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Transient elevation of carbon dioxide modifies the microbial community composition in a semi-arid grassland

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Abstract

Using open-top chambers (OTC) on the shortgrass steppe in northern Colorado, changes of microbial community composition were followed over the latter 3 years of a 5-year study of elevated atmospheric CO_2 as well as during 12 months after CO_2 amendment ended. The experiment was composed of nine experimental plots: three chambered plots maintained at ambient CO_2 levels of $360 \pm 20\,\mu mol\,mol^{-1}$ (ambient treatment), three chambered plots maintained at $720 \pm 20\,\mu mol\,mol^{-1}$ CO_2 (elevated treatment) and three unchambered plots. The abundance of fungal phospholipid fatty acids (PLFAs) shifted in the shortgrass steppe under the influence of elevation of CO_2 over the period of 3 years. Whereas the content of the fungal signature molecule (18:2ω6) was similar in soils of the ambient and elevated treatments in the third year of the experiment, CO_2 treatment increased the content of 18:2ω6 by around 60% during the two subsequent years. The shift of microbial community composition towards a more fungal dominated community was likely due to slowly changing substrate quality; plant community forage quality declined under elevated CO_2 because of a decline of N in all tested species as well as shift in species composition towards greater abundance of the low forage quality species (*Stipa comata*). In the year after which CO_2 enrichment had ceased, abundances of fungal and bacterial PLFAs in the post-CO_2 treatment plots shifted slowly back towards the control plots. Therefore, quantity and quality of available substrates had not changed sufficiently to shift the microbial community permanently to a fungal dominated community. We conclude from PLFA composition of soil microorganisms during the CO_2 elevation experiment and during the subsequent year after cessation of CO_2 treatment that a shift towards a fungal dominated system under higher CO_2 concentrations may slow down C cycling in soils and therefore enhance C sequestration in the shortgrass steppe in future CO_2-enriched atmospheres.

Keywords: Carbon dioxide; Climate change; PLFA; Shortgrass steppe; Soil fungi; Soil bacteria

1. Introduction

Increasing atmospheric CO_2 concentrations have the potential to alter carbon cycling of terrestrial ecosystems, because elevation of CO_2 can stimulate plant photosynthesis, enhance carbon allocation belowground, increase plant nutrient uptake and improve water use efficiency (Hu et al., 1999). Due to closely coupled plant–microbe interactions, soil microorganisms are predicted to respond to the CO_2 enrichment by increasing their activity due to higher C input or due to changes in chemical composition of the litter and root material (Kandeler et al., 1998; Freeman et al., 2004). Little information is available about the response of microbial community composition to the elevation of CO_2 (Wiemken et al., 2001; Montealegre et al., 2002; Ebersberger et al., 2004). Shifts in microbial community composition could have important implications for ecosystem-scale processes. For example, increasing the abundance of fungi relative to

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bacteria should favor soil C accrual, because fungi assimilate higher proportion of C, and because they are composed of more recalcitrant C compounds than bacteria (Allison et al., 2005; Six et al., 2006). Increased community metabolic efficiency due to higher relative abundances of fungi could be the primary mechanism leading to enhanced C storage in soils (Allison et al., 2005). Therefore, accounting for changes in microbial community composition may improve the prediction of ecosystem response to environmental change (Balser and Firestone, 2005; Schroter et al., 2004).

An open-top chamber (OTC) experiment on the shortgrass steppe in northern Colorado gave us the opportunity to follow possible changes of microbial community composition during a transient elevation of CO_2. The shortgrass steppe is a semi-arid grassland along the western edge of the Great Plains of the United States, stretching from south-eastern New Mexico and western Texas north to the Colorado–Wyoming border at 41°N (Lauenroth and Milchunas, 1991). Vegetation is dominated by warm-season, C_4 grasses (*Bouteloua* and *Buchloe* spp.), and contains also cool-season, C_3 grasses (e.g. *Pascopyrum* and *Stipa* spp.) as well as a variety of C_3 forbs and woody vegetation, C_4 forbs and woody vegetation are uncommon (Morgan et al., 2001). Consistent and strong CO_2-induced production responses were observed mainly due to preferentially enhanced growth of one of the dominant grasses, the C_3, *Stipa comata* (Morgan et al., 2004). Elevated CO_2 reduced stomatal conductance and promoted soil moisture storage in the shortgrass steppe (Morgan et al., 2001; Pendall et al., 2003). Greater root growth was coupled with greater loss of roots under elevated CO_2 over a period of 5 years (Milchunas et al., 2005a). Using growth-to-loss ratio regressions, the authors calculated very long root turnover times of 5.3–7.0 years. While we have some information about the function of soil microbial community in the shortgrass steppe that shows increase of enzyme activities (protease, xylanase, invertase, alkaline phosphatase, arylsulfatase) in the upper 5 cm of the soil under elevation of CO_2 (Kandeler et al., 2006), no results are available—to our knowledge—on microbial community composition.

In this study, we ask whether shifts in microbial community structure occur in a semi-arid grassland during the latter 3 years of a 5-year study of elevated atmospheric CO_2 as well as the post effect during 12 months after CO_2 amendment. The estimation of phospholipid fatty acid (PLFA) pattern should give information about the contribution of bacterial and fungal PLFAs within the soil microbial community (Frostegård et al., 1993, 1991). We hypothesized that the observed changes in plant metabolism under elevation of CO_2 would be accompanied by a concomitant change in microbial community composition of soils. Since the PLFA technique has proven to be a sensitive and reliable tool for assessment of changes in microbial community structure in soil samples (Johansen and Olsson, 2005; Potthoff et al., 2006; Williams et al., 2006), we used this method to measure the composition of the soil microbial community in bulk soil under two atmospheric CO_2 levels.

