Oecologia (2000) 124:589–598

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Soil microbiota in two annual grasslands: responses to elevated atmospheric CO_2

Received: 19 March 1999 / Accepted: 23 March 2000

Abstract We measured soil bacteria, fungi, protozoa, nematodes, and biological activity in serpentine and sandstone annual grasslands after 4 years of exposure to elevated atmospheric CO_2. Measurements were made during the early part of the season, when plants were in vegetative growth, and later in the season, when plants were approaching their maximum biomass. In general, under ambient CO_2, bacterial biomass, total protozoan numbers, and numbers of bactivorous nematodes were similar in the two grasslands. Active and total fungal biomasses were higher on the more productive sandstone grassland compared to the serpentine. However, serpentine soils contained nearly twice the number of fungivorous nematodes compared to the sandstone, perhaps explaining the lower standing crop of fungal biomass in the serpentine and suggesting higher rates of energy flow through the fungal-based soil food web. Furthermore, root biomass in the surface soils of these grasslands is comparable, but the serpentine contains 6 times more phytophagous nematodes compared to the sandstone, indicating greater below-ground grazing pressure on plants in stressful serpentine soils. Elevated CO_2 increased the biomass of active fungi and the numbers of flagellates in both grasslands during the early part of the season and increased the number of phytophagous nematodes in the serpentine. Elevated CO_2 had no effect on the total numbers of bactivorous or fungivorous nematodes, but decreased the diversity of the nematode assemblage in the serpentine at both sampling dates. Excepting this reduction in nematode diversity, the effects of elevated CO_2 disappeared later in the season as plants approached their maximum biomass. Elevated CO_2 had no effect on total and active bacterial biomass, total fungal biomass, or the total numbers of amoebae and ciliates in either grassland during either sampling period. However, soil metabolic activity was higher in the sandstone grassland in the early season under elevated CO_2, and elevated CO_2 altered the patterns of use of individual carbon substrates in both grasslands at this time. Rates of substrate use were also significantly higher in the sandstone, indicating increased bacterial metabolic activity. These changes in soil microbiota are likely due to an increase in the flux of carbon from roots to soil in elevated CO_2, as has been previously reported for these grasslands. Results presented here suggest that some of the carbon distributed below ground in response to elevated CO_2 affects the soil microbial food web, but that these effects may be more pronounced during the early part of the growing season.

Key words Elevated CO_2 · Soil food web · Community structure · Nematodes · Protozoa

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Introduction

Elevated atmospheric CO_2 usually stimulates plant photosynthesis, and often increases the flux of carbon from roots to soil (Van Veen et al. 1991; Canadell et al. 1996). Root-derived carbon is a key energy source for soil microorganisms, so an increased flux of carbon to soil in response to elevated CO_2 could increase the soil microbial biomass. By reducing plant transpiration, elevated CO_2 can increase soil water content (Field et al. 1995), which

can also affect soil biota (Hamdi 1971; Kuikman et al. 1991). Elevated CO_2 has been found to increase total soil microbial biomass in some cases (Díaz et al. 1993; Zak et al. 1993; Rice et al. 1994; Hungate et al. 1997a, 1997b), but to have no effect in others even when elevated CO_2 is known to stimulate carbon input below ground (Niklaus and Körner 1996; Jones et al. 1998). In these cases, elevated CO_2 might alter the turnover and composition of the soil microbial assemblage – changes that could occur without an expansion in total microbial biomass.

In several microcosm and monoculture experiments, elevated CO_2 has been shown to increase the density of protozoa (Treonis and Lussenhop 1997; Lussenhop et al. 1998), nematodes (Runion et al. 1994; Yeates et al. 1997), and collembola (Jones et al. 1998), changes that are likely to be associated with increased turnover of soil bacteria and fungi. Other studies emphasize distinct below-ground trophic pathways, showing that elevated CO_2 can increase hyphal length of mycorrhizal fungi but depress non-mycorrhizal fungi and associated collembolan grazers (Klironomos et al. 1997; Rillig et al. 1999), depending on soil nutrient status (Klironomos et al. 1996). Elevated CO_2 can also alter the composition and activity of the soil bacterial assemblage, for example, increasing the density of nitrogen-fixing *Rhizobium* in the rhizosphere of *Trifolium repens* (Schortenmeyer et al. 1996), and altering bacterial utilization of different classes of carbon compounds (Rillig et al. 1997).

In 1992, we began a CO_2-enrichment experiment in serpentine and sandstone California annual grasslands (Field et al. 1996). The sandstone grasslands, the more common in California, are dominated by exotic annual C3 grasses introduced from Europe over the last two centuries. Serpentine grasslands are rarer, found on ridge tops near the coast, and are dominated by annual C3 forbs and grasses mostly native to California. Also, though the serpentine is less productive than the sandstone, carbon allocation below ground as a proportion of above-ground productivity is higher on the serpentine than the sandstone, as indicated by measurements of below-ground respiration (Luo et al. 1996). Differences in the structure of the below-ground food web between the serpentine and sandstone may contribute to these differences in carbon cycling. Elevated CO_2 increases carbon flow below ground in both the serpentine and sandstone grasslands (Luo et al. 1996; Hungate et al. 1997a, 1997b), increases soil moisture, especially in the sandstone (Hungate et al. 1997a; Fredeen et al. 1997), and also alters mycorrhizal colonization, increasing colonization in some plant species and decreasing it in others (Rillig et al. 1997).

