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Effects of elevated atmospheric CO_2 concentration on C and N pools and rhizosphere processes in a Florida scrub oak community

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Abstract

The effect of elevated atmospheric CO_2 concentration (C_a) on soil carbon and nitrogen accumulation and soil microbial biomass and activity in a native Florida scrub oak community was studied. The plant community, dominated by *Quercus myrtifolia* Willd. and *Q. geminata* Small, was exposed for 2 years to elevated C_a in open-top chambers. Buried subsoil bags were retrieved after 1 year of exposure to elevated C_a. In addition, soil cores were taken twice from the chambers within two weeks in July 1998 (the first after a long dry spell and the second after 25 mm of rainfall) and divided into rhizosphere and bulk soil. Soil organic matter accumulation (excluding roots) into the buried subsoil bags was lower in elevated than in ambient C_a. Concentrations of soluble carbon and ninhydrin-reactive nitrogen (N_{ninh}) in the rhizosphere soil were reduced by elevated C_a for the first sampling date and unaffected for the second sampling date. Microbial activity, measured as fluorescein diacetate (FDA) hydrolysis, decreased in elevated C_a for the first sampling date. Microbial biomass carbon and nitrogen in the bulk soil were unaffected by elevated C_a. There was no effect of elevated C_a on bacterial numbers in the rhizosphere.

Keywords: elevated CO_2, microbial activity, phyllosphere, *Quercus* spp., rhizosphere, soil microbes

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Introduction

A wide range of environmental constraints affects the response of individual plants, communities and ecosystems to a future, CO_2-enriched atmospheric environment (Idso & Idso 1994). It has been suggested that plants growing in nutrient-poor environments are less responsive to elevated C_a than plants in fertile environments (Poorter *et al*. 1996). Similarly, available water resources may limit the CO_2 response of a plant community. However, C_a alters resource requirements of plants. For example, carbon assimilation in elevated C_a requires less water than in ambient C_a, due to greater stomatal closure (Drake *et al*. 1997 and References cited therein) and also operates at lower leaf nitrogen concentrations, due to lower Rubisco contents (Conroy & Hocking 1993). On the other hand, enhanced plant growth in elevated C_a requires a greater absolute amount of water and nutrients. By increased depletion of these resources, increased exploration of the soil by roots (Rogers *et al*. 1994), altered chemical composition of plant tissues (Cotrufo *et al*. 1998) and altered amounts and composition of root exudates (Paterson *et al*. 1997), the response of the vegetation to elevated C_a will modify its abiotic, microbial and faunal environment (Drake *et al*. 1997; Sadowsky & Schortemeyer 1997). This, in turn, will have a feedback effect on plant performance. The most pronounced responses of soil microbial communities to elevated C_a can be expected in the immediate vicinity of the roots, i.e. the rhizosphere. In their review of root and rhizosphere responses to elevated C_a, Rogers *et al*. (1994)

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postulated an increase in rhizodeposition (the release of cells, exudates, mucilages and other compounds into the rhizosphere by roots) following increasing photoassimilation in elevated C_a. Experimental evidence for this, however, is difficult to obtain (Sadowsky & Schortemeyer 1997).

Little is known about how C and N concentrations in the rhizosphere are affected by CO_2 enrichment in field experiments. Moreover, there are conflicting findings as to whether the microbial biomass pool in the soil is affected by elevated C_a: the field studies of Rice *et al*. (1994), Hungate *et al*. (1997a,b) and Niklaus (1998) report an increase in microbial biomass, while Rice *et al*. (1994; for a second year of the same study), Niklaus & Körner (1996) and Schortemeyer *et al*. (1996) do not detect a change in microbial biomass. The response of the soil microbial biomass pool will affect the N availability for plants as soil microbes may enhance mineralization of soil N (Zak *et al*. 1993) or immobilize soil N (Díaz *et al*. 1993), thus creating positive or negative feedbacks for plant N uptake. On the other hand, altered N uptake by plants exposed to elevated C_a and changed rhizosphere conditions may affect the available N pool for soil microbes.