2. Materials and methods

2.1. Study site

The study site is located at the USDA-ARS Central Plains Experimental Range (CPER), 40°40′N, 104°45′W, in the shortgrass steppe region of northeastern Colorado, about 56 km northeast of Fort Collins, CO (Lauenroth and Milchunas, 1991). The soil at the experimental site is a Remmit fine sandy loam (Ustollic Camborthids according to the USDA classification system) containing 0.89% total carbon, 0.10% total nitrogen and a pH (H_2O) value of 7.2 in the upper 20 cm layer (Mosier et al., 2002). The soil holds 18% water at field capacity and 4% at the permanent wilting point. The semi-arid grassland site is at 1650 m elevation and has a long-term mean annual precipitation of 320 mm, with the majority occurring in May, June and July. A variety of seasonal precipitation patterns typical for shortgrass steppe region occurred during the 5-year study (Milchunas et al., 2005b). The third year of the experiment (1999) had nearly twice the average annual precipitation due to both wet spring and autumn periods. Precipitation in year 4 of the experiment (2000) was a little below annual average precipitation, but a very long drought occurred from spring until late summer, when a few very heavy storms resulted in what appeared as a spring flush of growth in autumn. Year 5 was only slightly above the annual precipitation, but was very different seasonally. A long drought during the entire latter half of the growing season occurred in year 5, but the very wet spring elevated the annual amount received. Mean monthly air temperature ranges from 22 °C in July to below 0 °C in January. Frost-free days average 133 days.

The experimental site had a vegetative basal cover of 25–35% of the soil, and was composed of over 25 species of forbs and grasses, but dominated by three grass species: *Bouteloua gracilis* (H.B.K.) Lag. (a warm season, C_4 grass, accounts for approximately 42% of total aboveground biomass), *Pascopyrum smithii* (Rydb.) A. (C_3 grass, 26% of total aboveground biomass) and *Stipa comata* (C_3 grass, 26% of total aboveground biomass). Root biomass (including crowns) is responsible for around 70% of net primary production (NPP) in the ecosystem: belowground biomass NPP averages $223\,g\,m^{-2}\,yr^{-1}$, root crowns contribute $57\,g\,m^{-2}\,yr^{-1}$, while aboveground biomass NPP averages $109\,g\,m^{-2}\,yr^{-1}$ (Milchunas and Lauenroth, 2001). Nine experimental plots of similar botanical composition and similar chemical and physical soil properties were selected for the OTC experiment. The field had been grazed by cattle at a light to moderate intensity (about 30% annual forage removal) until the start of the experiment in March 1997.

2.2. Experimental design

From 1997 to 2001, open-top chambers (4.5 m diameter × 3 m height) were used to evaluate the effects of increased atmospheric CO_2 on the shortgrass steppe ecosystem (CO_2 enrichment experiment). After shutting

off the CO_2 enrichment, the experiment was prolonged for a further year to investigate the resilience of the CO_2 effects on the soil ecosystem (post-CO_2 experiment). A detailed description of the experiment is given by Morgan et al. (2001). Briefly, the experiment was composed of nine experimental plots: three chambered plots maintained at ambient CO_2 levels of $360 \pm 20\,\mu mol\,mol^{-1}$ (ambient treatment), three chambered plots maintained at $720 \pm 20\,\mu mol\,mol^{-1}$ CO_2 (elevated treatment) and three unchambered plots for evaluation of any chamber effect. The chambers, similar in design to Owensby et al. (1993), were installed in early spring each year and remained in place for the entire growing season until mid-October. Chambers were removed in the winter (from November to March) when vegetation was dormant. Sampling was conducted each fall at the same time as the chambers were removed. After shutting off the CO_2 enrichment and removing the chambers the experiment was prolonged.

Each chamber was sub-divided into a northern and southern half. The northern half of the chambers was reserved for all of the destructive sampling and most of the periodic trace gas exchange and soil water measurements. Ten steel cylinders (20 cm diameter × 60 cm length) were pushed into the soil in this half at the beginning of the experiment for destructive sampling.

2.3. Soil sampling

Two of the intact soil cylinders (20 cm diameter × 60 cm deep) were removed from each experimental plot at the end of the 1999, 2000 and 2001 growing seasons. The sampling dates in autumn were chosen to avoid high year-to-year variations in temperature or moisture conditions of the soil. The cores were transported to Fort Collins where they were stored at 4 °C until they were destructively sampled during the following 3 days. Sampling consisted of cutting the steel containment cylinder and sectioning the cylinder into 0–5, 5–10, 10–20, 20–30, 30–40 and 40–60 cm depth increments from the soil surface. After the transport of samples to Germany, each soil section was sieved through a 1 cm screen to remove large roots. The sieved soil was mixed and subsampled to provide samples for the various analyses.