We measured the responses of soil biota and soil activity to elevated CO_2 in serpentine and sandstone annual grasslands after 4 years of exposure to elevated CO_2. We postulated that elevated CO_2 would increase the numbers and activity of soil organisms on both grasslands, due to increased carbon flow below ground and to increased soil moisture. Because the increase in soil moisture under elevated CO_2 is most apparent on the sandstone and near-peak season (Fredeen et al. 1998), we expected that moisture-driven changes in soil biota would be most pronounced on the sandstone at this time. By contrast, we expected that carbon-driven responses of soil microbiota to elevated CO_2 would be similar on the serpentine and sandstone because the CO_2-induced increase in carbon flow below ground in these two systems – at least as captured by soil respiration (Luo et al. 1996), microbial biomass (Hungate et al. 1997a), and root biomass (Hungate et al. 1997b; Rillig et al. 1999) – appears to be similar in magnitude. To test these hypotheses, we examined soil biota and activity during early and peak season on the serpentine and sandstone grasslands after 4 years of CO_2 treatment.

Materials and methods

This research occurred on sandstone and serpentine grasslands at the Jasper Ridge Biological Preserve near Stanford University, in central coastal California. The Jasper Ridge Elevated CO_2 Experiment comprises adjacent tracts of sandstone and serpentine grassland. Open-top chambers, each 0.3 m^2, were placed over 20 plots on each grassland in January 1992, with 10 chambers at ambient CO_2 and 10 at ambient+350 µl l^{-1} CO_2 for each grassland. (For further details of the experimental setup, see Field et al. 1996.) The measurements described here took place during the 1994/1995 growing season, the fourth after the CO_2 manipulation began.

In January 1995, near the beginning of the growing season, we collected one 3-cm-diameter by 15-cm-deep soil core from each plot for measurements of microbial biomass and activity, returning the cores to the laboratory on ice. Soils were sorted by hand to remove rocks, large pieces of organic matter, and large roots. A subsample of soil from each core was removed and sent to the Soil Microbial Biomass Service at Oregon State University, for measurements of the biomass of bacteria, fungi, protozoa, and nematodes. Total bacteria were measured by fluorescein isothiocyanate staining and epifluorescence microscopy (Babiuk and Paul 1970), and active bacteria and fungi by fluorescein diacetate and epifluorescence microscopy (Ingham and Horton 1987). Total fungal hyphae were determined by fluorescein diacetate staining and light microscopy, respectively (Ingham and Klein 1984). Protozoa were measured by a most probable number (MPN) method (Ingham 1993). Nematodes were enumerated by extracting them from a saturated soil sample using a Baermann funnel and counting and typing under a microscope. Nematodes were identified to genus and were grouped into four functional feeding groups: phytophagous, bactivorous, fungivorous, and predatory.

The remaining soil was processed in the laboratory for 2-(p-iodo-phenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) reduction, Biolog, and gravimetric water content. Soil microbial metabolic activity (dehydrogenase) was measured in incubated soil slurries. Activity was indicated by the reduction of INT to iodonitrotetrazolium formazan (INT-formazan), and detected colorimetrically, as described in Trevors et al. (1982). The Biolog microplate system consists of gram-negative and gram-positive microplates, each with 96 wells. Each of 95 of these wells contains a unique carbon substrate, nutrients and tetrazolium dye, which turns the well purple in the presence of respiring bacteria. One well contains only nutrients and dye, to detect the introduction of carbon sources in the inoculum. Combining the gram-negative and gram-positive plates, one can assess the ability of a microbial community to use 128 different carbon sources in 6 functional guilds of compounds: amines and amides, amino acids, carbohydrates, carboxylic acids, polymers, and miscellaneous compounds (Zak et al. 1994). For the Biolog assay, we removed the equivalent of 1 g of dry soil, and performed serial dilutions in phosphate buffer. For each sample, we added 150 µl of the 10^{-4} dilution to each well in

both gram-negative and gram-positive Biolog plates. We analyzed the plates for color development at 590 nm 24, 48, 72, 96, and 120 h after inoculation. The control wells showed no color development over this interval, indicating that there was little soluble organic matter and background color in the inoculum. However, fungal growth and dye precipitation occurred at 120 h, so we focussed the analysis on the reading after 96 h. (Patterns observed after 96 h were similar for the 72- and 48 h readings.)

To examine CO_2 and soil differences in total microbial activity, we calculated total activity for each plate by summing the absorption in each well at 590 nm. We also compared activity by guild, summing the wells in each plate for each guild of compounds. Total activity and activity for each guild were compared statistically using analysis of variance (ANOVA). To examine CO_2 and soil differences in the qualitative pattern of sole carbon source use, we used principal component analysis (PCA) to describe overall patterns of substrate use. PCA was conducted on the relative use of carbon sources after 96 h (relative use calculated as absorption in each well divided by total absorption in the plate). We excluded 33 substrates because they showed activity in 8 or fewer of the 40 samples. The first four principal components were then analyzed by ANOVA to examine differences between soils and CO_2 treatments, and component loadings (correlation of individual substrates with the principal components) were then examined to determine which compounds were responsible for observed differences.

On 18 April (in the serpentine) and 28 May (in the sandstone), we collected another set of cores (5 cm diameter×15 cm deep). These dates corresponded to the time of maximum above-ground biomass on each grassland. For this peak season harvest, samples were sent to the Soil Microbial Biomass Service at Oregon State University for measurements of the biomass of bacteria, fungi, protozoa, and nematodes, and were analyzed as described above. We did not conduct the dehydrogenase and Biolog assays at this time, but we did measure gravimetric soil water content.