The aim of the present study was to investigate the effect of elevated C_a on C and N accumulation in soil, the availability of C and N in rhizosphere soil and in bulk soil (i.e. soil not directly influenced by roots), and microbial parameters in both rhizosphere and bulk soil in an environment that is severely limited by water and N availability. For this reason, the study was carried out in a natural, unfertilized Florida scrub-oak system on very nutrient-poor soil. The Florida scrub studied is subject to an 8–12 years fire return cycle and was burned prior to starting the experiment. Burning should initially increase the availability of soil nutrients. However, most of the N in above-ground biomass should be combusted by a fire, and the system remains N-limited. The limited water-holding capacity in the sandy soils of the Florida scrub exposes the system to frequent drought stress.

Materials and methods

Site description

The experiment was located at Kennedy Space Center on Merritt Island, a barrier island off the East Coast of Florida (28°38′N, 80°42′W). The ecosystem studied is an oak-saw palmetto scrub, described in more detail by Schmalzer & Hinkle (1992). The dominating species in this system are oaks (myrtle oak, *Quercus myrtifolia* Willd., sand live oak, *Q. geminata* Small, and Chapman oak, *Q. chapmannii* Sarg.) and saw palmetto (*Serenoa repens* (Bartram) Small). The leguminous vine, *Galactia elliottii* Nuttall (milk-pea), is also present in all plots. *Q. myrtifolia* is the most abundant species in the system. The soil is a Pomello sand (arenic haplohumod), a moderately well-drained sandy soil with low nutrients and low pH (3.9–4.1, Huckle *et al*. 1974). The soil has a marked organic layer on top. The organic matter content is mostly restricted to the top 30 cm of the soil profile (7% for 0–15 cm and 2% for 15–30 cm depth, Schmalzer & Hinkle 1992), where the majority of the roots are found (Day *et al*. 1996). The community is exposed to a natural fire return cycle of 8–12 years. Half of the experimental site was burned in August 1995, and the other half in January 1996. All oak species regrew into the experimental area by resprouting. As Florida scrub is generally limited by water as well as nutrients, annual biomass increase is slow with ~60 g m^{-2} for vegetation grown in ambient C_a (Drake, unpubl. data).

The open-top chamber experiment

After the initial burn, 16 octagonal open-top chambers (each covering an area of 9.65 m^2) were erected on the experimental site. The chambers were similar in design to those described by Drake (1992). Eight chambers received ambient CO_2 concentrations (with the actual CO_2 concentration in the chambers being ~410 µmol mol^{-1}), while the other eight chambers received elevated CO_2 concentrations (~757 µmol mol^{-1}) continuously. The chamber layout and technology are described in detail by Stiling *et al*. (1999). CO_2 fumigation was started in May 1996.

Measurement of changes in C and N pool sizes

Soil from the C horizon (0.8–1.0 m depth) of the experimental site was homogenized and samples of > 50 cm^3 volume were put into mesh bags (5 mm mesh size). The bags were buried in February 1997 in 15 cm depth in the CO_2-enriched and control chambers. Subsamples of the homogenized subsoil were dried and stored. In December 1997, one bag per chamber was retrieved, all visible root segments were removed, and the soil samples were dried and analysed together with the initial subsamples for total C and N on a Perkin–Elmer 2400 CHN analyser.