Additional soil samples were taken from the 0 to 5 cm soil layer in March, June and October 2002, 5, 8 and 12 months after the end of the CO_2 treatment, to test the reversibility of the microbial response to elevated CO_2. In general, soils for microbial analyses were immediately frozen after samples were weighed and were kept frozen until time of analyses. The frozen soils were express mailed to the University of Hohenheim under dry ice. For soil microbiological analyses, soils were allowed to thaw for 2 days at +4 °C. This storage and pretreatment preserves about 90% of microbial properties and is recommended for most soil microbial analyses (Schinner et al., 1996). Sieved soil samples (<2 mm) were analyzed in duplicate. Visible fine roots were removed before using the sieved samples for microbiological analyses.

2.4. Phospholipid fatty acid (PLFA) profiles

Lipids were extracted from soil, fractionated and quantified as described by Bardgett et al. (1996), who used a procedure based on that of Bligh and Dyer (1959). Separated fatty acid methyl-esters were identified by chromatographic retention time and mass spectral comparison with a mixture of standard qualitative bacterial acid methyl-ester and fatty acid methyl-ester (Fa. Supelco) that ranged from C11 to C20. For each sample, the abundance of individual fatty acid methyl-esters was expressed per unit dry weight. The analytical results were also verified by GC–MS (mass spectrometry) using a 3400/Saturn4 Diontrap GC/MS system (Varian, Darmstadt, Germany). The nomenclature for PLFAs is that of Frostegård et al. (1993). PLFA bioindicators were selected according to Federle (1986), Vestal and White (1989), Frostegård et al. (1993), Zelles and Bai (1994) and Haubert et al. (2006). The fatty acids i15:0, a15:0, i16:0, 16:1ω7, i17:0, cy17:0, 18:1ω7 and cy19:0 were regarded as bacterial PLFAs ($PLFA_{bac}$). Those representing Gram-positive bacterial PLFAs (G+) were i15:0, a15:0, i16:0, i17:0 and those representing Gram-negative bacterial PLFAs (G−) were cy17:0 and cy19:0. Signature PLFA for fungi was 18:2ω6. Total microbial PLFA ($PLFA_{mic}$) content was calculated as the sum of the bacterial fatty acids and 18:2ω6. We determined soil water content gravimetrically by drying moist soil samples to constant mass at 105 °C.

2.5. Statistical analyses

PLFA data were calculated on an oven-dry weight (105 °C) basis. Normal distribution of the data was tested by the Kolmogorov–Smirnov goodness-of-fit test and homogeneity of the variances was tested by Levene's test. Differences of the means of the microbial measurements between treatments (ambient, elevated and control) were tested by analyses of variance followed by the Student–Newman–Keuls test. The effect of CO_2 and experimental time on the PLFA was quantified using simple two-factorial analysis of variance (ANOVA; CO_2 and sampling year) including soil moisture as covariate. We did not perform a discriminant analysis because number of samples are in a similar range than number of single PLFAs.

3. Results

3.1. Response of soil microbial community composition during to CO_2 exposure phase (1999–2001)

The total content of microbial PLFAs of soils did not change under elevation of CO_2 (Table 1). Comparing treatments, there were only few consistent trends over time for individual PLFAs (Table 1). Repeated soil sampling gave evidence that 18:2ω6c content of top soils (5 cm layer) increased under elevation of CO_2. Although only marginal differences were detectable in the third year of the

Table 1
Amount of fatty acids (nmol g^{-1}) of the 0–5 cm soil layer in the unchambered control plot, and under ambient and elevated CO_2 in 1999–2001