For both sampling dates, we removed only one soil core per plot to characterize the soil microbial assemblage. Taking additional subsamples from each plot may have captured spatial heterogeneity more accurately, thereby reducing the variance associated with our measurements. However, our sampling design was necessary to minimize soil disturbance to our relatively small (0.3 m^2) plots, and thus to avoid interference with other sampling requirements in this experiment. Additionally, the relatively high level of replication (n=10) within each treatment was sufficient to detect statistically significant differences between treatments for some of the parameters measured (see Results and Discussion). Thus, we feel that the sampling design was adequate to capture spatial heterogeneity.

We compared numbers, biomass, and activity between soils and CO_2 treatments. Where appropriate, we used repeated measures analysis of variance (RMA), with CO_2 and soil as main effects and time as the repeated measure, to test for overall differences between CO_2 treatments, soil types, and differences between the two sampling dates, followed by t-tests to compare particular pairs of means. In some cases, severe departures from the assumption of homogeneity of variances required treating the sampling dates separately. To characterize nematode diversity, we examined rank/abundance relationships for each treatment and sampling date and calculated Simpson's E and D indices (Begon et al. 1990). Because all nematode genera in a treatment did not occur in each plot in that treatment, determining rank abundance relationships by summing across all replicates in a treatment (rather than by individual replicate) more accurately portrayed nematode diversity within that treatment. Determining rank abundance relationships by individual plot and then calculating treatment means did not qualitatively alter the patterns we observed, but caused D and E to be severely underestimated (data not shown).

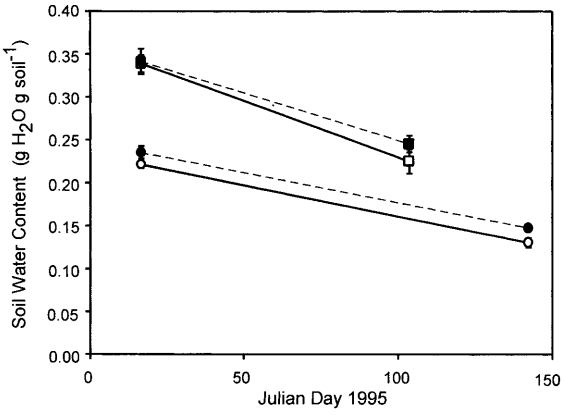

Fig. 1 Gravimetric soil water content on the serpentine (*squares*) and sandstone (*circles*) grasslands in the ambient (*unfilled symbols*) and elevated (*filled symbols*) CO_2 treatments. Elevated CO_2 did not alter water content in the early season (P=0.401) or in the serpentine in April, but increased water content on the sandstone in May (P=0.007). Values shown are means±standard errors

Results

Soil water content

Soil water content was higher on the serpentine than the sandstone, and decreased from the first to the second sampling period on both grasslands (Fig. 1). Elevated CO_2 had no effect on soil water content on either grassland in the early season (two-way ANOVA, P=0.401) or on the serpentine in April (one-way ANOVA, P=0.251), but increased soil water content in May on the sandstone (one-way ANOVA, P=0.007).

Bacteria

Total bacterial biomass was similar in the two grasslands and declined markedly during the season, from 425–550 μg g^{-1} in the early season to 175–220 μg g^{-1} at peak plant biomass (Fig. 2, RMA effect of time P<0.001). Active bacterial biomass was also similar for the two grasslands, but in contrast to total bacterial biomass, active bacterial biomass increased through the season, from 60–85 μg g^{-1} at the first harvest to 95–105 μg g^{-1} by the second harvest (Fig. 2, effect of time P<0.001). Thus, the proportion of total bacteria that were active increased through time, composing around 15% of total bacterial biomass in the first harvest and over 50% by the second. Elevated CO_2 had no effect on active or total bacterial biomass for either harvest date (Fig. 2, P>0.5).

Fungi

In contrast to bacterial biomass, fungal biomass differed between the two grasslands, with more active fungal biomass in the early season on the sandstone compared to the serpentine (t-test, P=0.013) and greater total fungal bio-

