ELSEVIER

Soil Biology & Biochemistry 39 (2007) 517–525

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Elevated CO_2 differentially alters belowground plant and soil microbial community structure in reed canary grass-invaded experimental wetlands

Jenny Kao-Kniffin[a], Teri C. Balser[a,b,*]

[a]*Division of Land Resources, Gaylord Nelson Institute for Environmental Studies, University of Wisconsin, Madison, WI 53706, USA*
[b]*Department of Soil Science, University of Wisconsin, Madison, WI 53706, USA*

Received 6 June 2006; received in revised form 26 August 2006; accepted 31 August 2006
Available online 2 October 2006

Abstract

Several recent studies have indicated that an enriched atmosphere of carbon dioxide (CO_2) could exacerbate the intensity of plant invasions within natural ecosystems, but little is known of how rising CO_2 impacts the belowground characteristics of these invaded systems. In this study, we examined the effects of elevated CO_2 and nitrogen (N) inputs on plant and soil microbial community characteristics of plant communities invaded by reed canary grass, *Phalaris arundinacea* L. We grew the invasive grass under two levels of invasion: the invader was either dominant (high invasion) at >90% plant cover or sub-dominant (low invasion) at <50% plant cover. Experimental wetland communities were grown for four months in greenhouses that received either 600 or 365 $\mu l l^{-1}$ (ambient) CO_2. Within each of three replicate rooms per CO_2 treatment, the plant communities were grown under high (30 $mg l^{-1}$) or low (5 $mg l^{-1}$) N. In contrast to what is often predicted under N limitation, we found that elevated CO_2 increased native graminoid biomass at low N, but not at high N. The aboveground biomass of reed canary grass did not respond to elevated CO_2, despite it being a fast-growing C3 species. Although elevated CO_2 had no impact on the plant biomass of heavily invaded communities, the relative abundance of several soil microbial indicators increased. In contrast, the moderately invaded plant communities displayed increased total root biomass under elevated CO_2, while little impact occurred on the relative abundance of soil microbial indicators. Principal components analysis indicated that overall soil microbial community structure was distinct by CO_2 level for the varying N and invasion treatments. This study demonstrates that even when elevated CO_2 does not have visible effects on aboveground plant biomass, it can have large impacts belowground.

Keywords: Microbial community composition; Invasion; Elevated CO_2; Nitrogen; Reed canary grass; Lipid analysis

1. Introduction

The replacement of diverse plant communities with single invasive plant species accelerates the loss of biodiversity, as well as altering ecosystem functioning (Ehrenfeld, 2003; Zedler and Kercher, 2004). In addition, many species of invasive plants also alter belowground properties and processes (such as soil microbial community composition and activity) (Kourtev et al., 2002; Hawkes et al., 2005). Given that microorganisms mediate important biogeochemical cycles, shifts in the composition of the soil microbial community can alter ecosystem nutrient cycling processes (Schimel, 1995; Zogg et al., 1997; Schimel and Gulledge, 1998; Balser et al., 2001; Balser and Firestone, 2005).

As increasing ecological impacts of invasions are realized, some researchers are examining whether changes in environmental parameters or resources are intensifying the impacts of plant invasions. Dukes and Mooney (1999) suggested that some factors of global change, such as elevated CO_2 and N deposition, influence the magnitude

*Corresponding author. Department of Soil Science, 1525 Observatory Drive, University of Wisconsin, Madison, WI 53706-1299 USA.
Tel.: +1 608 262 0132; fax: +1 608 265 2595.
E-mail address: tcbalser@wisc.edu (T.C. Balser).

0038-0717/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2006.08.024

and direction of plant invasions. These factors also play important roles in influencing overall plant community composition and ecosystem functioning (Potvin and Vasseur, 1997; Mack and D'Antonio, 2003; Windham and Ehrenfeld, 2003; Horz et al., 2004; Niklaus and Korner, 2004). Other, more recent, studies have shown that increased concentrations of atmospheric CO_2 favor the growth, reproduction, and competitive abilities of several invasive species (Smith et al., 2000; Ziska, 2003; Belote et al., 2004; Nagel et al., 2004). However, elevated CO_2 may have additional impacts on invasion, beyond biomass increases, that are less understood, such as altering belowground chemical and microbial properties. For example, for many plant species, the quantity and quality of root exudation are influenced by atmospheric CO_2 concentrations (Paterson et al., 1996; Pendall et al., 2004), and such changes in exudation patterns may alter soil microbial composition and activity.

Furthermore, researchers have been testing the effects of multiple factor influences of global change, sometimes finding unexpected outcomes (Zavaleta et al., 2003; Norby and Luo, 2004; Dijkstra et al., 2005; Xie et al., 2005). For example, CO_2 and N levels can interact to influence the amount of new C inputs, potentially altering C storage in soils (Xie et al., 2005). However, few studies have examined the effects of plant invasions on belowground properties, as influenced by both elevated CO_2 and N inputs.

The replacement of diverse plant communities with monocultures of fast-growing, invasive C3 plants may lead to large-scale changes in belowground properties and processes. Many of the most aggressive invaders of wetlands are fast- growing C3 species, such as reed canary grass (*Phalaris arundinacea* L.), common reed (*Phragmites australis* Cav.), and hybrid cattail (*Typha x glauca* Godr.) (Galatowitsch et al., 1999; Zedler and Kercher, 2004). The fast-growing C3 species tend to show larger growth increases under elevated CO_2 than their slow-growing C3 counterparts, while C4 species show even smaller increases (Poorter, 1993; Poorter and Navas, 2003). The greater stimulation of fast-growing C3 plants by elevated CO_2, combined with the large belowground biomass of many invasive clonal dominants, may therefore lead to significant changes in belowground properties that can impact ecosystem processes.

In this study, we selected reed canary grass as an invasive species because of its significance as an aggressive weed in the northern US (Lavergne and Molofsky, 2004). Native populations of the species do exist in the US, but some researchers believe that the introduced, aggressive form has spread across North America (Galatowitsch et al., 1999). Reed canary grass can form hectares of monospecific stands, displacing native sedge meadows and other diverse wetland communities (Maurer et al., 2003). The scale of invasion by the grass species is so extensive that land managers currently use Landsat imagery to track reed canary grass cover (Helmuth, 2003).

