner C (1996) The response of complex multispecies systems to elevated CO_2. In: *Global Change and Terrestrial Ecosystems. IGBP Series No. 2* (eds Walker BH, Steffen WL), pp. 20–42. Cambridge University Press, Cambridge.

Lal R (2000) Soil aggregation and C sequestration. In: *Global Climate Change and Tropical Ecosystems* (eds Lal R, Kimble JM, Stewart BA), pp. 317–329. CRC Press, Boca Raton.

Lauber W, Körner C (1997) *In situ* stomatal responses to long-term CO_2 enrichment in calcareous grassland plants. *Acta Oecologica*, **18**, 221–229.

Leadley PW, Niklaus PA, Stocker R *et al.* (1997) Screen-aided CO_2 control (SACC): a middle ground between FACE and open-top chambers. *Acta Oecologica*, **18**, 207–219.

Leadley PW, Niklaus PA, Stocker R *et al.* (1999) A field study of the effects of elevated CO_2 on plant biomass and community structure in a calcareous grassland. *Oecologia*, **118**, 39–49.

Liljeroth E, van Veen JA, Miller HJ (1990) Assimilate translocation to the rhizosphere of two wheat lines and subsequent utilization by rhizosphere microorganisms at two soil nitrogen concentrations. *Soil Biology and Biochemistry*, **22**, 1015–1021.

Lund CP, Riley WJ, Pierce LL *et al.* (1999) The effects of chamber pressurization on soil-surface CO_2 efflux and the implications for NEE measurements under elevated CO_2. *Global Change Biology*, **5**, 269–281.

Lussenhop J, Treonis A, Curtis PS *et al.* (1998) Response of soil biota to elevated atmospheric CO_2 in poplar model systems. *Oecologia*, **113**, 247–251.

Megonigal JP, Schlesinger WH (1997) Enhanced CH_4 emissions from a wetland soil exposed to elevated CO_2. *Biogeochemistry*, **37**, 77–88.

Merckx R, Dijkstra A, den Hartog A *et al.* (1987) Production of root-derived material and associated microbial growth in soil at different nutrient levels. *Biology and Fertility of Soils*, **5**, 126–132.

Moore JC, de Ruiter PC, Hunt HW (1993) Influence of productivity on the stability of real and model ecosystems. *Science*, **261**, 906–908.

Morison JIL (1985) Sensitivity of stomata and water use efficiency to high CO_2. *Plant, Cell and Environment*, **8**, 467–474.

Neilson RP, Drapek RJ (1998) Potentially complex biosphere responses to transient global warming. *Global Change Biology*, **4**, 505–521.

Newton PCD, Clark H, Bell CC *et al.* (1996) Interaction of soil moisture and elevated CO_2 on the above-ground growth rate, root length density and gas exchange of turves from temperate pasture. *Journal of Experimental Botany*, **47**, 771–779.

Niklaus PA (1998) Effects of elevated atmospheric CO_2 on soil microbiota in calcareous grassland. *Global Change Biology*, **4**, 451–458.

Niklaus PA, Glöckler E, Siegwolf R *et al.* (2001a) Carbon allocation in calcareous grassland under elevated CO_2: a combined ^{13}C pulse-labelling/soil physical fractionation study. *Functional Ecology*, **15**, 43–50.

Niklaus PA, Körner C (1996) Responses of soil microbiota of a late successional alpine grasslands to long term CO_2 enrichment. *Plant and Soil*, **184**, 219–229.

Niklaus PA, Leadley PW, Schmid B *et al.* (2001b) A long-term field study on biodiversity × elevated CO_2 interactions in grassland. *Ecological Monographs*, **71**, 341–356.

Niklaus PA, Leadley PW, Stöcklin J *et al.* (1998a) Nutrient relations in calcareous grassland under elevated CO_2. *Oecologia*, **116**, 67–75.

Niklaus PA, Spinnler D, Körner C (1998b) Soil moisture dynamics of calcareous grassland under elevated CO_2. *Oecologia*, **117**, 201–208.

Niklaus PA, Stocker R, Körner C *et al.* (2000) CO_2 flux estimates tend to overestimate ecosystem carbon sequestration at elevated CO_2. *Functional Ecology*, **14**, 546–559.

Niklaus PA, Wohlfender M, Siegwolf R *et al.* (2001c) Effects of six years atmospheric CO_2 enrichment on plant, soil, and soil microbial C of a calcareous grassland. *Plant and Soil*, **233**, 189–202.

Oades JM (1978) Mucilages at the root surface. *Soil Science*, **29**, 1–16.

Oades JM (1984) Soil organic-matter and structural stability – mechanisms and implications for management. *Plant and Soil*, **76**, 319–337.

Owensby CE, Ham JM, Knapp AK *et al.* (1999) Biomass production and species composition change in a tallgrass prairie ecosystem after long-term exposure to elevated atmospheric CO_2. *Global Change Biology*, **5**, 497–506.

Owensby CE, Ham JM, Knapp AK *et al.* (1997) Water vapour fluxes and their impact under elevated CO_2 in a C_4-tallgrass prairie. *Global Change Biology*, **3**, 189–195.

Parton WJ, Scurlock JMO, Ojima DS *et al.* (1995) Impact of climate change on grassland production and soil carbon worldwide. *Global Change Biology*, **1**, 13–22.