Fatty acid	1999			2000			2001		
	Control	Ambient	Elevated	Control	Ambient	Elevated	Control	Ambient	Elevated
$PLFA_{mic}$	22.37 (1.37)a	28.21 (1.59)b	32.16 (1.58)b	15.77 (1.65)	17.77 (1.86)	18.25 (1.13)	17.69 (1.36)	18.17 (1.22)	20.59 (1.32)
14:0	0.90 (0.15)	1.16 (0.18)	1.46 (0.78)	0.09 (0.03)ab	0.17 (0.13)b	0.00 (0.00)a	0.55 (0.07)	0.61 (0.12)	0.58 (0.11)
i15:0	4.05 (0.24)	5.06 (1.35)	5.30 (1.36)	1.49 (0.23)ab	1.72 (0.74)b	0.63 (0.14)a	3.40 (0.37)	3.45 (0.61)	3.45 (0.63)
a15:0	2.70 (0.15)	3.14 (0.57)	3.23 (0.52)	0.99 (0.18)ab	1.12 (0.48)b	0.33 (0.10)a	1.52 (0.18)	1.79 (0.36)	1.53 (0.32)
15:0	0.19 (0.09)	0.09 (0.21)	1.82 (2.37)	0.22 (0.05)ab	0.32 (0.11)b	0.14 (0.05)a	0.46 (0.04)	0.45 (0.10)	0.47 (0.08)
i16:0	1.85 (0.15)a	2.40 (0.93)ab	2.93 (0.49)b	1.86 (0.24)	1.95 (0.68)	1.26 (0.23)	2.19 (0.18)	2.19 (0.47)	2.25 (0.32)
16:1ω7	3.63 (0.23)a	4.26 (0.61)b	4.91 (0.69)b	2.14 (0.25)	2.41 (0.74)	1.67 (0.35)	2.40 (0.17)	2.46 (0.45)	2.61 (0.44)
16:0	8.32 (0.48)a	10.57 (1.42)b	11.53 (1.68)b	5.27 (0.36)	5.92 (1.52)	4.97 (0.76)	5.70 (0.44)	5.99 (0.97)	6.67 (0.93)
i17:0	0.00 (0.00)	0.01 (0.04)	0.13 (0.18)	0.84 (0.09)	0.82 (0.17)	0.77 (0.08)	0.93 (0.06)	0.94 (0.14)	0.99 (0.11)
17:1	0.96 (0.07)a	1.30 (0.28)b	1.37 (0.30)b	1.07 (0.13)	1.25 (0.27)	1.04 (0.15)	0.34 (0.16)	0.51 (0.42)	0.37 (0.47)
cy17:0	1.09 (0.08)a	1.68 (0.28)b	1.53 (0.34)b	0.96 (0.10)	0.95 (0.24)	0.92 (0.13)	0.98 (0.08)	0.93 (0.19)	0.92 (0.22)
17:0	1.56 (0.78)	0.62 (0.23)	1.28 (1.21)	0.42 (0.05)	0.41 (0.10)	0.38 (0.10)	0.33 (0.02)a	0.33 (0.05)a	0.42 (0.09)b
18:1ω9c	4.84 (0.35)a	6.68 (0.65)b	7.27 (1.18)b	4.78 (0.40)a	5.03 (0.83)a	6.11 (0.71)b	4.28 (0.22)a	4.34 (0.52)a	5.15 (0.57)b
18:1ω7c	5.84 (0.45)a	7.70 (1.08)b	8.71 (1.32)b	4.77 (0.45)a	5.29 (0.89)a	7.39 (1.28)b	3.88 (0.27)a	4.09 (0.50)a	5.32 (0.73)b
18:0	2.12 (0.28)	2.22 (0.34)	2.29 (0.48)	2.08 (0.43)	1.67 (0.41)	1.76 (0.19)	1.38 (0.10)	1.40 (0.24)	1.52 (0.19)
cy19:0	0.80 (0.09)	0.31 (0.32)	0.80 (0.66)	0.66 (0.15)	0.44 (0.10)	0.46 (0.06)	0.37 (0.03)	0.34 (0.06)	0.38 (0.07)
20:0	0.00 (0.00)	0.00 (0.00)	0.08 (0.19)	0.39 (0.03)	0.45 (0.12)	0.41 (0.07)	0.30 (0.02)	0.35 (0.08)	0.33 (0.06)

Given are means and their standard errors (S.E.). Different letters indicate significant differences between treatments.

experiment (Fig. 1), elevated CO_2 significantly increased 18:2ω6c content of the 5 cm layer by 60% during the fourth and fifth year (Fig. 1). Bacterial PLFAs were not significantly influenced by the elevation of CO_2 during the course of the study (Table 1). Consequently, the bacterial-to-fungal ratio decreased significantly under elevated CO_2 (Fig. 1). Simple two-factorial ANOVA revealed that both factors (time and CO_2 treatment) significantly influenced 18:2ω6c content and the ratio of bacPLFA/fungPLFA (ratio of bacterial to fungal PLFAs), but the factors did not interact with one another (Table 2). The effect of time and treatment could not be explained by differences in soil moisture content (Table 2).

Gram-positive bacterial PLFAs as well as the ratio of G+/G− of the elevated CO_2 treatment were lower than in the ambient treatment in the fourth year of the experiment (Fig. 1). Univeriate statistical analysis revealed a chamber effect for bacterial fatty acids in the third year of the experiment and for Gram-positives for the fourth year (Fig. 1). Temporal variation of bacterial fatty acids and Gram-positive fatty acids in soils was much higher than the variation of fungal and Gram-negative fatty acids.

3.2. Reversibility of the carbon dioxide effect on soil microbial community structure after the end of CO_2 enhancement (post-CO_2 effect)

PLFA patterns were studied in March, June, and October 2002 after the end of the CO_2 enhancement in autumn 2001 to determine whether the microbial community composition had returned to its original composition of the control plot (Table 3). Simple two-factorial ANOVA using time and treatment as factors revealed that PLFAs were significantly different in the elevated CO_2 plot after shutting off CO_2 enrichment (Table 2). Sampling date and moisture content of soils did not have any significant effects on microbial community composition. There was only one exception from this general trend: moisture influenced bacterial PLFAs (Table 2).

Whereas the contents of bacterial and fungal fatty acids of ambient and elevated treatments were different from the control treatment in March, we detected a shift back towards the values of the control treatment within the season (Fig. 2). PLFA contents of Gram-positives and fungi of the former elevated treatments reached its original level in June. Univariate statistical analyses showed no significant differences between treatments in October.

4. Discussion

4.1. Response of soil microbial community composition during CO_2 exposure phase (1999–2001)

Soil microbial community plays an important role in organic matter decomposition and nutrient cycling of semi-arid grassland. This study focused on the upper soil layer, since approximately 60% of total root biomass occurred in the top 20 cm of the shortgrass steppe (LeCain et al., 2006) and soil microorganisms followed their substrate availability within the soil profile of the semi-arid grassland (Kandeler et al., 2006). Until now, no evidence was available on how elevated CO_2 would change total abundance of soil microbial biomass in this ecosystem. In the present study we estimated microbial PLFA contents as an independent measure of microbial biomass (Table 1). Elevated CO_2 did not change the total amount of microbial PLFAs, although rhizodeposition was roughly doubled in elevated compared with ambient CO_2 treatment over the