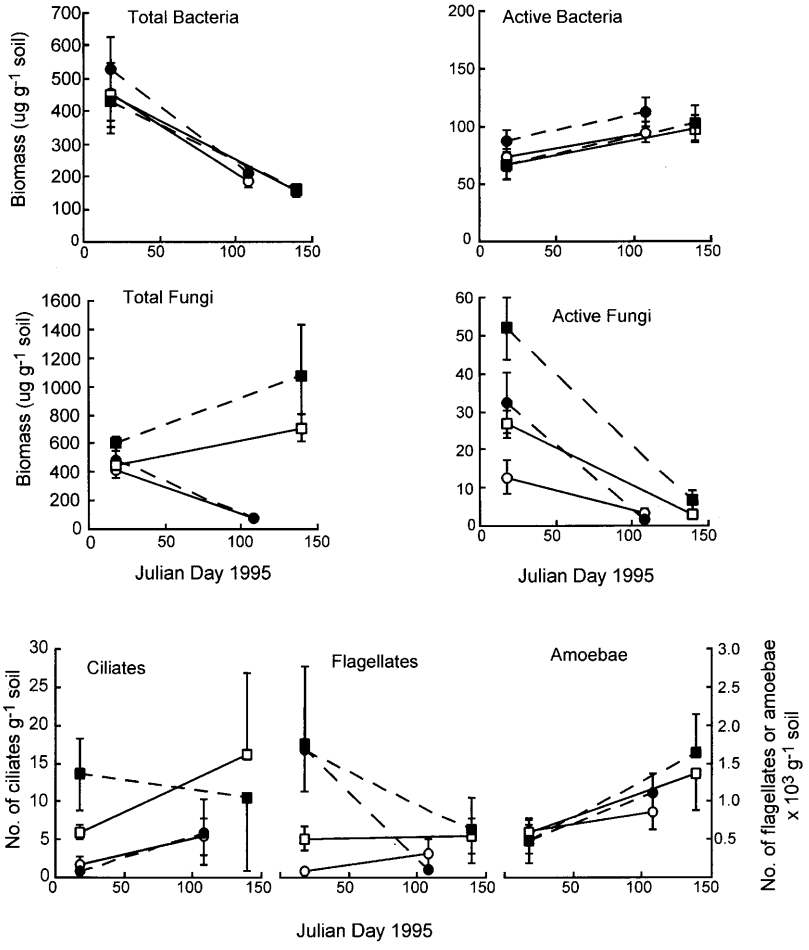

Fig. 2 Biomass of total and active bacteria and fungi in soil, and numbers of soil protozoa during the 1995 growing season for the serpentine and sandstone grasslands treated with ambient and elevated CO_2 (*symbol* definitions are given in the legend to Fig. 1). Values shown are means±standard errors (for clarity, only 1 standard error is shown in some cases)

mass during the late season on the sandstone than on the serpentine (*t*-test, $P<0.001$). Changes in fungal biomass from early to late season also differed between the two grasslands. While active fungal biomass was lower on both grasslands at the peak season harvest (Fig. 2; RMA, time, $P<0.001$), total fungi declined through time in the serpentine but increased through time on the sandstone (Fig. 2; RMA, soil×time, $P<0.001$). During the early season, elevated CO_2 significantly increased active fungal biomass on both grasslands (RMA CO_2, $P=0.002$). CO_2 did not alter active fungal biomass during the late season (RMA, CO_2×time, $P=0.002$) and had no effect on total fungal biomass for either harvest (RMA CO_2, $P=0.20$).

Ratios of fungal to bacterial biomass were around 1 in both grasslands during the early season, but changed markedly by late season and in different directions for the two grasslands. On the serpentine, the ratio of fungal to bacterial biomass decreased to 0.5 in the late season, whereas on the sandstone, this ratio increased to 5. Thus, in the late season fungi constituted a larger proportion of total microbial biomass on the sandstone than the serpentine.

Protozoa

The sandstone grassland tended to have more ciliates than the serpentine ($P=0.078$); flagellate numbers were highly variable and differences between the two grasslands were not significant ($P>0.2$). Numbers of amoebae were less variable than ciliates and flagellates and were not significantly different between the two grasslands ($P>0.35$), increasing through time on both (RMA time, $P<0.001$). Elevated CO_2 increased flagellate numbers for the first harvest (*t*-test, $P=0.033$), but had no effect for the second harvest (*t*-test, $P>0.5$). Elevated CO_2 affected the numbers of neither ciliates nor amoebae ($P>0.8$).

Nematodes

On both grasslands, fungivorous nematodes were the most numerous group, followed by bactivorous, phytophagous (Fig. 3), and predatory (data not shown). Numbers of nematodes increased from the early to the late season on both grasslands (RMA, $P<0.001$), and were generally higher on the serpentine grassland than on the sandstone (RMA, $P=0.025$). Thus, nematodes were the only component of the microbial assemblage that was greater on the serpentine compared to the sandstone. Elevated CO_2 had no effect on the numbers of fungivorous (RMA, $P=0.422$) or bactivorous (RMA, $P=0.551$) nematodes (Fig. 3). However, elevated CO_2 increased phytophagous nematodes (RMA, $P=0.016$) in

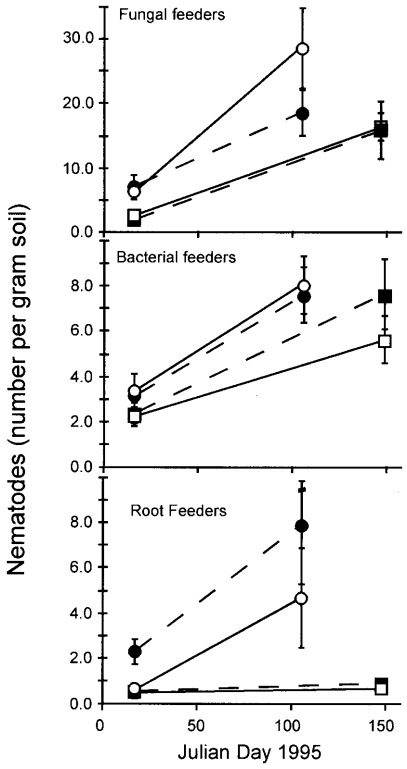

Fig. 3 Numbers of soil nematodes on the serpentine and sandstone grasslands during the 1995 growing season, broken down into trophic groups based on their feeding apparatus (*symbol* definitions are given in the legend to Fig. 1). Values shown are means±standard errors