We examined the importance of elevated CO_2, N addition, and invasion level in influencing aboveground and belowground ecosystem properties. Our objective was to determine the impact of elevated CO_2 and N inputs on plant biomass and soil microbial community structure in experimental wetlands invaded by reed canary grass at varying levels. We define invasion level in this study as the overall density of the invasive grass within the mesocosm. We tested whether N fertilization impacts the magnitude and direction of CO_2 influence on plant and microbial communities, and whether reed canary grass in either monoculture or polyculture conditions further determines shifts in microbial community composition. Specifically, we hypothesized that elevated CO_2 would increase the abundance of gram-negative bacteria in high N treatments, while low N would minimize the CO_2 effect. Gram-negative bacteria tend to be faster growing bacteria (as compared to gram-positives) that favor low molecular weight C compounds. Additionally, we hypothesized that the increase in gram-negative bacteria would be greater under the high N monoculture communities, as a result of an increase in the biomass of CO_2-stimulated reed canary grass roots.

2. Materials and methods

2.1. Experimental design

We grew experimental plant communities under elevated CO_2 in a controlled greenhouse facility located on the University of Wisconsin-Madison campus. The CO_2 treatments included three replicate rooms at $600\,\mu l\,l^{-1}$ (elevated CO_2) and an additional three rooms at $365\,\mu l\,l^{-1}$ (ambient CO_2) (Fig. 1). Nitrogen treatments were nested within each room, so that half of the mesocosms received $5\,mg\,l^{-1}$ of NO_3^-–N as potassium nitrate (KNO_3) and the other half received $30\,mg\,l^{-1}$. Wetlands in the Midwestern US receive stormwater inputs of nitrate typically between the ranges of 20–40 $mg\,l^{-1}$ (Zucker and Brown, 1998). The number of mesocosms in the experiment totaled 120; each N and invasion level combination contained five replicate mesocosms. All mesocosms (dimension: $36 \times 47 \times 16$ cm) received Hoagland's solution once each week, with N levels adjusted to maintain our N treatment levels. Daily room temperatures ranged from 24 °C for 14 h to 17 °C for 10 h.

The soil used in this experiment was derived from a wet meadow community located near Dickeyville, Wisconsin, USA (42°39′21″N, 90°34′39″W). We added equal parts of topsoil (Liesener Soils Inc., Jackson, WI) to wet meadow soil and homogenized the medium on large soil mixing counters using shovels, prior to placement in the mesocosms. To provide a larger pool of wet prairie soil microorganisms, we inoculated each mesocosm with an additional 150 g of soil from Lower Greene Prairie, a wet prairie located at the University of Wisconsin-Madison Arboretum (43°1′40″N, 89°26′15″E). For both the wet

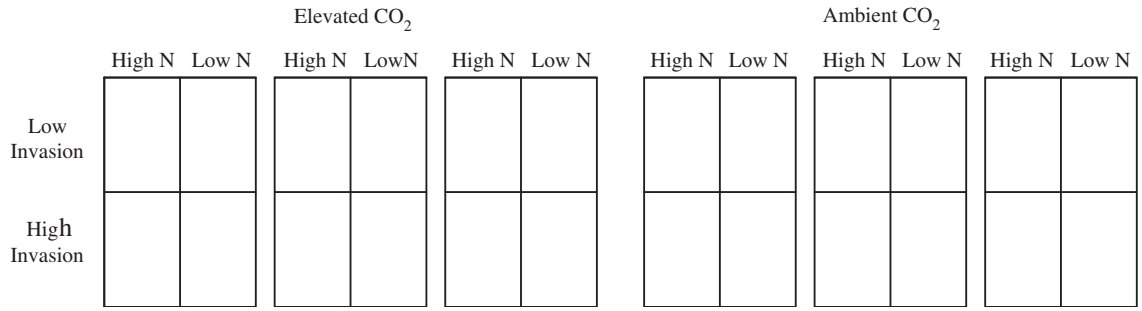

Fig. 1. Diagram of the CO_2 greenhouse rooms, indicating the N and plant invasion treatments. Each quadrant, or N and invasion level combination, consisted of five replicates.

meadow and wet prairie soils, we sampled soil to at least 30 cm depth. The mesocosms were then flooded to a water level that was 5 cm above the soil surface.

The experimental wetland plant communities consisted of two levels of invasion by the aggressive invader, reed canary grass (*Phalaris arundinacea* L.) (Lavergne and Molofsky, 2004). Reed canary grass was either dominant (greater than 90% cover) or sub-dominant (less than 50% cover) within the experimental plant communities. We seeded half of the total number of mesocosms with a monoculture of 1900 seeds m^{-2} to establish reed canary grass as the dominant species. For the other half of the mesocosms, we established reed canary grass as a sub-dominant species by maintaining a polyculture community consisting of 10 additional species at a seeding density of 200 seeds m^{-2} per species.

The polyculture community included the following 10 species that are native to the US: swamp milkweed, forb (*Asclepias incarnata* L.); cup plant, forb (*Silphium perfoliatum* L.); blue vervain, forb (*Verbena hastata* L.); Illinois tick trefoil, legume (*Desmodium illinoense* A. Gray); big bluestem, C4 graminoid (*Andropogon gerardii* Vit.); blue-joint grass, C3 graminoid (*Calamagrostis canadensis*); fowl mannagrass, C3 graminoid (*Glyceria striata* Lam.); green bulrush, C3 graminoid (*Scirpus atrovirens* Willd.); fox sedge, C3 graminoid (*Carex vulpinoidea* Michx.); and tussock sedge, C3 graminoid (*Carex stricta* Lam.). The legume *D. illinoense* germinated early on in the experiment, but did not survive; therefore, biomass data for the species are not reported. For data analysis, we grouped species into three categories: graminoids, forbs, and reed canary grass (invasive).

2.2. Soil and plant samples

After four months, we destructively harvested all soil and plants from the mesocosms. We manually removed soil adhering to the roots using spatulas. The rhizosphere soil was homogenized, frozen at −20 °C, and lyophilized prior to analysis. We separated aboveground plant biomass from belowground plant biomass to determine CO_2-stimulation of plants and changes in biomass allocation. The plants were washed, dried at 60 °C until constant weight, and weighed.