1998 Sampling and extraction

On 9 July 1998, after a long dry period (26 mm rainfall in the three months before sampling), and on 23 July 1998, after the first rainfalls of the summer season, four soil cores (to 15 cm depth) were taken from each chamber and pooled. Soil moisture contents were 1.1% and 2.8% (w/w) for the first and second sampling, respectively. Average soil moisture did not differ between C_a treatments. All visible fine roots with the adhering soil were picked from

the composite soil samples and shaken for 30 min in 50 mL 0.15% (w/v) sodium pyrophosphate in 100 mL nalgene bottles. As most live roots formed rhizosheaths, 'rhizosphere soil' was relatively easy to define. After shaking, the now soil-free roots were removed and dried for 48 h at 70 °C. Neither root dry weight of the samples (nor the amount of rhizosphere soil extracted per g root dry weight) differed between C_a treatments. Root samples were analysed for total C and N concentrations using a Perkin–Elmer 2400 CHN analyser.

An inevitable problem with root sampling from soil cores in mixed communities is that the species the roots belong to is often unclear. Here, we tried to core close to specimens of *Q. myrtifolia*, and assume that the majority of the roots sampled belong to this species. An additional problem in this experiment was that there were only a few specimens of *S. repens* shoots growing in the chambers. However, *S. repens* has extensive rhizomes which reach from the outside into the chambers and a disproportional number of *S. repens* roots (which could be clearly distinguished) were found in the chamber area. These roots were removed from the samples.

The root-free soil was passed through a 2-mm sieve and cleared of large organic debris. This soil was considered 'bulk soil'. Bulk soil samples of 10 g fresh weight were extracted and analysed in the same way as the rhizosphere soil samples. For the rhizosphere soil samples, the suspension was dried after removal of extract samples for analysis and the dry weight of the soil extracted was determined.

Determination of microbial activity

Microbial activity was measured as fluorescein diacetate (FDA) hydrolysis according to Schnürer & Rosswall (1982). FDA is hydrolysed by a number of enzymes, such as lipases, proteases, and esterases. Four replicate subsamples of the rhizosphere and bulk soil suspensions were incubated with FDA reagent (final concentration: 10 mg L^{-1}). The amount of FDA hydrolysed was measured spectrophotometrically at 490 nm. Readings were taken after 0 and 240 min of incubation and the reaction stopped by addition of $HgCl_2$. For a number of samples, the reaction was stopped hourly until 4 h after FDA addition. The amount of FDA hydrolysis increased linearly for the time period measured. Results are expressed as the absorption at 490 nm after 4 h, minus the absorption of the unincubated samples at 490 nm and 0 h, divided by the weight of the soil extracted.

Determination of ninhydrin-reactive nitrogen and soluble carbon

After centrifugation of the soil suspension, four replicate subsamples were taken from each rhizosphere and bulk soil sample and incubated with ninhydrin reagent at 100 °C for 25 min as described by Joergensen & Brookes (1990). The concentration of ninhydrin-reactive nitrogen (N_{ninh}, which consists of ammonium-N and -amino-N) was measured spectrophotometrically at 570 nm against $(NH_4)_2SO_4$ and L-leucine standards. Results are expressed as μmol N g^{-1} soil extracted.

Soluble organic carbon contents of the rhizosphere and bulk soil extracts were measured after centrifugation and acidification with H_2SO_4 using a Shimadzu TOC-5050 total organic carbon analyser.

Determination of microbial biomass carbon and nitrogen in bulk soil

Bulk soil samples were analysed for microbial biomass by a modified fumigation–extraction method described by Vance *et al*. (1987). The bulk soil samples were moistened to field capacity, and four subsamples of 10 g fresh soil from each sample were placed into desiccators lined with moist paper towels, sealed and kept at 25 °C for 24 h. A fifth soil sample was oven-dried at 105 °C for 24 h to correct for dry weight. After 24 h of incubation, two subsamples were extracted in 50 mL of 0.5 m K_2SO_4. The other two subsamples were exposed to ethanol-free $CHCl_3$ vapour for 24 h, the fumigant was removed, and the soils were extracted under the same conditions as the nonfumigated samples. For determination of C, the extracts were acidified and measured using a Shimadzu TOC-5050 total organic carbon analyser. Biomass C was estimated from the difference between organic C extracted from the fumigated and unfumigated soil (E_C) using the equation: Biomass $C = E_C \times 2.22$, with 2.22 being the efficiency of extraction constant (Wu *et al*. 1990). Biomass N was measured by determining ninhydrin-reactive N (N_{ninh}) in extracts from fumigated and unfumigated samples, and using the equation: Biomass $N = E_N \times 5$ (Joergensen & Brookes 1990), where E_N is the difference between N_{ninh} values from fumigated and unfumigated samples.