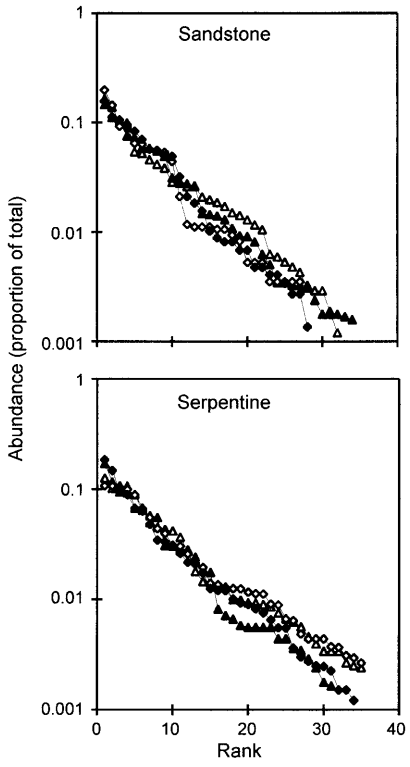

Fig. 4 Rank-abundance curves for nematode genera on the sandstone (*top panel*) and serpentine (*bottom panel*) grasslands in the ambient (*unfilled symbols*) and elevated (*filled symbols*) CO_2 treatments for the early (*triangles*) and peak (*diamonds*) season sampling dates. Curves shown were generated by summing (rather than averaging) across all replicates in a treatment (see Materials and methods)

the early season on the serpentine (*t*-test, P=0.007). The pattern continued into peak season (Fig. 3), but was not significant (*t*-test, P=0.185).

In total, 52 genera of nematodes were identified on the serpentine and 49 on the sandstone (Table 1). The fungal feeding and bacterial feeding groups contained the most genera, whereas the root feeding and predatory groups contained relatively few (Table 1). Most genera occurred on both grasslands, but several were found only on one of the two grasslands (Table 1). Numbers of genera identified in ambient and elevated CO_2 treatments were similar on the sandstone grassland, but fewer genera were found in elevated CO_2 on the serpentine (Fig. 4), and the rank abundance relationship indicated reduced nematode diversity on the serpentine for both sampling dates (Table 2).

Soil activity

During the early season, soil metabolic activity (as determined by the dehydrogenase assay) was higher under elevated CO_2 in the sandstone (*t*-test P<0.001), but unchanged in the serpentine (*t*-test P=0.561, Table 3, Fig. 5). Similarly, the rate of tetrazolium dye reduction in the Biolog microplates, summed across all substrates, was higher under elevated CO_2 in the sandstone (*t*-test P=0.010), but unchanged in the serpentine (*t*-test P=0.975, Table 3, Fig. 5). Elevated CO_2 increased the rate at which bacteria in the sandstone consumed amino acids, carbohydrates, polymers, and miscellaneous compounds, decreased the rate of use of amines and amides, and had no effect on the rate of use of carboxylic acids (Table 3, Fig. 6). None of these effects were observed in the serpentine, where rates of substrate use were similar under ambient and elevated CO_2 (Fig. 6). Under ambient CO_2, soil metabolic activity was higher in the serpentine than the sandstone (Fig. 5).

After collapsing the relative rates of use of all 95 compounds in the Biolog microplate assay, principle component 1 (PC1) explained 29.8% of the variance, and very clearly distinguished the CO_2 treatments on the sandstone (Table 3, Fig. 6). PC1 values for both CO_2 treatments on the serpentine fell in a narrower range, between the ambient and elevated CO_2 treatments for the sandstone. Thus, for the variance summarized by PC1, the differences in patterns of use of carbon substrates caused by elevated CO_2 on the sandstone soil were larger than differences between the two contrasting ecosystems. PC2 explained another 17.3% of the variance, and clearly separated the serpentine and sandstone soils, while also distinguishing the ambient and elevated CO_2 treatments on the serpentine. Together, PC3–5 explained

Table 1 Nematode genera listed by trophic classes (bacterial feeders, fungal feeders, root feeders, predatory), and by occurrence on the sandstone and serpentine grassland

Occurrence	Bacterial feeders	Fungal feeders	Root feeders	Predatory
Sandstone and serpentine				
	Achromadora	*Aphelenchoides*	*Meloidogyne*	*Clarkus*
	Acrobeles	*Aphenlenchus*	*Neosilenchus*	*Coomansus*
	Acrobeloides	*Aporcellaimellus*	*Paratylenchus*	*Prionchulus*
	Cephalobus	*Bitylenchus*		
	Chiloplacus	*Coslenchus*		
	Eucephalobus	*Ditylenchus*		
	Eumonhystera	*Epidorylaimus*		
	Heterocephalobus	*Eucorylaimus*		
	Odontolaimus	*Filenchus*		
	Panagrolaimus	*Malenchus*		
	Plectus	*Mesodorylaimus*		
	Prismatolaimus	*Microdorylaimus*		
	Rhabditus	*Pratylenchus*		
	Rhabdolaimus	*Seinura*		
	Tripyla	*Thonus*		
	Wilsonema	*Tylencholaimellus*		
		Tylencholaimus		
		Tylenchorhynchus		
		Tylenchus		
Sandstone only				
	Alaimus	*Laimydorylaimus*	*Helicotylenchus*	
	Cervidellus	*Prodorylaimus*	*Psilenchus*	
		Scutylenchus		
		Thornia		
Serpentine only				
	Anaplectus	*Aprutides*	*Gracilicus*	*Iotonchus*
	Mesorhabditus	*Dorylaimoides*	*Xiphinema*	
	Microlaimus	*Paraxonchium*		
	Tetratocephalus	*Pungentus*		

Table 2 Simpson's Diversity ('*D*')and Evenness ('*E*') indices calculated from the relative abundance of nematode genera on the serpentine and sandstone grasslands for the early and peak season sampling times under ambient and elevated CO_2. Because not all genera occurred in each replicate for a treatment, values shown were calculated by summing across replicates in a treatment for a given sampling date (see Materials and methods)