2.3. Microbial community analysis

We used a hybrid procedure of phospholipid fatty acid (PLFA) and fatty acid methyl ester (FAME) to analyze microbial community composition. The procedure is based on the extraction of 'signature' lipid biomarkers from soil organisms (White and Ringelberg, 1998). Samples are homogenized, frozen, and then freeze-dried before analysis. All glassware is baked at 550 °C for 3 h. Lipids are then extracted, purified and identified using steps from a modified Bligh and Dyer (1959) technique for lipid extraction, combined with FAME as described by Microbial ID Inc. (Hayward, CA). We extracted lipids from 3 g of freeze-dried soil using a chloroform-methanol extraction with a phosphate buffer [potassium phosphate, 0.1 M and pH 7 (3.6 ml), methanol (8 ml), and $CHCl_3$ (4 ml)] in 25-ml glass tubes, shaken for 1 h and centrifuged. Supernatant was then decanted to 30-ml tubes and potassium phosphate buffer and $CHCl_3$ were re-added and the tubes were vortexed for 30 s. The phases were allowed to separate overnight at room temperature. The top layer was aspirated off (saving the chloroform phase), and volume was reduced in a RapidVap. We then followed the procedure for FAME as given by Microbial ID Inc.; sodium hydroxide is added for saponification and the solution heated in a water bath for 30 min, followed by methanolysis.

A 2 μl injection of the methyl-ester derivatives of the extracted lipid was analyzed using a Hewlett-Packard 6890 Gas Chromatograph equipped with a flame ionization detector and split/splitless inlet and a 25 m × 0.2 mm inside diameter × 0.33 μm film thickness Ultra 2 (5%-phenyl, 95% methyl) capillary column (Agilent) using hydrogen as the carrier gas, N as the make up gas, and air to support the flame. Gas chromatograph conditions are set by the MIDI Sherlock program (MIDI, Inc. Newark, DE). Peaks were identified with bacterial fatty acid standards and Sherlock peak identification software (MIDI, Inc. Newark, DE). Fatty acids were quantified by comparisons of peak areas from the sample compared to peak areas of two internal standards, 9:0 (nonanoic methyl ester) and 19:0 (nonadeconoic methyl ester), of known concentration. In all subsequent analyses we used only

fatty acids that were identifiable and present at >0.5 mol%.

Lipids cannot confidently be used to represent specific strains or species but are more commonly assigned to functional guilds. Terminology to describe fatty acids is described by 'A:B*w*C' where 'A' indicates the total number of C atoms, 'B' the number of double bonds (unsaturations), and '*w*' indicates the position of the double bond from the methyl end of the molecule. The prefixes '*i*' and '*a*' refer to iso and anti-iso methyl branching. Monounsaturated fatty acids labeled with a '*c*' or '*t*' refer to cis or trans forms. Hydroxy groups are indicated by 'OH'. Cyclopropyl groups are denoted by '*cy*' (Arao, 1999; Bååth and Anderson, 2003; Steenwerth et al., 2003).

2.4. Statistical analysis

Our experimental design is a split-plot ANOVA with CO_2 levels as the whole plot treatment and N and invasion levels as the split-plot treatments. We analyzed our data using the 'proc mixed' procedure in SAS, version 8.0 (SAS Institute Inc., Cary, NC). CO_2 (365 and 600 $\mu l\,l^{-1}$), N (5 and 30 $mg\,l^{-1}$ of NO_3^-–N), and invasion level (monoculture and polyculture of reed canary grass) are the fixed factors, while greenhouse rooms are the random factor. We determined the effects of elevated and ambient CO_2 on microbial relative abundance and plant biomass at each N and invasion level using Fisher's LSD. Relative abundance refers to the proportion of an indicator lipid relative to the total of microbial lipids in a sample. We tested each variable used in our analysis for normality using the Shapiro-Wilk's statistic, and used arcsine transformations of microbial mol% data and log-transformations of plant biomass data. For multivariate analysis of microbial lipid data, we performed principal components analysis (PCA) on the arcsine transformed mol fractions of individual lipids using JMP software, version 5.0 (SAS Institute Inc., Cary, NC).

3. Results

3.1. Elevated CO_2 and soil microorganisms

Principal components analysis indicated that the overall soil microbial community structure differed between ambient and elevated CO_2 (Fig. 2). Principal components (PC) 1 and 2 explained 63% of the variation, with the CO_2 effect most distinct along PC 2 (Fig. 2). Under N-limiting conditions, the soil microbial community structure differed by CO_2 level, while invasion level (or plant composition) showed no effect (Fig. 2a). However, when N was abundant, the composition of the soil microbial community was distinct among CO_2 levels as well as invasion level (or plant composition) (Fig. 2b).

The relative abundance of microbial lipid biomarkers also differed by CO_2 levels, but was further influenced by N inputs and invasion level (Tables 1 and 2). The lipid

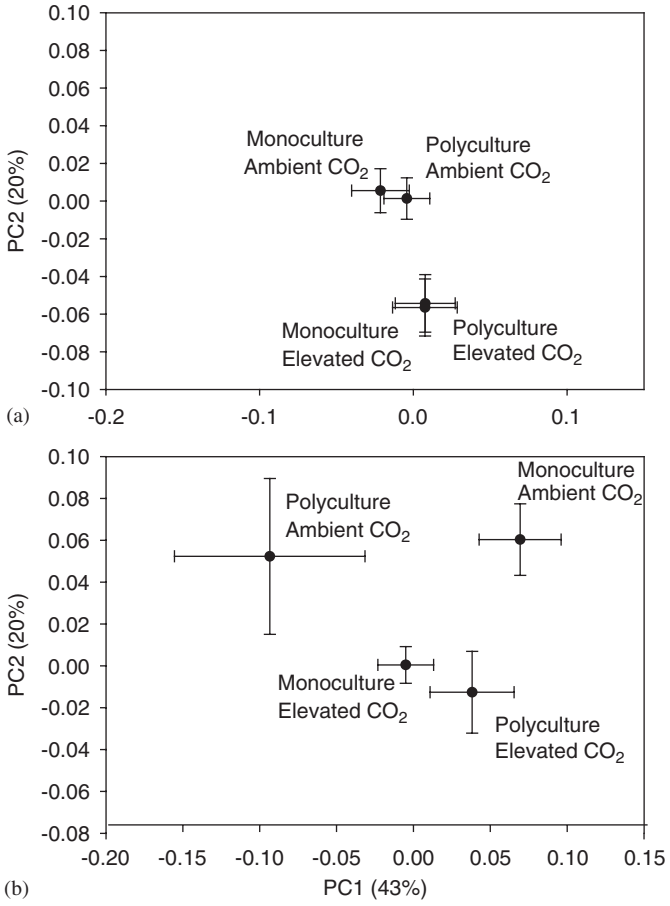

Fig. 2. Principal components analysis of microbial lipids from elevated vs. ambient CO_2 and low (polyculture) vs. high (monoculture) invasion treatments under: (a) low N and (b) high N. Error bars are ± 1 SE of the mean ($n = 15$). The first and second principal components account for 63% of the variability.