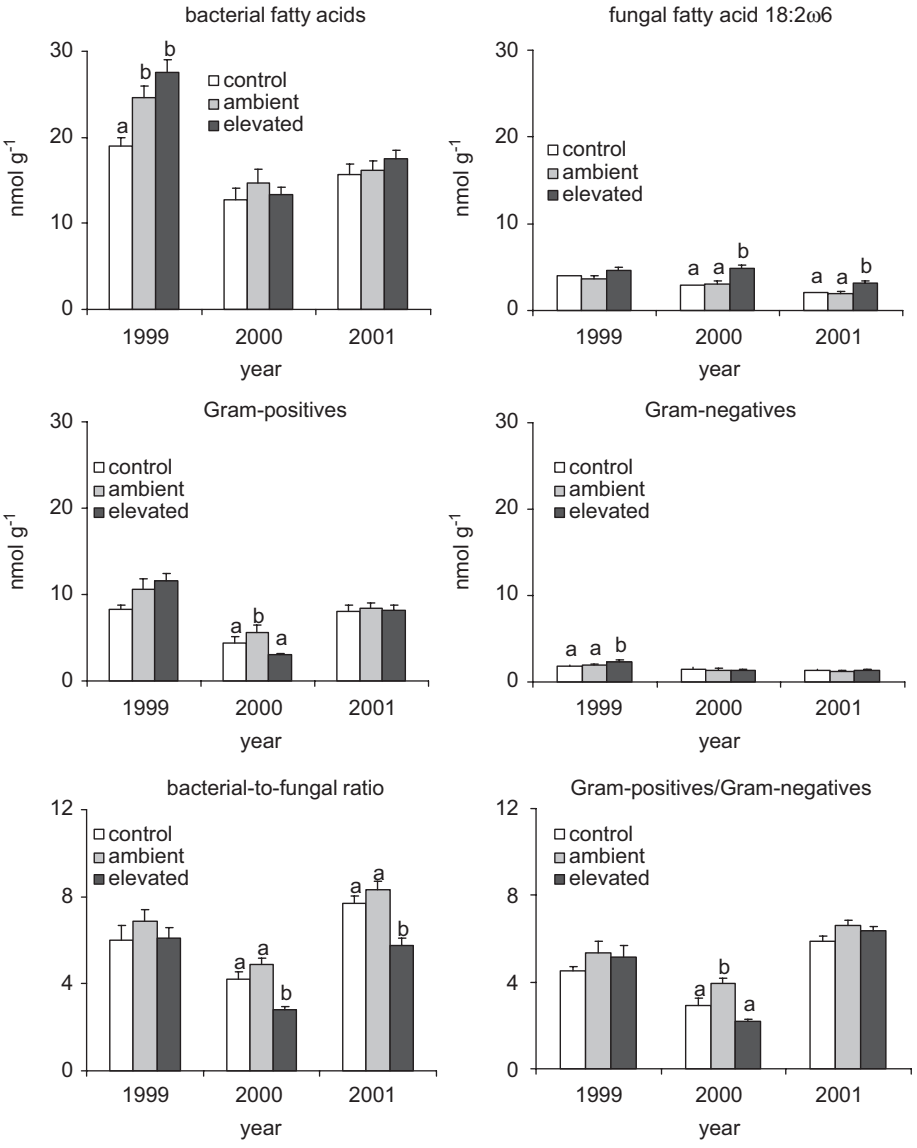

Fig. 1. Concentration of phospholipid fatty acids (PLFAs) in soil characteristic for all bacteria, Gram-positive, Gram-negative or fungi (nmol g^{-1} soil) and ratios of Gram-positive to Gram-negative bacteria and bacteria to fungi in the 0–5 cm soil layer of a shortgrass steppe after 3–5 years of elevation of atmospheric CO_2. Letters (a, b) indicate significant differences between treatments at $P \leqslant 0.05$.

Table 2
Significant differences between means of soil microbial components estimated by simple two-factorial ANOVA (time of sampling and CO_2 treatment)

	CO_2 run				Post-CO_2 run			
	Year (d.f. = 2)	Treatment (d.f. = 2)	Year × treatment (d.f. = 4)	Moisture (d.f. = 1)	Month (d.f. = 2)	CO_2 (d.f. = 2)	Month × treatment (d.f. = 4)	Moisture (d.f. = 1)
bacPLFA	24.80^{***}	7.04^{**}	3.54^{*}	2.71	0.27	20.68^{***}	2.11	4.64^{*}
18:2ω6	17.73^{***}	16.78^{***}	0.46	1.29	0.73	26.97^{***}	4.62^{**}	0.15
Gram-positives	26.58^{***}	2.64	3.38^{*}	2.51	0.49	9.60^{***}	1.35	1.97
Gram-negatives	24.39^{***}	1.01	1.49	1.73	0.05	6.07^{**}	1.18	1.18
bacPLFA/fungPLFA	43.52^{***}	13.36^{***}	1.86	0.11	2.83	12.04^{***}	1.97	1.16
G+/G−	78.13^{***}	8.84^{**}	3.19^{*}	10.33^{**}	1.42	4.12^{*}	0.74	0.40

Soil moisture was included as co-variate. Given are the *F*-values and level of significance (significance of the model is $P<0.05$), $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

Table 3
Amount of fatty acids (nmol g^{-1}) of the 0–5 cm soil layer in the unchambered control plot, and under ambient and elevated CO_2 in the post-CO_2 run 2002