Paul EA, Clark FE (1996) *Soil Microbiology and Biochemistry*, 2nd edn. Academic Press, San Diego.

Penuelas J, Estiarte M (1998) Can elevated CO_2 affect secondary metabolism and ecosystem function? *Trends in Ecology and Evolution*, **13**, 20–24.

Phillips RL, Whalen SC, Schlesinger WH (2001a) Influence of atmospheric CO_2 enrichment on methane consumption in a temperate forest soil. *Global Change Biology*, **7**, 557–563.

Phillips RL, Whalen SC, Schlesinger WH (2001b) Influence of atmospheric CO_2 enrichment on nitrous oxide flux in a temperate forest ecosystem. *Global Biogeochemical Cycles*, **15**, 741–752.

Poorter H, Roumet C, Campbell BD (1996) Interspecific variation in the growth response of plants to elevated CO_2: a search for functional types. In: *Carbon Dioxide, Populations and Communities* (eds Körner C, Bazzaz FA), pp. 375–412. Academic Press, San Diego.

Pregitzer KS, Zak DR, Maziasz J *et al.* (2000) Interactive effects of atmospheric CO_2 and soil-N availability on fine roots of *Populus tremuloides*. *Ecological Applications*, **10**, 18–33.

Rillig MC, Field CB, Allen MF (1999a) Soil biota responses to long-term atmospheric CO_2 enrichment in two California annual grasslands. *Oecologia*, **119**, 572–577.

Rillig MC, Wright SF, Allen MF *et al.* (1999b) Rise in carbon dioxide changes soil structure. *Nature*, **400**, 628.

Ross DJ, Saggar S, Tate KR *et al.* (1996) Elevated CO_2 effects on carbon and nitrogen cycling in grass/clover turves of a Psammaquent soil. *Plant and Soil*, **182**, 185–198.

Ross DJ, Tate KR, Newton PCD (1995) Elevated CO_2 and temperature effects on soil carbon and nitrogen cycling in ryegrass/white clover turves of an Endoaquept soil. *Plant and Soil*, **176**, 37–49.

Scheu S (1992) Automated measurement of the respiratory response of soil microcompartments – active microbial biomass in earthworm feces. *Soil Biology and Biochemistry*, **24**, 1113–1118.

Schläpfer M, Zoller H, Körner C (1998) Influences of mowing and grazing on plant species composition in calcareous grassland. *Botanica Helvetica*, **108**, 57–67.

Sessitsch A, Weilharter A, Gerzabek MH *et al.* (2001) Microbial population structures in soil particle size fractions of a long-term fertilizer field experiment. *Applied and Environmental Microbiology*, **67**, 4215–4224.

Smith JL, Paul EA (1990) The significance of soil microbial biomass estimations. In: *Soil Biochemistry* (eds Bollag J, Stotzky G), pp. 357–393. Marcel Dekker, New York.

Sohlenius B (1979) A carbon budget for nematodes, rotifers and tardigrades in a Swedish coniferous forest soil. *Holarctic Ecology*, **2**, 30–40.

Stocker R, Leadley PW, Körner C (1997) Carbon and water fluxes in a calcareous grassland under elevated CO_2. *Functional Ecology*, **11**, 222–230.

Tisdall JM, Oades JM (1982) Organic matter and water-stable aggregates in soils. *Journal of Soil Science*, **33**, 141–163.

Treonis AM, Lussenhop JF (1997) Rapid response of soil protozoa to elevated CO_2. *Biology and Fertility of Soils*, **25**, 60–62.

Van Vuuren MMI, Robinson D, Scrimgeour CM *et al.* (2000) Decomposition of C-13-labelled wheat root systems following growth at different CO_2 concentrations. *Soil Biology and Biochemistry*, **32**, 403–413.

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, **19**, 703–707.

Volk M, Niklaus PA, Körner C (2000) Soil moisture effects determine CO_2-responses of grassland species. *Oecologia*, **125**, 380–388.

Wallace HR (1958) Movement of eelworms. II. A comparative study of the movement in soil of Heterodera schachtii (Schmidt) and of Ditylenchus dipsaci (Kühn). *Annaly of Applied Biology*, **46**, 86–94.

Yeates GW, Bongers T, de Goede RGM *et al.* (1993) Feeding habits in soil nematode families and genera – an outline for soil ecologists. *Journal of Nematology*, **25**, 315–331.

Yeates GW, Dando JL, Shepherd TG (2002) Pressure plate studies to determine how moisture affects access of bacterial-feeding nematodes to food in soil. *European Journal of Soil Science*, **53**, 355–365.

Yeates GW, Newton PCD, Ross DJ (1999) Response of soil nematode fauna to naturally elevated CO_2 levels influenced by soil pattern. *Nematology*, **1**, 285–293.

Yeates GW, Tate KR, Newton PCD (1997) Response of the fauna of a grassland soil to doubling of atmospheric carbon dioxide concentration. *Biology and Fertility of Soils*, **25**, 307–315.

Zak DR, Pregitzer KS, Curtis PS *et al.* (1993) Elevated atmospheric CO_2 and feedback between carbon and nitrogen cycles. *Plant and Soil*, **151**, 105–117.