biomarkers 12:0 (LSD: $P<0.001$), 14:0 ($P<0.001$), *cy*19:0 ($P<0.001$), 19:1*w*8t ($P<0.01$), and 20:0 ($P<0.001$) significantly increased with elevated CO_2, under heavily invaded communities that received high N inputs. When N inputs were low in the high invasion communities, biomarker relative abundance were similar as under high N, but less significant for 12:0 (LSD, $P<0.1$), 14:0 ($P<0.01$), *cy*19:0 ($P<0.1$), although not for 20:0 ($P<0.001$). In addition, the biomarker 19:1*w*8t showed no significant differences between varying CO_2 levels when N inputs were low. The methanotroph lipid biomarker, 18:1*w*7c (Knief et al. 2003; Bowman et al. 1991), was the only lipid biomarker to decrease in relative abundance under elevated CO_2 for both N levels of the high invasion communities (at low N, $P<0.01$ and at high N, $P<0.0001$). Under the low invasion (polyculture) communities, the CO_2 effect was relatively minor, with only two lipid biomarkers, 17:1*w*7c (at low N, $P<0.1$ and at high N, $P<0.01$) and 18:02 OH (at low N, $P<0.05$ and at high N, *ns*), showing increases in relative abundance under elevated CO_2 levels.

Table 1
Effects of elevated CO_2 on relative abundance in mol% of microbial lipid indicators under ambient and elevated CO_2, low and high N inputs, and low and high invasion by reed canary grass

Lipids	Low invasion (Polyculture)				High invasion (Monoculture)			
	Low nitrogen		High nitrogen		Low nitrogen		High nitrogen	
	Amb. CO_2	Elev. CO_2	Amb. CO_2	Elev. CO_2	Amb. CO_2	Elev. CO_2	Amb. CO_2	Elev. CO_2
Saturated								
12:0	0.89 (0.06)	1.05 (0.05)	0.81 (0.05)	1.14 (0.05)	0.88 (0.08)†	1.20 (0.19)†	0.73 (0.08)***	1.06 (0.10)***
14:0	3.05 (0.13)	3.70 (0.13)	2.78 (0.12)	3.59 (0.10)	3.03 (0.14)**	2.96 (0.47)**	2.75 (0.10)***	3.29 (0.11)***
20:0	1.75 (0.09)	2.03 (0.09)	1.53 (0.14)	2.15 (0.08)	1.82 (0.12)***	2.38 (0.20)***	1.57 (0.10)***	2.06 (0.09)***
Monounsaturated								
17:1*w*7c	0.11 (0.08)†	0.28 (0.11)†	0.06 (0.06)**	0.30 (0.11)**	0.00	0.24 (0.11)	0.04 (0.04)	0.43 (0.02)
18:1*w*7c	0.90 (0.06)	0.72 (0.04)	0.98 (0.05)	0.70 (0.04)	0.96 (0.09)**	0.84 (0.04)**	1.06 (0.12)***	0.80 (0.04)***
19:1*w*8t	3.48 (0.30)	3.97 (0.22)	2.82 (0.29)	4.21 (0.15)	3.35 (0.34)	4.66 (0.36)	2.88 (0.23)**	3.90 (0.21)***
Hydroxy								
18:02OH	1.14 (0.07)*	1.23 (0.05)*	1.05 (0.07)	1.31 (0.04)	1.07 (0.09)	1.52 (0.13)	0.95 (0.06)	1.21 (0.05)
Cyclo								
*cy*19:0	2.19 (0.08)	2.32 (0.10)	2.09 (0.11)	2.53 (0.10)	2.19 (0.12)†	2.72 (0.26)†	1.97 (0.09)***	2.26 (0.08)***

Asterisk (*) indicates significance at $P<0.05$, (**) at $P<0.01$, (***) at $P<0.001$, and dagger (†) indicates significance at $P<0.1$ (Fisher's LSD).Error bars are ± 1 SE of the mean ($n = 15$).

Table 2
ANOVA *F*- and *P*- values for microbial lipids and plant biomass

Microbial lipids or plant biomass	Low invasion (Polyculture)			High Invasion (Monoculture)		
	CO_2	N	$CO_2 \times N$	CO_2	N	$CO_2 \times N$
12:0	n.s.	n.s.	n.s.	8.9*	n.s.	3.3†
14:0	n.s.	n.s.	n.s.	25.6**	n.s.	n.s.
*cy*19:0	n.s.	n.s.	n.s.	8.9*	n.s.	5.4*
19:1*w*8t	n.s.	n.s.	n.s.	5.8†	n.s.	n.s.
20:0	n.s.	n.s.	n.s.	12.4*	n.s.	3.3†
18:1*w*7c	n.s.	n.s.	n.s.	23.98**	n.s.	n.s.
17:1*w*7c	9.9*	n.s.	n.s.	n.s.	n.s.	n.s.
18:02 OH	4.7†	7.2*	n.s.	n.s.	n.s.	n.s.
Graminoid shoot biomass	6.7†	21.0***	n.s.	n.s.	n.s.	n.s.
Root biomass	4.9†	114.4***	n.s.	n.s.	n.s.	n.s.

Asterisk (*) indicates significance at $P<0.05$, (**) at $P<0.01$, (***) at $P<0.001$, and (†) dagger indicates significance at $P<0.1$ for fixed effects CO_2, N, and $CO_2 \times N$.

3.2. Elevated CO_2 and plant dynamics

Elevated CO_2 led to changes in plant biomass only under low invasion (polyculture) communities (Fig. 3, Table 2). The total belowground biomass of the low invasion communities increased significantly under elevated CO_2 with low N conditions ($P<0.05$), and to a lesser extent under high N ($P<0.1$). The aboveground biomass of native graminoids (grasses/sedges/bulrush) also increased significantly under low N ($P<0.01$), while the native forbs and the invasive grass showed no changes (Fig. 3a). The high invasion (monoculture) communities showed no CO_2-induced changes in either aboveground or belowground biomass (Fig. 3).