Determination of culturable heterotrophic bacteria in rhizosphere soil

Serial dilutions of the rhizosphere soil extracts were plated onto R2A agar (a nonselective, low-nutrient medium) and incubated for 3 d at 35 °C. Plates yielding between 20 and 200 colonies were selected for counts of total culturable heterotrophic bacteria. Numbers are expressed as colony forming units (CFU) g^{-1} soil extracted.

Results

Soil C concentrations in the buried bags were initially low (~0.1 mmol C g^{-1} soil), as could be expected for the

local subsoil. During one year, the soil C in the buried bags exposed to elevated C_a accumulated to more than double the initial concentration and to more than four times the initial concentration when exposed to ambient C_a (Table 1). Soil N concentrations in the buried bags did not change during the year 1997. There was no effect of C_a on N concentrations in the buried bags (Table 1).

The total carbon concentration of the root material isolated from the soil cores was 48 ± 1% for both sampling dates on 9 and 23 July (Table 2). For the first set of samples, root N concentrations were around 0.77% and did not differ between C_a treatments. For the second sampling date, however, root N concentrations exceeded those of the first sampling date, and were increased in elevated C_a (0.98%, compared with 0.86% at ambient C_a, Table 2). This resulted in a significantly decreased C:N ratio of the root material exposed to elevated C_a and sampled on 23 July (Table 2).

Table 1 Total carbon and nitrogen in subsoil bags buried for 1 year in the top soil horizon. Data presented as mean ± SE ($n = 8$)

		1 year (Dec 1997)		
	Initial (Feb 1997)	Ambient C_a	Elevated C_a	
µmol C g^{-1} soil DW	108 ± 25	492 ± 117	275 ± 25	$P = 0.12$
µmol N g^{-1} soil DW	15.8 ± 2.5	18.3 ± 5.0	15.0 ± 2.5	n.s.

Table 2 Carbon and nitrogen concentrations and C:N ratios of roots taken from 0 to 15 cm soil depth at two sampling dates. Data presented as in Table 1.

	9 July 1998			23 July 1998		
	Ambient C_a	Elevated C_a		Ambient C_a	Elevated C_a	
% C	49.0 ± 0.6	48.6 ± 0.8	n.s.	48.0 ± 0.6	47.4 ± 0.8	n.s.
% N	0.78 ± 0.02	0.76 ± 0.04	n.s.	0.86 ± 0.05	0.98 ± 0.02	$P = 0.04$
C:N	63 ± 2	65 ± 3	n.s.	57 ± 4	49 ± 2	$P = 0.06$

Table 3 Concentrations of soluble C in rhizosphere and bulk soil, ninhydrin-reactive nitrogen (N_{ninh}) in rhizosphere and bulk soil, microbial activity (measured as FDA hydrolysis – values are calculated from extinction readings at 490 nm after 4 h of incubation) in rhizosphere and bulk soil, total heterotrophic bacteria (enumerated as colony forming units (CFU) on R2A agar) in rhizosphere soil, and microbial biomass C and N in bulk soil. Data presented as in Table 1.