Fatty acid	March 2002			June 2002			October 2002		
	Control	Ambient	Elevated	Control	Ambient	Elevated	Control	Ambient	Elevated
$PLFA_{mic}$	25.65 (2.16)a	40.05 (2.41)b	36.62 (2.77)b	26.72 (2.78)a	47.57 (0.69)b	33.64 (3.00)a	31.69 (3.28)	38.61 (2.31)	31.87 (2.10)
14:0	0.97 (0.07)a	1.56 (0.08)b	1.34 (0.10)b	0.92 (0.09)a	1.68 (0.21)b	1.20 (0.13)a	1.01 (0.11)	1.25 (0.07)	0.94 (0.09)
i15:0	5.28 (0.35)a	6.73 (0.29)b	6.44 (0.46)b	4.71 (0.46)	6.48 (0.42)	5.25 (0.58)	5.54 (0.50)	6.33 (0.34)	5.28 (0.43)
a15:0	2.72 (0.33)a	3.99 (0.21)b	3.52 (0.31)ab	3.01 (0.40)a	5.03 (0.25)b	3.28 (0.71)a	3.59 (0.41)	3.89 (0.19)	3.10 (0.30)
15:0	0.53 (0.05)a	0.77 (0.07)b	0.76 (0.07)b	0.58 (0.04)a	0.94 (0.06)b	0.66 (0.06)a	0.61 (0.06)	0.75 (0.07)	0.58 (0.05)
i16:0	3.39 (0.21)a	4.39 (0.23)b	4.01 (0.26)ab	3.43 (0.38)a	4.97 (0.28)b	3.77 (0.26)a	4.02 (0.43)	4.22 (0.34)	3.57 (0.23)
16:1ω7	4.21 (0.41)a	7.16 (0.61)b	6.29 (0.63)b	4.34 (0.48)a	8.38 (0.31)b	6.20 (0.47)c	5.22 (0.65)a	6.97 (0.46)b	5.19 (0.42)a
16:0	7.22 (0.55)a	11.55 (0.63)b	10.13 (0.60)b	7.69 (0.85)a	14.38 (0.57)b	9.30 (0.80)a	8.89 (1.01)	10.78 (0.71)	8.61 (0.59)
i17:0	1.09 (0.07)a	1.36 (0.07)b	1.27 (0.09)ab	1.12 (0.10)a	1.46 (0.05)b	1.16 (0.09)a	1.18 (0.10)	1.36 (0.07)	1.15 (0.06)
cy17:0	1.22 (0.07)	1.54 (0.09)	1.44 (0.12)	1.24 (0.12)	1.67 (0.13)	1.18 (0.18)	1.45 (0.14)	1.60 (0.12)	1.23 (0.09)
17:0	0.36 (0.02)a	0.52 (0.03)b	0.47 (0.03)b	0.38 (0.04)a	0.68 (0.02)b	0.43 (0.04)a	0.47 (0.05)	0.54 (0.03)	0.45 (0.03)
18:1ω9c	5.38 (0.42)a	7.86 (0.37)b	7.53 (0.60)b	5.65 (0.52)a	9.55 (0.19)b	7.18 (0.35)c	6.29 (0.64)	7.95 (0.41)	6.76 (0.37)
18:1ω7c	5.10 (0.43)a	9.07 (0.75)b	8.95 (0.95)b	5.94 (0.58)a	11.66 (0.41)b	8.77 (0.64)c	6.63 (0.65)	8.69 (0.61)	7.37 (0.40)
18:0	1.67 (0.09)a	2.34 (0.11)b	2.08 (0.09)b	1.87 (0.19)ab	2.91 (0.14)b	1.95 (0.12)a	1.98 (0.17)	2.22 (0.14)	1.80 (0.10)
cy19:0	0.43 (0.02)	0.51 (0.02)	0.53 (0.05)	0.51 (0.05)a	0.66 (0.03)b	0.57 (0.03)ab	0.49 (0.04)	0.51 (0.06)	0.44 (0.03)
20:0	0.26 (0.03)a	0.38 (0.02)b	0.35 (0.03)b	0.39 (0.05)a	0.62 (0.05)b	0.45 (0.05)a	0.37 (0.03)	0.42 (0.03)	0.32 (0.03)

Given are means and their standard errors (S.E.). Different letters indicate significant differences between treatments.

last 4 years of the same experiment (Pendall et al., 2004). Modified turnover times of the microbial biomass or possible changes of composition and function of the soil microbial community might have caused this effect. The abundance of fungal PLFAs increased by around 60% under the influence of elevated CO_2 over the latter 3 years of the 5-year study in comparison to control and ambient treatments. As a consequence, the bacterial-to-fungal ratio decreased significantly in 2000 and 2001. Results from previous studies clearly demonstrated that the response of fungi to elevated CO_2 depends on plant species and duration of CO_2 exposure. In the past, the majority of studies showed a trend for higher relative fungi abundance in various forest ecosystems under elevation of CO_2 (Janus et al., 2005; Wiemken et al., 2001; Hu et al., 2005), whereas the relative abundance of fungi was not influenced by the elevated CO_2 in grassland ecosystems (Ebersberger et al., 2004). There are some exceptions from this general trend: fungi were responsible for the stimulation of enzymatic activities in a free-air CO_2 enrichment experiment in southern California (Lipson et al., 2005), whereas fungal species richness, defined by the number of terminal restriction fragments, was not significantly affected by CO_2 treatment in a coastal oak forest soil (Klamer et al., 2002). The shift of microbial community composition towards a more fungal dominated community in the shortgrass steppe can be discussed on the basis of slowly changing substrate quality over a period of 5 years. It is well-known that low quality substrates (high carbon-to-nitrogen ratio (C/N)) favor fungi and high quality substrates (low C/N) favor bacteria (Bossuyt et al., 2001). Morgan et al. (2004) and LeCain et al. (2006) found that elevated CO_2 enhanced productivity of this shortgrass steppe mainly due to increased phytomass of a single species, *S. comata*. The relative amount of litter from this low forage quality species (*S. comata*) increased, while the relative amount of litter from the dominant, high forage quality species, *B. gracilis*, decreased under elevated CO_2 (Morgan et al., 2004). *S. comata* was characterized by higher lignin and lower crude protein and total nitrogen content than *B. gracilis* (Milchunas et al., 2005c; King et al., 2004). Furthermore, N concentration of all plant species tested and grown at the elevated CO_2 concentration declined. As the result, lower quality litter mainly stimulated fungal decomposition during the fourth and fifth year of the experiment.