4. Discussion

4.1. Above- and belowground invasion dynamics under elevated CO_2

Our results suggest that atmospheric CO_2 levels and N inputs influence belowground plant and microbial parameters. We observed an effect of elevated CO_2, N availability and plant invasion level on microbial community structure in general, as well as for individual microbial lipid indicators.

Principal components analysis (PCA) revealed that atmospheric CO_2 levels, N inputs, and degree of invasion all influenced the overall structure of the soil microbial

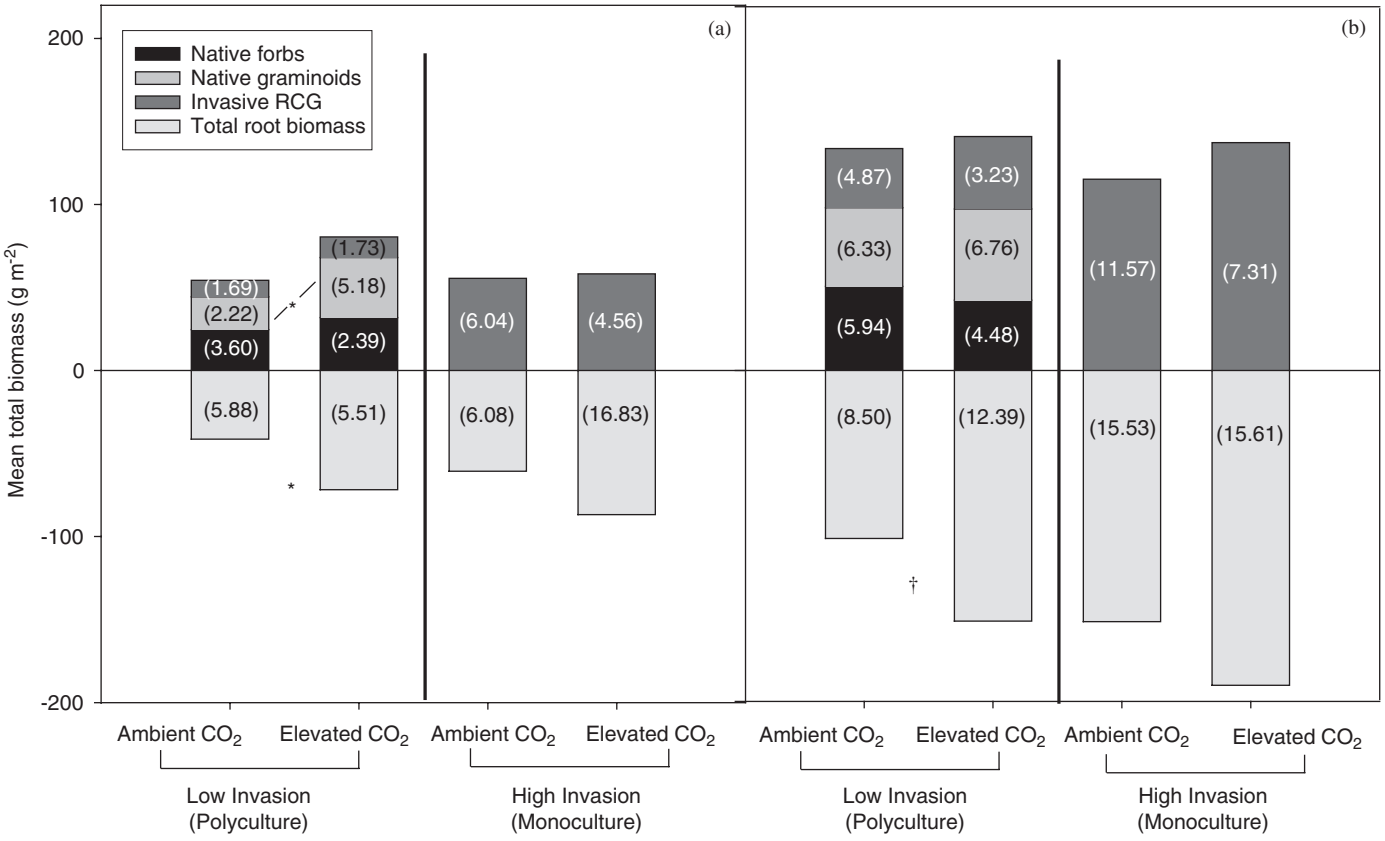

Fig. 3. Effects of elevated CO_2 on plant aboveground and belowground biomass in low invasion (polyculture) and high invasion (monoculture) communities grown under: (a) low N and (b) high N. Asterisk (*) indicates significance at $P<0.05$ and dagger (†) indicates significance at $P<0.1$ (Fisher's LSD) for total aboveground biomass, native graminoids biomass, and total belowground biomass. Standard errors are located on the bars within parentheses.

community. In this study, under both high and low N, the general structure of microbial communities exposed to elevated CO_2 was distinctly different from those exposed to ambient CO_2 (Fig. 2). In contrast, Ebersberger et al. (2004) and Zak et al. (1996, 2000) found no impact of CO_2 on the PLFA profiles of microbial communities in grasslands exposed to elevated CO_2. The contrasting findings may be due to differences in sampling method. We carefully removed soil directly from plant roots to characterize rhizosphere microorganisms, while other studies more often describe microorganisms found in bulk soils. Rhizosphere microorganisms are more likely to be influenced by elevated CO_2 as a result of CO_2-induced changes in root photosynthate (Pendall et al., 2004). However, inconsistencies may also arise because often the sole environmental manipulation is atmospheric CO_2 concentration, while the response of ecosystems to elevated atmospheric CO_2 has been shown to be highly dependent on interactions among soil fertility, water, and temperature (Zak et al., 2000; Niklaus et al., 2003). In this study we manipulated three factors: CO_2, N, and plant composition. We observed that the availability of N altered the magnitude of the CO_2 effect on plant biomass, microbial lipid relative abundance, and microbial community structure.

Plant composition (or in this study, invasion level) also played an important role in influencing microbial lipid relative abundance and overall microbial community structure (at high N) under elevated CO_2. This finding is consistent with many studies showing that plant composition determines or heavily influences the composition of the soil microbial community (Kourtev et al., 2002; Kourtev et al., 2003; Montealegre et al., 2002). In this study, N availability influenced the degree to which plant composition altered soil microbial community structure. At low N, atmospheric CO_2 was the major determinant of microbial community structure, while at high N, both atmospheric CO_2 and invasion level (or plant composition) played important roles. Although we did not include a zero percent invasion level community in this study, we found that the addition of other species into the mesocosms (polyculture community) diminished the impact of elevated CO_2 on the relative abundance of several microbial lipids.