	9 July 1998			23 July 1998		
	Ambient C_a	Elevated C_a		Ambient C_a	Elevated C_a	
Soluble C (rhiz. soil) [µmol g^{-1} soil DW]	431 ± 44	300 ± 44	$P = 0.053$	298 ± 51	265 ± 32	n.s.
Soluble C (bulk soil) [µmol g^{-1} soil DW]	71 ± 11	69 ± 5	n.s.	51 ± 4	64 ± 4	$P = 0.035$
N_{ninh} (rhiz. soil) [µmol g^{-1} soil DW]	2.80 ± 0.41	1.89 ± 0.27	$P = 0.086$	1.71 ± 0.28	1.67 ± 0.21	n.s.
N_{ninh} (bulk soil) [µmol g^{-1} soil DW]	0.40 ± 0.03	0.33 ± 0.03	$P = 0.091$	0.27 ± 0.03	0.30 ± 0.02	n.s.
Microbial activity (rhiz. soil) [A_{490} mg^{-1} soil DW]	202 ± 25	143 ± 31	$P = 0.058$	95 ± 11	97 ± 12	n.s.
Microbial activity (bulk soil) [A_{490} mg^{-1} soil DW]	22 ± 4	19 ± 2	$P = 0.056$	27 ± 3	25 ± 2	n.s.
Heterotrophic bacteria [CFU g^{-1} soil DW] (x 10^5)	2.84 ± 0.62	4.16 ± 0.79	n.s.	4.05 ± 0.98	2.77 ± 0.33	n.s.
Microbial biomass C [µg C g^{-1} soil DW]	149 ± 14	140 ± 26	n.s.	285 ± 42	255 ± 23	n.s.
Microbial biomass N [µg N g^{-1} soil DW]	6.5 ± 0.9	4.9 ± 1.3	n.s.	11.6 ± 2.8	9.4 ± 1.7	n.s.

The concentrations of soluble C analysed in rhizosphere soil were an order of magnitude higher than those in bulk soil (Table 3). For the second sampling on 23 July, soluble C concentrations in both rhizosphere and bulk soil were slightly lower than for the first sampling on 9 July. For that sampling date, elevated C_a significantly reduced the amount of soluble C in the rhizosphere, while there was no effect on bulk soil C concentrations (Table 3). In contrast, rhizosphere C concentrations were unaffected by C_a on 23 July, while bulk soil C concentrations were slightly increased by elevated C_a (Table 3).

As was the case for soluble C concentrations, ninhydrin-reactive N (N_{ninh}) concentrations were 8–10 fold higher in rhizosphere than in bulk soil samples (Table 3). On the first sampling date, N_{ninh} concentrations were decreased by elevated C_a in both rhizosphere and bulk soil samples. For the second sampling date, numbers were generally lower than for the first sampling date, and there was no effect of C_a on either bulk soil or rhizosphere soil N_{ninh} concentrations (Table 3).

Microbial activity (measured as FDA hydrolysis of soil extracts) exhibited similar patterns, as did the C and N concentrations: a tenfold higher activity was measured in rhizosphere soil compared with bulk soil (Table 3). FDA hydrolysis in both rhizosphere and bulk soil samples was reduced in elevated C_a for the first sampling date (Table 3). For the second sampling date, there was no effect of C_a on FDA hydrolysis (Table 3).

Microbial numbers in the rhizosphere, as enumerated by plate counts on R2A agar, represent only a fraction of the total microbial population in the soil. However, it should be possible to make comparative statements about the C_a treatments. There was no statistically significant C_a effect on the number of colony-forming units (CFU) on R2A agar at either sampling date (Table 3).

Microbial biomass C and N of root-free bulk soil were measured for both sampling dates. Both microbial biomass C and N increased by almost 100% for the second sampling date (Table 3). There was no significant effect of C_a on microbial biomass C or N, although there was a tendency for decreased microbial biomass *N*-values in elevated C_a (Table 3).