Fungi responded to the changing litter quality under elevated CO_2, while bacterial composition shifted only temporarily due to changing quantity and quality of easily available substrates or due to difference in microclimatic conditions. We detected an increase in the relative proportion of Gram-negative bacteria under elevated CO_2 in 2000 (Fig. 1). Since the same effect did not occur in the previous and the penultimate years, we hypothesize that the extended spring drought in the fourth year caused the reduced abundance of Gram-positives. Similar abundance of Gram-positives in the unchambered control and the elevated treatment support this view (Fig. 1). An alternative explanation is based on differences in resource availability. Since rhizodeposition of the shortgrass steppe was roughly doubled in elevated compared with ambient CO_2 treatment over the last 4 years of the experiment (Pendall et al., 2004), easily available, low molecular weight C substrates derived from turnover of fine roots, root hairs, and mycorrhizal secretion of soluble root exudates, and turnover of rhizosphere-associated microbial biomass specifically favored Gram-negative bacteria (Montealegre et al., 2002; Milchunas et al., 2005a; Drissner et al., 2006).

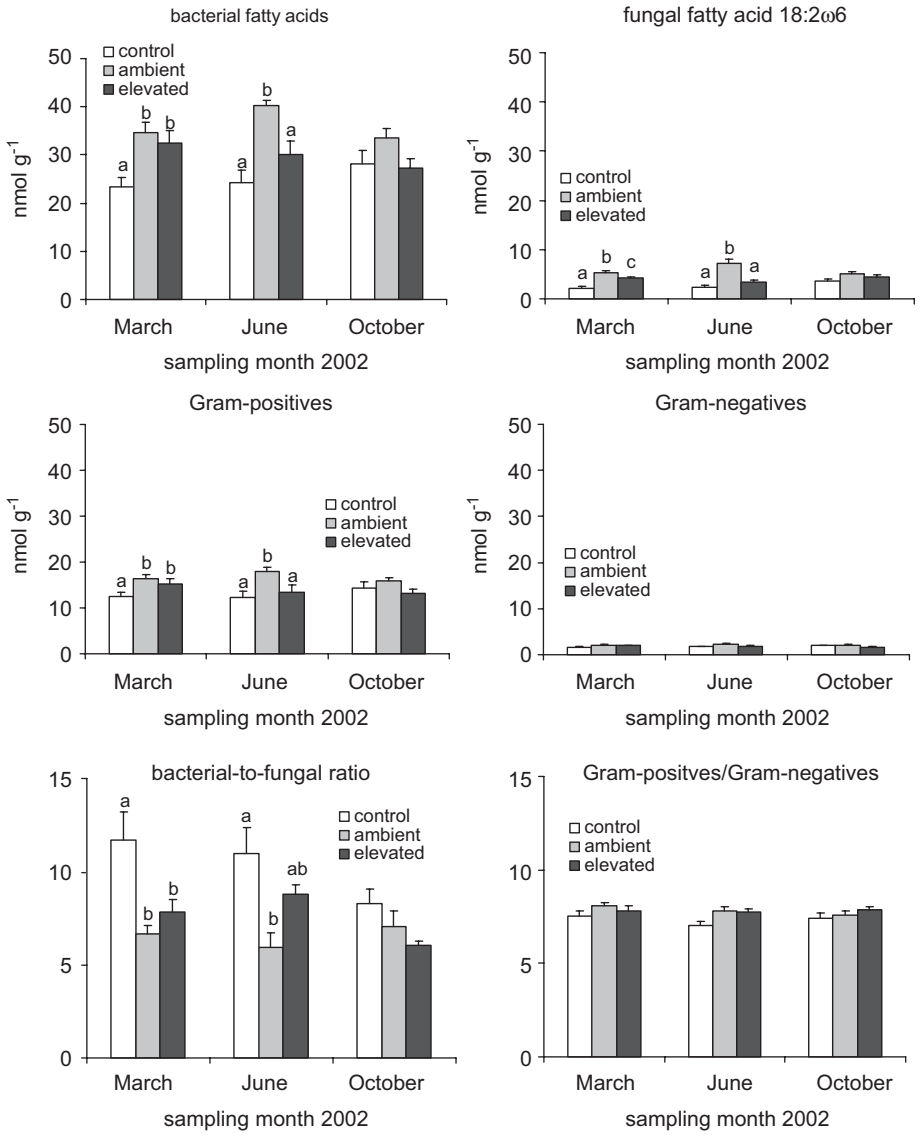

Fig. 2. Concentration of phospholipid fatty acids (PLFAs) in soil characteristic for all bacteria, Gram-positive, Gram-negative or fungi (nmol g^{-1} soil) and ratios of Gram-positive to Gram-negative bacteria and bacteria to fungi in the 0–5 cm soil layer of a shortgrass steppe during a period of 12 months after CO_2 amendment ended. Letters (a, b, c) indicate significant differences between treatments at $P \leqslant 0.05$.