In addition to altering general microbial community 'fingerprints', we found that elevated CO_2 also altered plant community characteristics. Exposure to elevated CO_2 increased total belowground biomass in plant communities moderately invaded by reed canary grass. However, only the aboveground biomass of the native graminoids increased, while the invasive grass and native forbs did

not change. The native graminoids likely caused an increase in total community belowground biomass when exposed to elevated CO_2 and low N, due to increased competition for mineral nutrients between the plants and microorganisms (Schimel and Bennett, 2004). The native graminoids included species that are found in relatively low N environments, such as sedge meadows, wet prairies, or wet meadows (Galatowitsch et al., 1999; Woo and Zedler, 2002). When communities received high inputs of N, the native graminoids showed no changes in biomass with elevated CO_2. Furthermore, as atmospheric CO_2 concentrations increase in the future, the native graminoids may be more competitive with the invasive reed canary grass, as plant aboveground and belowground biomass increase in relation to the invaders. However, although the biomass of reed canary grass did not change under elevated CO_2, the structure of the soil microbial community was altered in such a way that the relative abundance of lipid biomarkers thought to indicate gram-negative bacteria (17:1*w*7c, 18:1*w*7c, and 18:1*w*8t) and anaerobic bacteria (*cy*19:0) did change in relation to the total microbial community, with all except 18:1*w*7c increasing. This finding reveals the limitations of using plant biomass to indicate the potential effects of elevated CO_2 on ecosystem processes. Instead, other plant traits that are also influenced by atmospheric CO_2 levels, such as rhizodeposition and tissue plant chemistry, may be impacting soil microbial community structure, with potential long-term impacts on nutrient and C cycles.

Several studies have indicated that increased atmospheric CO_2 leads to greater exudation of labile C from plant roots, which stimulates microbial growth (Hutchin et al.,1995; Paterson et al., 1996; Inubushi et al., 2003; Freeman et al., 2004). For example, Butler et al. (2003) found using ^{13}C PLFA that gram-negative bacteria increased in relative abundance when rhizodeposition was more readily available during the later stages of plant growth. Similarly, Bünemann et al. (2004) and Tscherko et al. (2004) found that changes in C availability (organic matter) or substrate quality (lower soil C:N ratio), respectively, promoted the growth of gram-negative bacteria. We also found in this study that CO_2-induced changes in soil microbial community structure were further impacted by N availability. Correspondingly, Martín-Olmedo et al. (2002) found that N availability influenced the growth of microorganisms under elevated CO_2 conditions (albeit with greater microbial biomass when N was limiting). However, other elevated CO_2 studies have shown high variability in effects on soil microbial composition, biomass, and activity (Sadowsky and Schortemeyer, 1997).

Shifts in the abundance of gram-negative bacteria, or other microbial ecological guilds, within the soil microbial community may have important consequences for ecosystem functioning (Waldrop and Firestone, 2004). In general, many gram-negative bacteria are fast-growing species that tend to utilize simple C substrates, whereas many of the gram-positive bacteria are slower-growing species that tend to specialize on more complex C substrates (Paul and Clark, 1989; Atlas and Bartha, 1993). A decrease in the relative abundance of gram-positive bacteria may result in reduced decomposition of complex C in the soil, which consequently could increase soil C storage (Balser, 2005). Nevertheless, the effect of shifting the composition of the microbial community to one of increasing abundance of fast-growing gram-negative bacteria could lead to more rapid cycling of C and N (Fraterrigo et al., 2006). This is one of the first studies to report distinct effects of elevated CO_2 on ecologically significant groups of bacteria in wetland soils.

Although this study was short-term, we consider the experiment a good starting point for investigating the impacts of elevated CO_2 on an aggressive invading wetland plant. Some studies point out that plants may respond initially to CO_2 enrichment, but that plant acclimation occurs several years later (Oren et al., 2001; Calfapietra et al., 2003). However, the longest running CO_2 experiment (17 yr) indicates that plants sustain positive responses to an increase in atmospheric CO_2 (Rasse et al., 2005). The researchers suggest that studies lasting from 3 to 7 yr are not long enough to assess whether acclimation continues. They found that shoot density and biomass responses continued to increase beyond the initial acclimation period. Furthermore, given that few studies of CO_2 enrichment in wetland systems exist (Saarnio et al., 2000; Inubushi et al., 2003; Rasse et al., 2005), the results from our study shed some light on how CO_2 enrichment impacts plants and soil microorganisms in flooded environments.

5. Conclusion

This study indicates that combinations of global change factors (elevated CO_2 and N addition) influence the magnitude and direction of ecological impacts resulting from plant invasions. We found that belowground properties, such as soil microbial community structure and root biomass, were impacted by varying CO_2, N, and invasion levels, despite a general lack of change in aboveground parameters. Although aboveground plant physical traits of an invasive species may not appear to change under increased CO_2 concentrations, alterations in plant chemistry, root exudation, or other non-physical traits influenced by atmospheric CO_2 levels may impact ecosystem functioning. Furthermore, our results suggest that when CO_2 concentrations rise in the future, wetland plant communities comprised of native graminoids may be better able to hinder reed canary grass invasion, particularly under low N environments. Most studies that compare invasion dynamics under elevated CO_2 levels focus only on aboveground parameters, such as aboveground biomass and seed density (Dukes, 2002; Smith et al., 2000). Instead, the most significant impacts of atmospheric CO_2 concentrations on ecosystem properties occurred belowground in this study.

Acknowledgments

We thank LiPing Wei, Chao Liang, and Melissa Veit for their assistance in the greenhouse, Peter Crump for help with statistics, Harry Read for assistance with MIDI Sherlock analysis, and Bradley Banford (Wisconsin Department of Transportation) for locating soil used in this study. This study was supported by grants from the Department of Energy National Institute for Global Environmental Change and the University of Wisconsin-Madison Graduate School.

References

Arao, T., 1999. In situ detection of changes in soil bacterial and fungal activities by measuring ^{13}C incorporation into soil phospholipid fatty acids from ^{13}C acetate. Soil Biology & Biochemistry 31, 1015–1020.

Atlas, R.M., Bartha, R., 1993. Microbial Ecology: Fundamentals and Applications. Benjamin Cummings, Redwood City, CA.

Bååth, E., Anderson, T.H., 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. Soil Biology & Biochemistry 35, 955–963.