Discussion

Carbon

Net changes in the soil carbon pool, as affected by elevated C_a, are generally difficult to detect due to the large background soil C pools and high spatial variability in soil C concentrations (Hungate *et al*. 1996). This problem has been overcome by use of stable C isotope techniques (Hungate *et al*. 1996; Ineson *et al*. 1996; Nitschelm *et al*. 1997) or very C-poor growth substrates, such as homogenized subsoil material (Lutze & Gifford 1998). The present study made use of the latter technique and measured a significant increase in buried subsoil bags over a one-year period, in both ambient and elevated C_a (Table 1). That significantly less C ($P = 0.12$) was accumulated in the soil in elevated C_a is an unexpected result. Most other studies of elevated C_a effects have observed significant increases in soil C (Rice *et al*. 1994; Ineson *et al*. 1996; Lutze & Gifford 1998) or trends for increased C sequestration in soil (Wood *et al*. 1994). The two major sources for carbon accumulation in soil are, apart from standing root biomass, decomposed root material and root exudates. Decreased root growth in elevated C_a is unlikely to have contributed to the lower C sequestration recorded in elevated C_a, as root growth for the same site has been reported to be enhanced by elevated C_a (Day *et al*. 1996).

The concentration of soluble carbon in the rhizosphere was either reduced or unaffected by elevated C_a (Table 3). In several experiments, increased root exudation has been inferred from results of radiolabelling studies. These studies often find an enhanced translocation of recently assimilated C into the soil in elevated C_a (Norby *et al*. 1987; Billes *et al*. 1993; Rattray *et al*. 1995; Paterson *et al*. 1996). Most of this increase can be attributed to larger root systems that have developed in elevated C_a. However, several studies also report a relative increase in C release (on a root dry matter basis) into the rhizosphere, especially of C3-grasses (Rattray *et al*. 1995; Paterson *et al*. 1996). One other study (Hodge *et al*. 1998) reports a negative effect of elevated C_a on C release into the rhizosphere of *Lolium perenne*. Although we could not measure root exudation rates *per se* in the present field study, our data suggest that in the Florida scrub ecosystem studied, there is no effect of elevated C_a.

Nitrogen

Ninhydrin reacts with N bound in amino compounds or in ammonium. An 8–10 fold higher concentration of N_{ninh} in the rhizosphere compared with that in bulk soil indicates that most of the rhizosphere N_{ninh} is likely to originate from amino acids and amides exuded by roots. The accumulation of amino acids in the rhizosphere is the net result of two processes: the loss of amino acids from the roots into the surrounding soil and their recapture by active uptake (Jones & Darrah 1994). The same authors also suggest that the uptake of previously exuded N compounds becomes more important for plants as soil N resources become more limiting. A lower concentration of N_{ninh} found in the rhizosphere could thus reflect a greater uptake of N previously lost by roots.

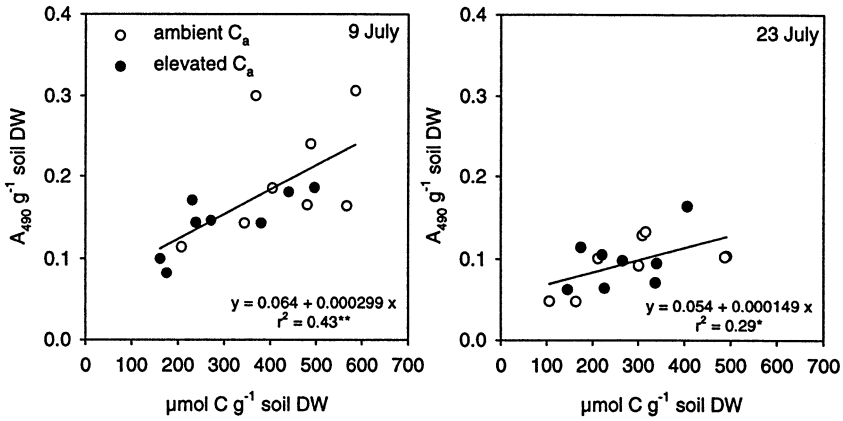

Fig. 1 Relationship between soluble C concentrations and microbial activity (measured as FDA hydrolysis) in rhizosphere soil. Each datum represents one sample. Open symbols represent samples from ambient C_a chambers, and closed symbols represent samples from elevated C_a chambers.