A significant chamber effect was detected by statistical analysis (Fig. 1). This effect may be caused by differences in microclimatic conditions in the different treatments. Morgan et al. (2004) showed that canopy air and soil temperatures averaged 2.6 and 1.25 °C warmer inside than outside the chambers, respectively. Analyses of PLFA pattern of below-ground communities of an N-limited model ecosystem gave strong evidence that an increase in atmospheric temperature changes microbial community composition (Bardgett et al., 1999). The chamber effect in our experiment not only stimulated microbial turnover (Kandeler et al., 2006) but also changed microbial community composition. Separation between ambient and elevated treatments were not caused by differences in soil moisture (Table 2). This soil moisture effects were proposed, because elevated atmospheric CO_2 led to increased soil–water conservation via reduced stomatal conductance in the shortgrass steppe (Ferretti et al., 2003; LeCain et al., 2003).

4.2. *Reversibility of the carbon dioxide effect after the end of the CO_2 enhancement (post-CO_2 effect)*

After completion of the CO_2 enrichment experiment, we expected that the function and composition of the soil microbial community of ambient and elevated treatments would begin to shift back to its original composition of the control treatment. Since we do not expect atmospheric CO_2 levels to decrease anytime soon, we viewed the second part of our study as a way of measuring recovery of microbial community from disturbance (elevated CO_2, increased soil moisture, decreased substrate quality, increased labile

substrate quantity). Indeed, bacterial and fungal PLFAs were significant different from control treatments in the post CO_2 treatments in March 2002 (Fig. 2). Therefore, microbial communities of the elevated and ambient plots could have adapted to the changed quantity and quality of substrates as well as to the changed microclimatic environments under their respective treatment conditions. Microbial community composition of the elevated treatment shifted faster towards the control treatment than the ambient treatment (Fig. 2). Although rhizodeposition was roughly doubled under CO_2 enrichment (Pendall et al., 2004), fewer low molecular weight C substrates derived from turnover of fine roots, root hairs and mycorrhizal secretion of soluble root exudates would likely have been available to rhizosphere microorganisms in the elevated CO_2 treatment in the year following the experiment. Furthermore, soil microorganisms of ambient and elevated treatments no longer benefited from a surplus of soil moisture in 2002 such as occurred in the previous years of the experiment due to more efficient transpiration of plants under elevated CO_2 (see Table 2; Nelson et al., 2004; LeCain et al., 2003; Ferretti et al., 2003). Both of these changes might have accounted for the post-CO_2 treatment responses in 2002.

In this study, we detected a higher abundance of fungal PLFAs during the last 3 years of the CO_2 elevation experiment. An increase in fungi would be expected to alter soil physical properties, since fungal extracellular polysaccharides and hyphae are primarily responsible for the formation of soil macro-aggregates, which protect plant-derived soil organic matter (Six et al., 2006; Thiet et al., 2006). Had CO_2 enrichment continued, we expect that the microbial community shift would have continued to move towards an increased presence of fungi, and the predicted changes in soil properties would have followed. However, cessation of the CO_2 treatment resulted in a shift of bacterial-to-fungal ratio towards the control treatment (Figs. 1 and 2). After 5 years of elevated CO_2, the available substrates apparently had not changed sufficiently to shift the microbial community permanently to a fungal dominated community. In the future, studies should focus on higher taxonomical resolution of soil microorganisms to clarify whether either symbiotic fungi colonizing plant roots and having direct access to easily degradable carbohydrates or saprotrophic fungi responsible for the primary attack of the recalcitrant C in litter (cell wall compounds of dead plant tissues) were responsible for the temporary increase of fungal PLFAs during the enrichment experiment.

In conclusion, this study showed for the first time that microbial community composition of a shortgrass steppe shifted towards a more fungal-dominated composition during the latter 3 years of a 5-year study of elevated atmospheric CO_2. Differences of moisture regimes between ambient and elevated CO_2 treatments could not explain the variation of fungal abundance. Since fungi are unique in their ability to translocate and utilize spatially separated nutrient resources (Poll et al., 2006) and fungi are composed of more complex recalcitrant compounds (Allison et al., 2005), long-term dominance of a fungal-dominated microbial community may result in greater C sequestration than in systems with lower fungal abundance (Six et al., 2006). We conclude from PLFA composition of soil microorganisms during the CO_2 elevation experiment and during another year after cessation of CO_2 that the higher abundance of fungi could be a primary mechanism leading to enhanced C storage in shortgrass steppe, if elevation of atmospheric CO_2 continues in the future. The shift in microbial community composition towards a fungal-dominated microbial community will occur in other ecosystems only if either litter quality of the vegetation changes towards a lower quality material or if elevation of CO_2 favors the growth of a low forage quality plant (e.g. *S. comata*) within a plant community.

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