Balser, T.C., 2005. Humification. In: Hillel, D., Hatfield, J., Powlson, D., Rosenzweig, C. (Eds.), Encyclopedia of Soils in the Environment, vol. 2. Elsevier, Oxford, UK, pp. 195–207.

Balser, T.C., Firestone, M.K., 2005. Linking microbial community composition and soil processes in a California annual grassland and mixed-conifer forest. Biogeochemistry 73, 395–415.

Balser, T.C., Kinzig, A.P., Firestone, M.K., 2001. Linking soil microbial communities and ecosystem functioning. In: Kinzig, A., Pacala, A.S., Tilman, D. (Eds.), The Functional Consequences of Biodiversity. Princeton University Press, Princeton, NJ, pp. 265–293.

Belote, R.T., Weltzin, J.F., Norby, R.J., 2004. Response of an understory plant community to elevated [CO_2] depends on differential responses of dominant invasive species and is mediated by soil water availability. New Phytologist 161, 827–835.

Bligh, E.G., Dyer, W.J., 1959. A rapid method for total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911–917.

Bowman, J.P., Skerratt, J.H., Nichols, P.D., Sly, L.I., 1991. Phospholipid fatty acid and lipopolysaccharide fatty acid signature lipids in methane-utilizing bacteria. FEMS Microbiology Ecology 85, 15–22.

Bünemann, E.K., Bossiob, D.A., Smithson, P.C., Frossarda, E., Obersona, A., 2004. Microbial community composition and substrate use in a highly weathered soil as affected by crop rotation and P fertilization. Soil Biology & Biochemistry 36, 889–901.

Butler, J.L., Williams, M.A., Bottomley, P.J., Myrold, D.D., 2003. Microbial community dynamics associated with rhizosphere carbon flow. Applied and Environmental Microbiology 69, 6793–6800.

Calfapietra, C., Gielen, B., Sabatti, M., De Angelis, P., Miglietta, F., Scarascia-Mugnozza, G., Ceulemans, R., 2003. Do above-ground growth dynamics of poplar change with time under CO_2 enrichment? New Phytologist 160, 305–318.

Dijkstra, F.A., Hobbie, S.E., Reich, P.B., Knops, J.M.H., 2005. Divergent effects of elevated CO_2, N fertilization, and plant diversity on soil C and N dynamics in a grassland field experiment. Plant and Soil 272, 41–52.

Dukes, J.S., 2002. Comparison of the effect of elevated CO_2 on an invasive species (*Centaurea solstitialis*) in monoculture and community settings. Plant Ecology 160, 225–234.

Dukes, J.S., Mooney, H.A., 1999. Does global change increase the success of biological invaders? Trends in Ecology & Evolution 14, 135–139.

Ebersberger, D., Wermbter, N., Niklaus, P.A., Kandeler, E., 2004. Effects of long term CO_2 enrichment on microbial community structure in calcareous grassland. Plant and Soil 264, 313–323.

Ehrenfeld, J.G., 2003. Effects of exotic plant invasions on soil nutrient cycling processes. Ecosystems 6, 503–523.

Fraterrigo, J.M., Balser, T.C., Turner, M.G., 2006. Microbial community variation and its relationship with nitrogen mineralization in historically altered forests. Ecology 87, 570–579.

Freeman, C., Fenner, N., Ostle, N.J., Kang, H., Dowrick, D.J., Reynolds, B., Lock, M.A., Sleep, D., Hughes, S., Hudson, J., 2004. Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. Nature 430, 195–198.

Galatowitsch, S.M., Anderson, N.O., Ascher, P.D., 1999. Invasiveness in wetland plants in temperate North America. Wetlands 19, 733–755.

Hawkes, C.V., Wren, I.F., Herman, D.J., Firestone, M.K., 2005. Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. Ecology Letters 8, 976–985.

Helmuth, L., 2003. Wisconsin Water Quality Assessment Report to Congress 2002, Wisconsin Department of Natural Resources Water Division, PUB-WT-254.

Horz, H.P., Barbrook, A., Field, C.B., Bohannan, B.J., 2004. Ammonia-oxidizing bacteria respond to multifactorial global change. Proceedings of the National Academy of Sciences 101, 15136–15141.

Hutchin, P.R., Press, M.C., Lee, J.A., Ashenden, T.W., 1995. Elevated concentrations of CO_2 may double methane emissions from mires. Global Change Biology 1, 125–128.

Inubushi, K., Cheng, W.G., Aonuma, S., Hoque, M.M., Kobayashi, K., Miura, S., Kim, H.Y., Okada, M., 2003. Effects of free-air CO_2 enrichment (FACE) on CH_4 emission from a rice paddy field. Global Change Biology 9, 1458–1464.

Knief, C., Lipski, A., Dunfield, P.F., 2003. Diversity and activity of methanotrophic bacteria in different upland soils. Applied and Environmental Microbiology 69, 6703–6714.

Kourtev, P.S., Ehrenfeld, J.G., Haggblom, M., 2002. Exotic plant species alter the microbial community structure and function in the soil. Ecology 83, 3152–3166.

Kourtev, P.S., Ehrenfeld, J.G., Haggblom, M., 2003. Experimental analysis of the effect of exotic and native plant species on the structure and function of soil microbial communities. Soil Biology & Biochemistry 35, 895–905.

Lavergne, S., Molofsky, J., 2004. Reed canary grass (*Phalaris arundinacea*) as a biological model in the study of plant invasions. Critical Reviews in Plant Sciences 23, 415–429.

Mack, M.C., D'Antonio, C.M., 2003. Exotic grasses alter controls over soil nitrogen dynamics in a Hawaiian woodland. Ecological Applications 13, 154–166.

Martín-Olmedo, P., Rees, R.M., Grace, J., 2002. The influence of plants grown under elevated CO_2 and N fertilization on soil nitrogen dynamics. Global Change Biology 8, 643–657.

Maurer, D.A., Lindig-Cisneros, R., Werner, K.J., Kercher, S., Miller, R., Zedler, J.B., 2003. The replacement of wetland vegetation by reed canarygrass (*Phalaris arundinacea*). Ecological Restoration 21, 116–119.

Montealegre, C.M., van Kessel, C., Russelle, M.P., Sadowsky, M.J., 2002. Changes in microbial activity and composition in a pasture ecosystem exposed to elevated atmospheric carbon dioxide. Plant and Soil 243, 197–207.