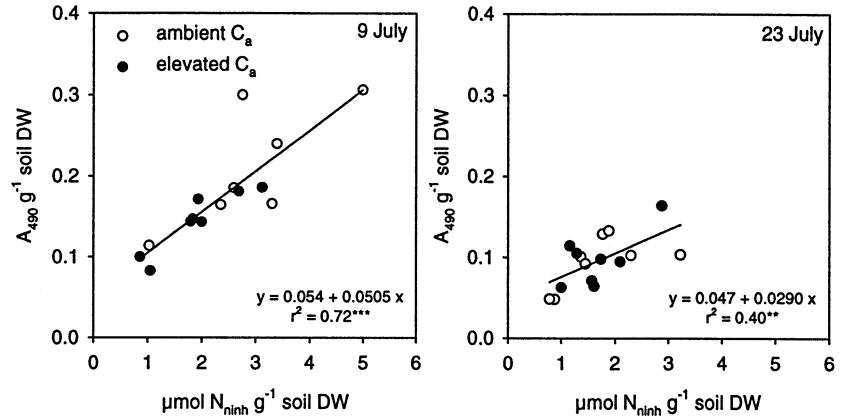

Fig. 2 Relationship between N_{ninh} concentrations and microbial activity (measured as FDA hydrolysis) in rhizosphere soil. Each datum represents one sample. Open symbols represent samples from ambient C_a chambers, and closed symbols represent samples from elevated C_a chambers.

For the same Florida study site, Hungate *et al*. (1999) found less ammonium-N in bulk soil under plants exposed to elevated C_a as well as reduced gross N mineralization rates. Also, the authors report no effect of C_a on ammonium recovery from resin lysimeters after one year of exposure, but reduced nitrate recovery in elevated C_a. The reduced soil N concentration is most likely a result of both increased plant N uptake, and subsequent immobilization of N in the standing biomass (Dijkstra, pers. comm.), as well as of decreased mineralization (Hungate *et al*. 1999). The reduced availability of mineral N could induce a greater re-capture of amino acids by roots and be responsible for the decreased concentrations of N_{ninh} in rhizosphere and bulk soil for the first sampling date (Table 3).

Between 9 and 23 July, some considerable rainfall events occurred (25 mm over the 14-d period, with 13.5 mm falling within 45 min on 18 July). This more than doubled the soil water content. The soil water content was not affected by C_a. The increase in soil moisture most likely triggered N mineralization. The lower concentrations of N_{ninh} in rhizosphere and bulk soil found for the second sampling date could be due either to enhanced root N uptake, to immobilization in microbial biomass (see below), or to leaching of the end-product of N mineralization and subsequent nitrification, i.e. nitrate (which would be facilitated by the sandy soil texture). There was no C_a effect on N_{ninh} concentrations for the second sampling date (Table 3). This could indicate that physical processes independent of plant N uptake, such as leaching, have contributed to the reduced N concentrations and evened out a possible C_a effect.

Microbial activity

FDA hydrolysis is an indicator for the metabolic activity of the soil microbial community. As FDA hydrolysis was greatly enhanced for rhizosphere soil extracts over bulk soil extracts (Table 3), this indicates a concentration of the soil microbial activity in the rhizosphere. Rhizosphere microbial activity was reduced by elevated C_a for the first sampling, and not affected by C_a for the second sampling (Table 3). The fact that FDA hydrolysis follows the same trends as do rhizosphere C and especially N concentrations, suggests a link between the availability of C and N as substrates for microbial activity and the FDA hydrolysis results. Figures 1 and 2 plot microbial activity against the concentrations of soluble C and N_{ninh} in the

rhizosphere for both sampling dates. For both dates, microbial activity is significantly correlated with the availability of C and N as microbial substrates. N availability is more closely correlated with microbial activity than is C availability (r^2 values of 0.72 and 0.40 vs. 0.43 and 0.29, respectively), and the slope of the regression between N_{ninh} and microbial activity is steeper than that of the regression between soluble C and microbial activity. For bulk soil, C availability is not correlated with microbial activity, and N availability is correlated with microbial activity only for the first sampling date ($r^2 = 0.56$, data not shown). Indeed, microbial activity in the rhizosphere depends on the availability of C and especially N.