Ecosystem	Early season		Peak season	
	Ambient CO_2	Elevated CO_2	Ambient CO_2	Elevated CO_2
Sandstone				
E	0.41	0.38	0.38	0.43
D	13.1	12.8	10.3	12.0
Serpentine				
E	0.42	0.38	0.43	0.33
D	14.6	11.7	15.0	11.1

another 20% of the variance, but did not distinguish the soil types or CO_2 treatments (data not shown). The compounds that were strongly (r>0.8) positively correlated to PC1 were several carbohydrates and two amino acids, whereas several amines and amides, carboxylic acids, and two amino acids were strongly (r <–0.8) negatively correlated to PC1 (Table 4). A number of carboxylic acids, an amino acid, and a carbohydrate were strongly negatively correlated to PC2, whereas one miscellaneous compound was strongly positively correlated to PC2. Overall, differences between soils and CO_2 treatments in the relative use of substrates were similar for compounds within a given guild, but this was not always the case for PC1, where compounds within a guild had both strong positive and strong negative correlations (Table 4).

In summary, the serpentine and sandstone grasslands were similar in bacterial biomass, total protozoan numbers, and numbers of bactivorous nematodes. The sandstone had higher fungal biomass, whereas the serpentine contained nearly twice the number of fungivorous nematodes and 6 times more phytophagous nematodes compared to the sandstone. Under ambient CO_2, soil metabolic activity was higher on the serpentine grassland compared to the sandstone, and patterns of use of individual carbon substrates differed between the two grasslands in both CO_2 treatments.

Table 3 Statistical results from the dehydrogenase and Biolog assays conducted on the January samples. *P*-values for the main effects of CO_2, Soil, and their interaction, are shown for each twoway ANOVA conducted

Variable	CO_2	Soil	CO_2×Soil
Dehydrogenase	0.009	0.260	0.084
Biolog total activity	0.019	0.807	0.021
Amines/amides	0.001	0.200	0.032
Amino acids	0.032	0.382	0.032
Carbohydrates	<0.001	0.893	<0.001
Carboxylic acids	0.633	0.070	0.742
Miscellaneous	<0.001	0.590	<0.001
Polymers	0.943	0.157	0.051
Biolog PC1	<0.001	0.407	<0.001
Biolog PC2	<0.001	<0.001	0.003

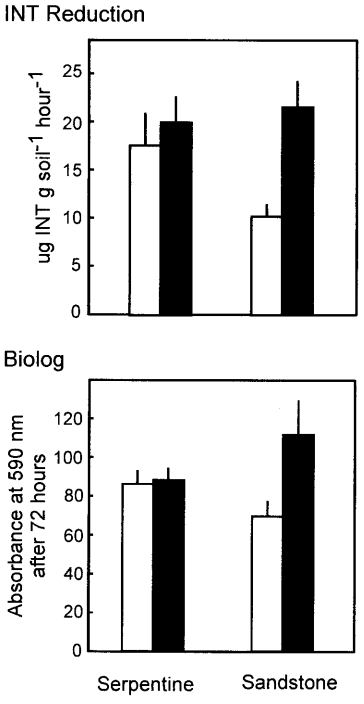

Fig. 5 Soil metabolic activity on the sandstone and serpentine grasslands under ambient (*unfilled bars*) and elevated (*filled bars*) CO_2 concentrations. The *top panel* shows total soil metabolic activity as indicated by the dehydrogenase assay in ug INT/g soil per hour. The *bottom panel* shows metabolic activity as indicated by the total color development (at 590 nm) in the Biolog microplates after 96 h. Values are means±standard errors

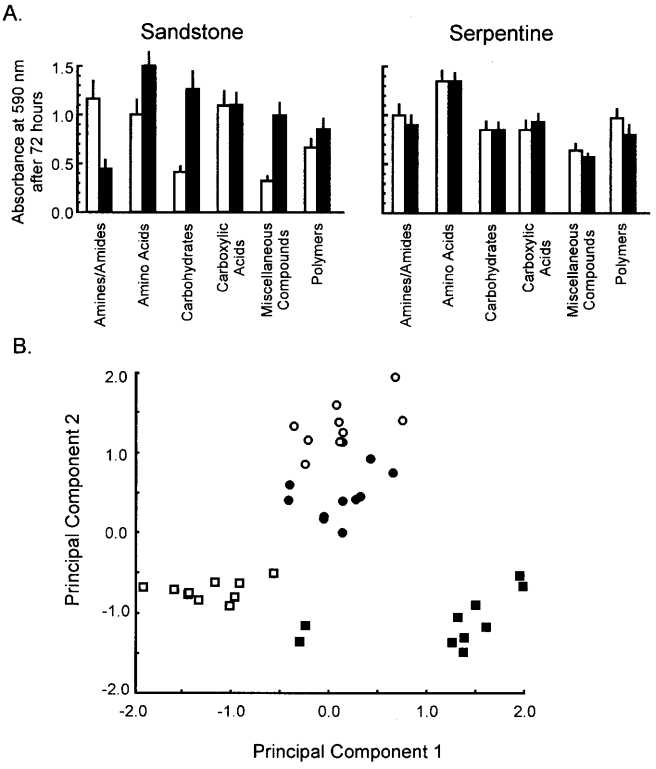

Fig. 6 **A** The rate of dye reduction in the Biolog microplates, broken down into chemical functional groups according to Zak et al. (1994), for the sandstone (*left panel*) and serpentine (*right panel*), for the ambient (*unfilled bars*) and elevated (*filled bars*) CO_2 treatments. Values are means±standard errors. **B** Principal components 1 and 2, summarizing the data on the relative rate of each of the 95 carbon sources included in the Biolog analysis, for both grasslands under ambient and elevated CO_2. *Symbols* definitions are given in the legend to Fig. 1

In January, when plants were in the vegetative growth phase, elevated CO_2 increased active fungal biomass and flagellate numbers on both the serpentine and sandstone grasslands, caused an increase in the number of root-feeding nematodes on the serpentine, and altered the community composition of nematodes on the serpentine, reducing nematode diversity. These effects disappeared later in the growing season, when plants were approaching their maximum above-ground biomass, though the reduction in nematode diversity was still apparent. Elevated CO_2 increased soil metabolic activity in the sandstone grassland in January, as indicated by two independent measures of soil metabolic activity. Furthermore, elevated CO_2 caused a qualitative shift in pattern of substrate use on both grasslands.