Nagel, J.M., Huxman, T.E., Griffin, K.L., Smith, S.D., 2004. CO_2 enrichment reduces the energetic cost of biomass construction in an invasive desert grass. Ecology 85, 100–106.

Niklaus, P.A., Korner, C., 2004. Synthesis of a six-year study of calcareous grassland responses to in situ CO_2 enrichment. Ecological Monographs 74, 491–511.

Niklaus, P.A., Alphei, J., Ebersberger, D., Kampichler, C., Kandeler, E., Tscherko, D., 2003. Six years of in situ CO_2 enrichment evoke changes in soil structure and soil biota of nutrient-poor grasslands. Global Change Biology 9, 585–600.

Norby, R.J., Luo, Y.Q., 2004. Evaluating ecosystem responses to rising atmospheric CO_2 and global warming in a multi-factor world. New Phytologist 162, 281–293.

Oren, R., Ellsworth, D.S., Johnsen, K.H., Phillips, N., Ewers, B.E., Maier, C., Schafer, K.V.R., McCarthy, H., Hendrey, G., McNulty, S.G., Katul, G.G., 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. Nature 411, 469–472.

Paterson, E., Rattray, S., Killham, K., 1996. Effect of elevated atmospheric CO_2 concentration on C-partitioning and rhizosphere C-flow for three plant species. Soil Biology Biochemistry 28, 195–201.

Paul, E.A., Clark, F.E., 1989. Soil Microbiology and Biochemistry. Academic Press, New York.

Pendall, E., Mosier, A.R., Morgan, J.A., 2004. Rhizodeposition stimulated by elevated CO_2 in a semiarid grassland. New Phytologist 162, 447–458.

Poorter, H., 1993. Interspecific variation in the growth-response of plants to an elevated ambient CO_2 concentration. Vegetation 104, 77–97.

Poorter, H., Navas, M.L., 2003. Plant growth and competition at elevated CO_2: on winners, losers and functional groups. New Phytologist 157, 175–198.

Potvin, C., Vasseur, L., 1997. Long-term CO_2 enrichment of a pasture community: species richness, dominance, and succession. Ecology 78, 666–677.

Rasse, D.P., Peresta, G., Drake, B.G., 2005. Seventeen years of elevated CO_2 exposure in a Chesapeake Bay wetland: Sustained but contrasting responses of plant growth and CO_2 uptake. Global Change Biology 11, 369–377.

Saarnio, S., Saarinen, T., Vasander, H., Silvola, J., 2000. A moderate increase in the annual CH_4 efflux by raised CO_2 or NH_4NO_3 supply in a boreal oligotrophic mire. Global Change Biology 6, 137–144.

Sadowsky, M.H., Schortemeyer, M., 1997. Soil microbial responses to increased concentrations of atmospheric CO_2. Global Change Biology 3, 217–224.

Schimel, J., 1995. Ecosystem consequences of microbial diversity and community structure. In: Chapin, F.S.I., Koerner, C. (Eds.), Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem Consequences. Springer, New York.

Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85, 591–602.

Schimel, J.P., Gulledge, J., 1998. Microbial community structure and global trace gases. Global Change Biology 4, 745–758.

Smith, S.D., Huxman, T.E., Zitzer, S.F., Charlet, T.N., Housman, D.C., Coleman, J.S., Fenstermaker, L.K., Seemann, J.R., Nowak, R.S., 2000. Elevated CO_2 increases productivity and invasive species success in an arid ecosystem. Nature 408, 79–82.

Steenwerth, K.L., Jackson, L.E., Calderón, F.J., Stromberg, M.R., Scow, K.M., 2003. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biology & Biochemistry 35, 489–500.

Tscherko, D., Hammesfahr, U., Marx, M.C., Kandeler, E., 2004. Shifts in rhizosphere microbial communities and enzyme activity of *Poa alpina* across an alpine chronosequence. Soil Biology & Biochemistry 36, 1685–1698.

Waldrop, M.P., Firestone, M.K., 2004. Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. Oecologia 138, 275–284.

White, D.C., Ringelberg, D.B., 1998. Signature lipid biomarker analysis. In: Burlage, R.S., Atlas, R., Stahl, D., Geesey, G., Sayler, G. (Eds.), Techniques in Microbial Ecology. Oxford University Press, New York.

Windham, L., Ehrenfeld, J.G., 2003. Net impact of a plant invasion on nitrogen-cycling processes within a brackish tidal marsh. Ecological Applications 13, 883–896.

Woo, I., Zedler, J.B., 2002. Can nutrients alone shift a sedge meadow towards dominance by the invasive *Typha x glauca*? Wetlands 22, 509–521.

Xie, Z.B., Cadisch, G., Edwards, G., Baggs, E.M., Blum, H., 2005. Carbon dynamics in a temperate grassland soil after 9 years exposure to elevated CO_2 (Swiss FACE). Soil Biology & Biochemistry 37, 1387–1395.

Zak, D.R., Ringelberg, D.B., Pregitzer, K.S., Randlett, D.L., White, D.C., Curtis, P.S., 1996. Soil microbial communities beneath *Populus grandidentata* crown under elevated atmospheric CO_2. Ecological Applications 6, 257–262.

Zak, D.R., Pregitzer, K.S., Curtis, P.S., Holmes, W.E., 2000. Atmospheric CO_2 and the composition and function of soil microbial communities. Ecological Applications 10, 47–59.

Zavaleta, E.S., Shaw, M.R., Chiariello, N.R., Thomas, B.D., Cleland, E.E., Field, C.B., Mooney, H.A., 2003. Grassland responses to three years of elevated temperature, CO_2, precipitation, and N deposition. Ecological Monographs 73, 585–604.

Zedler, J.B., Kercher, S., 2004. Causes and consequences of invasive plants in wetlands: opportunities, opportunists, and outcomes. Critical Reviews in Plant Sciences 23, 431–452.

Ziska, L.H., 2003. Evaluation of the growth response of six invasive species to past, present and future atmospheric carbon dioxide. Journal of Experimental Botany 54, 395–404.

Zogg, G.P., Zak, D.R., Ringelberg, D.B., MacDonald, N.W., Pregitzer, K.S., White, D.C., 1997. Compositional and functional shifts in microbial communities due to soil warming. Soil Science Society of America Journal 61, 475–481.

Zucker, L.A., and Brown, L.C., 1998. Agricultural drainage—water quality impacts and subsurface drainage studies in the Midwest. Ohio State University Extension Bulletin 871, 40.