Bacterial numbers

There was no significant effect on microbial numbers in rhizosphere soil extracts (Table 3). Similarly, Grayston *et al*. (1998), with a different definition of 'rhizosphere' (the whole upper 2.5 cm of the soil horizon) found no C_a effect on bacterial numbers in the rhizosphere of the grass *Danthonia richardsonii*. Schortemeyer *et al*. (1996) found contrasting effects of elevated C_a on bacterial numbers in the rhizosphere of *Lolium perenne* and *Trifolium repens* (increase, decrease, or no effect, depending on plant species and season). In any case, microbial numbers may not relate to the metabolic activity of the rhizosphere microflora.

Microbial biomass

The most striking effect on the amount of microbial biomass C and N measured in bulk soil was that of the sampling date. The water content of the topsoil, although still low, more than doubled between 9 and 23 July, and was unaffected by C_a. This increase in soil moisture probably gave rise to microbial biomass C and N in all treatments (Table 3). Interestingly, this increase in microbial biomass is not reflected by increased microbial activity in the bulk soil extracts (Table 3). The lack of a C_a response of microbial biomass C and N concentrations is in accordance with previous field studies which did not detect a C_a effect (Rice *et al*. 1994; Niklaus & Körner 1996; Schortemeyer *et al*. 1996). Other studies found evidence for increased microbial biomass C in elevated C_a (Rice *et al*. 1994; Hungate *et al*. 1997a,b; Niklaus 1998). For the same Florida site in July 1997, Hungate *et al*. (1999) report no effect of C_a on microbial biomass N. Our numbers for microbial biomass N range around 50% of those reported by Hungate *et al*. (1999), which is possibly due to the different method used for analysis.

Other factors than C_a, such as plant species (Schortemeyer *et al*. 1996) or soil moisture (this study) cause the largest variability in soil microbial biomass values. It is also possible that this variability masks any possible 'real' C_a effect on microbial biomass. Some studies that have detected increases in microbial biomass in elevated C_a (Díaz *et al*. 1993; Zak *et al*. 1993; Schenk *et al*. 1995; Lutze, pers. comm.) have used pot or monolith systems where the substrate was rather homogeneous and had a very low initial microbial biomass content, which should make the detection of changes easier. The absence of a C_a effect on microbial biomass in bulk soil cannot entirely be explained by reduced availability of resources for microbes: even if rhizodeposition rates per unit of root mass or length are unaffected by elevated C_a, it still considerably enhances root production (Rogers *et al*. 1994), but reduces turnover rates (Jongen *et al*. 1995). Jongen *et al*. (1995) attribute the slower root turnover to an increased C:N ratio of root material in elevated C_a, an observation that could not be confirmed for the oak roots in the present study. In a pilot study for this project, Day *et al*. (1996) found enhanced production of fine roots in the oak-palmetto scrub system when exposed to elevated C_a. As root N concentrations are not affected by C_a in this study (Table 2), this would mean increased immobilization of N in standing root biomass.

Conclusion

Our results show that there is no evidence of increased root exudation, microbial activity, and microbial biomass for a natural Florida scrub ecosystem after two years of CO_2 enrichment. Rather, reduced microbial activity has been observed for one sampling date. Limitations in available N, induced by lower mineralization rates and enhanced N immobilization in plant biomass, might be responsible for this. Heterogeneity in time and space and the rapid dynamics of most measured parameters caution us not to extrapolate or generalize these findings too far, as we only sampled the experimental site twice, and C_a effects have not been consistent over the two sampling dates.

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