Discussion

These results show that the composition of the belowground food web differs between the sandstone and serpentine grasslands. The sandstone, the more typical California annual grassland, was dominated by fungal biomass, with an average ratio of fungal to bacterial biomass of 3. This contrasts sharply with the serpentine, where the average ratio was around 0.8, similar to that previously reported for shortgrass prairie and alpine meadows (Hunt et al. 1987; Ingham et al. 1989). However, the lower proportion of fungal biomass on the serpentine may belie their importance, as fungal-feeding nematodes were more than twice as numerous on the serpentine than on the sandstone, indicating that energy flow through fungi in this grassland is greater than implied by their biomass. Furthermore, both fungi and bacterial biomass shifted throughout the season (Fig. 2), so our two

Table 4 Summary of high correlations from the principal components analysis on relative substrate use in the Biolog assay. For compounds with positive correlations, samples with greater response have higher PC scores; for negative correlations, samples with greater response have lower PC scores. Compound guild, compound name, and correlation (*r*) with PC1 or PC2 are shown

Guild	Compounds	*r* (PC1)	Compounds	*r* (PC2)
Amines/amides	2-Amino ethanol, glucuronamide, succinamic acid, putrescine, alanidamide	<–0.80	None	
Amino acid	L-Asparagine, L-phenylalanine L-alanine, δ-amino butyric acid	<–0.80 >0.88	L-Pyroglutamic acid	–0.91
Carboxylic acid	D-Glucuronic acid, succinic acid, L-malic acid, *N*-acetyl L-glutamic acid	<–0.83	D-Galacturonic acid, *cis*-aconitic acid, D-glucosminic acid, D-gluconic acid, *p*-hydroxyphenylacetic acid, L-lactic acid	<–0.90
Carbohydrate	D-Raffinose, D-fructose, D-galactose, D-ribose, α-D-glucose, D-mannitol, D-mannose, D-xylose	>0.84	D-Sorbitol	–0.94
Miscellaneous	None		D,L-α-Glycerol phosphate	0.87

samplings may not have been adequate to characterize the average ratio of fungi:bacteria. Indeed, sampling in April 1997, Rillig et al. (1999) found higher ratios of fungi:bacteria on the serpentine compared to the sandstone, a pattern opposite to that we found here (in 1995). Thus, ratios of fungi:bacteria are highly dynamic in these two systems and differences between them determined by a single sampling are likely to be misleading.

The greater numbers of fungal-feeding and phytophagous nematodes on the serpentine compared to the sandstone could contribute to the greater flux of carbon below ground in this ecosystem as a proportion of above-ground production (Hungate et al. 1996; Luo et al. 1996). However, assuming average nematode carbon mass of 0.025 µgC, growth efficiency of 8% (Anderson et al. 1981), and turnover of 10 times per year, nematodes would consume only about 14 gC m^{-2} $year^{-1}$ on the serpentine, compared to 7 gC m^{-2} $year^{-1}$ on the sandstone. Though higher on the serpentine, this flux amounts to only a small proportion of the annual carbon budget of these grasslands, where total below-ground respiration is around 346 g m^{-2} $year^{-1}$ on the serpentine and 485 g m^{-2} $year^{-1}$ on the sandstone (Luo et al. 1996).

Our measurements also show that elevated CO_2 alters the structure of the microbial assemblage in the serpentine and sandstone grasslands, but that the effects, overall, are moderate, and that they are more apparent when plants are in the vegetative growth phase than later in the growing season. Specifically, we observed increased active fungal biomass and flagellate numbers in both grasslands and increased phytophagous nematodes on the serpentine during the early season, but neither total fungal biomass, total and active bacterial biomass, ciliates, amoebae, bactivorous nematodes, nor fungivorous nematodes responded to elevated CO_2 during either sampling period. On the serpentine grassland, elevated CO_2 reduced the diversity of the nematode assemblage, apparent from the steeper rank-abundance curve (Fig. 4). Elevated CO_2, perhaps due to altered resource availability, favored the more abundant nematode genera over the rarer ones, causing a reduction, and in some cases, a loss, of the latter.

Increased carbon flow below ground is the most likely explanation for the changes we observed in the soil microbial assemblage. In these grasslands, elevated CO_2 increases photosynthesis at the leaf and canopy levels (Jackson et al. 1994, 1995; Fredeen et al. 1995; Field et al. 1996), below-ground respiration (Luo et al. 1996), microbial biomass (Hungate et al. 1997a; Rillig et al. 1999), and root biomass (Hungate et al. 1997b; Rillig et al. 1999). During the 1995 growing season, when the measurements reported here were made, root biomass was higher in the elevated CO_2 treatment on both grasslands, indicating that elevated CO_2 increased carbon flow below ground at this time (Higgins 1996). In these and other grassland ecosystems, elevated CO_2 has been shown to affect microbial processes through altered soil water content (Rice et al. 1994; Hungate et al. 1997a; Arnone and Bohlen 1998). However, because the changes in the microbial assemblage reported here occurred primarily during the early season, when CO_2 treatments did not differ in soil water content on either grassland, this mechanism is unlikely to explain the CO_2 effects we observed.

Taken together, the effects of elevated CO_2 on the microbial biomass and numbers were fairly small. Nevertheless, it is possible that elevated CO_2 enhanced the turnover of these organisms. Indeed, the increase in flagellate numbers observed during January suggests an increase in the rate of turnover of bacteria, the major food source for flagellates, and is consistent with results from *Brassica negra* monocultures (Treonis and Lussenhop 1997). Furthermore, a stimulation of carbon flow through the below-ground food web would not stop with the highest trophic levels examined in this study – protozoa and nematodes – as microarthropods consume protozoa and nematodes and thus constitute higher trophic levels in grassland ecosystems (Hunt et al. 1987). Rillig et al. (1999) found increased microarthropod numbers in response to elevated CO_2, more pronounced on the sandstone than on the serpentine. Additionally, the increase in soil metabolic activity and bacterial activity observed on the sandstone grassland in January (Fig. 4), combined with the observation of no large changes in standing mass of microorganisms, suggest greater rates of turnover through the microbial biomass.

Elevated CO_2 also altered patterns of use of individual carbon substrates in both grasslands, increasing the relative use of carbohydrates and decreasing that of amines and amides on the sandstone, while decreasing the relative use of carboxylic acids on both grasslands (Fig. 6). Though patterns of relative use changed in both ecosystems, elevated CO_2 caused changes in the absolute rate of substrate oxidation in the Biolog microplates only in soils from the sandstone grassland, decreasing use of amines and amides, causing no change in the use of carboxylic acids, and increasing rates of use of the other compound classes (Fig. 6). This is partly consistent with results for *Gutierrezia sarothrae* monocultures, where elevated CO_2 caused increased use of carbohydrates, amino acids, and carboxylic acids, but decreased use of polymers, with no change in amines and amides (Rillig et al. 1997). These results could be interpreted to mean that elevated CO_2 altered the rate of use of similar compounds in situ (e.g., Ellis et al. 1995). However, equating bacterial activity in situ with activity in the Biolog microplate is questionable (Haack et al. 1995; Garland 1997), and recent results show that qualitative shifts in individual carbon-source use are more likely to reflect differences in structure of the bacterial assemblage, rather than its function (Garland et al. 1997). The Biolog assay also distinguished the two grassland ecosystems in their patterns of sole carbon-source use. Given the differences between the serpentine and sandstone grasslands in plant species composition and soil characteristics (Field et al. 1996), it was surprising that the differences associated with most of the variance (i.e. PC1) were caused by elevated CO_2, rather than natural differences between the bacterial communities in these two strongly contrasting ecosystems.

Responses to elevated CO_2 of some components of the microbial biomass were similar in the two grasslands, but soil metabolic activity increased only in the sandstone (Fig. 3), and phytophagous nematodes increased only in the serpentine. The observed increases under elevated CO_2 in root biomass (Hungate et al. 1997b; Rillig et al. 1999) and in soil respiration (Luo et al. 1996) are similar for these two grasslands, so the different responses of soil biota in the two grasslands are not clearly explained by the amount of carbon distribution below ground in response to elevated CO_2. Alternatively, carbon distributed below ground may be partitioned to different parts of the soil microbial assemblage in the two grasslands. Specifically, Rillig et al. (1999) found that elevated CO_2 enhanced root length in the serpentine grassland but not in the sandstone, whereas arbuscular mycorrhizal hyphal length increased in the sandstone grassland but not in the serpentine. Thus, it is understandable that phytophagous nematodes would respond to elevated CO_2 in the serpentine, the system where CO_2 caused increased root length, but that they would not respond in the sandstone, where no such increase in root length occurred (Rillig et al. 1999). Additionally, carbon distributed below ground in the serpentine to root-feeding nematodes would likely not be apparent in assays of soil metabolic activity in laboratory incubations, particularly after roots have been removed. Thus, the nature of the below-ground response to elevated CO_2 may be governed, not only by how much elevated CO_2 increases carbon distribution below ground, but also by how that response is manifest, as increased root length, exudation, turnover, or mycorrhizal infection.

Despite these differences, our findings show that in both grasslands some of the extra carbon flow below ground in elevated CO_2 is transferred up the soil microbial food web, through bacteria and fungi, to higher decomposer trophic levels, such as protozoa and nematodes. In these annual grasslands, such effects may be confined to the early part of the growing season, when plants are in the vegetative growth phase (Figs. 2, 3). Evidence for carbon transfer up the decomposer food web has also been shown in previous work in agricultural systems (Runion et al. 1994), poplar monocultures (Lussenhop et al. 1998), pasture microcosms (Yeates et al. 1997), and model grasslands (Jones et al. 1998). Thus, in cases where elevated CO_2 increases carbon input to soil, such changes in the below-ground food web are likely to ensue.

Acknowledgements We thank Mary Firestone for use of the laboratory facilities, Teri Balser for assistance with the Biolog assay, Elaine Ingham and the Soil Microbial Biomass Service for sample analyses, and Rob Jackson for assistance with the field sampling. Diana Wall, Jane Marks, Valerie Eviner, and two anonymous reviewers provided valuable comments on the manuscript. This work was supported by the National Science Foundation (NSF DEB 90–20134, 90–20347, 90–20135, and 92–245235).

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