New Phytologist

Research

Grassland species effects on soil CO_2 flux track the effects of elevated CO_2 and nitrogen

Joseph M. Craine[1], David A. Wedin[2] and Peter B. Reich[3]

[1]Department of Integrative Biology, University of California Berkeley, Berkeley, CA 94720, USA; [2]School of Natural Resource Sciences, University of Nebraska, Lincoln, NE 68583, USA; [3]Department of Forest Resources, University of Minnesota, St. Paul, MN 55108 USA

Author for correspondence:
Joseph M. Craine
Tel: +1 612 434 5131
Fax: +1 612 434 7361
Email: jcraine@socrates.berkeley.edu

Received: *27 July 2000*
Accepted: *17 November 2000*

Summary

- Understanding and predicting the impacts of elevated atmospheric CO_2, elevated nitrogen deposition, and decreases in plant diversity require better understanding of the way in which plant species influence soil CO_2 flux.
- In experimental grassland plots where atmospheric CO_2, nitrogen supply, and species composition and diversity were manipulated, species effects on soil CO_2 flux during 19 sampling periods over 2 yr were determined for 16 grassland species.
- The average effect of a species on soil CO_2 flux was correlated with biomass of the species grown in monoculture, suggesting that effects of species on soil CO_2 flux are related to the potential productivity of a species and total belowground C allocation. During dry, warm conditions there is a greater effect of elevated atmospheric CO_2 on soil CO_2 flux and during these times deeper-rooted species contribute to soil CO_2 flux more than average.
- Although differences in responses to elevated CO_2 and nitrogen among species were not great, decreases in diversity can affect belowground carbon allocation depending on the plant traits of the species that are lost from ecosystems.

Key words: elevated CO_2, grasslands, N fertilization, plant diversity, soil CO_2 flux.

Introduction

Increasing atmospheric CO_2 concentrations, increasing rates of nitrogen (N) deposition, and changes in species composition of ecosystems are three major global changes caused by human activities. Understanding and predicting the impacts of these global changes on ecosystem processes requires a better understanding of the influence of these factors on belowground carbon (C) cycling, especially soil CO_2 flux, an integrated measure of root and microbial activity (Raich & Nadelhoffer, 1989).

Although plant species can affect ecosystem processes in different ways (Hobbie, 1992; Caldwell *et al.*, 1998), the effects of different species on soil CO_2 flux is not understood well. Global changes in ecosystem diversity and composition require ecologists to begin to understand the variation that may exist in species effects on soil CO_2 flux and search for ways to reduce the complexity that may exist among species in their effects on soil CO_2 flux.

Soil CO_2 flux varies over the year and temporal patterns of soil CO_2 flux are important to models of ecosystem C balance (Raich & Potter, 1995). Factors that affect soil CO_2 flux with similar temporal patterns are more likely to affect soil CO_2 flux through the same mechanism or interact with abiotic factors in the same way. Quantifying the relationships over time between soil CO_2 flux and the effects of resource supplies or species on soil CO_2 flux serves as a first step to understanding the mechanisms behind potential changes in belowground C cycling and simplifying models of ecosystem functioning.

We addressed issues of the effects of species on soil CO_2 flux and the response of soil CO_2 flux to changes in atmospheric CO_2 and N supply in 359 experimental plots of a grassland study that directly manipulates each factor in a well-replicated factorial manner. The 16 species in this experiment are drawn from four functional classifications (C_3 grass, C_4 grass, C_3 forb, and C_3 legume), but also vary in ecologically important traits that do not necessarily correspond to the most commonly used functional classifications (J. M. Craine, unpublished). In this paper, we utilize a multiple regression technique that determines the effects of species on soil CO_2 flux, adjusted for the presence of other species in a given plot while controlling for plot species diversity, CO_2 treatment and N treatment. A subset of this data was analyzed in Craine *et al.* (2001), focusing on the effects of CO_2, N, and diversity, with no analyses of species composition. We examine not only the average effects of species and treatments on soil CO_2 flux over 19 sampling periods over 2 yr, but also whether species and treatments differ in their seasonal and annual patterns of effects on soil CO_2 flux. We also examined the relationships among effects of the different treatments and species and their relationships to other measures such as average soil CO_2 flux, soil temperature, and soil moisture.

Materials and Methods

BioCON design and sampling

We addressed the effects of species on soil CO_2 flux in the BioCON (Biodiversity, CO_2 and Nitrogen) experiment. The BioCON experiment (Reich *et al.*, 2001) is located at the Cedar Creek Natural History Area on the Anoka sand plain in central Minnesota, USA. The portion of the BioCON experiment for which data were analyzed is composed of 359 plots, 2 × 2 m each. These plots were planted to various combinations of 1, 4, 9 or 16 prairie plant species chosen from 16 species in four plant functional groups (C_3 grass, C_4 grass, forb, legume). In the experiment, 296 plots were evenly divided among the four CO_2 × N combinations with 32, 15, 15, and 12 replicates for the 4 levels of biodiversity (1, 4, 9 and 16 species, respectively) at each treatment combination. For these 296 plots, there were two replicates of the monocultures for each species, with species composition determined randomly for the 4- and 9-species plots. In addition to the main 296 plots, 63 plots were sampled that were included in the experimental design to increase the ability to determine the differences among species in traits or responses. These 63 additional plots included 3 single 4-species plots for each of the four functional groups at each of the four CO_2 and N combinations (48 plots total). Three additional 4-species plots were included such that there were 5 replicates at each CO_2 and N combination of 2-functional group plots. The remaining 12 plots provided three additional replicates at each CO_2 and N combination of 4-functional group 4-species plots.

Plots were seeded in June 1997 at a rate of 12 g seed m^{-2}, with seed mass evenly divided among the species in a plot. Adding a constant amount of seed to each plot insures that plots of different diversity have the same total seed mass added. Half of all of the plots are fertilized at 4 g N m^{-2} $years^{-1}$ applied as NH_4NO_3 over three dates during the growing season (1.33 g N m^{-2} yr^{-1} $application^{-1}$) and the other plots are not fertilized. Work in other upland ecosystems at Cedar Creek has shown that N is the major limiting soil resource (Tilman, 1987; Reich *et al.*, 1997). Plots are evenly partitioned among six 14 m diameter circular experimental areas (rings), three of which are exposed to ambient CO_2 and three to elevated CO_2 mole fractions during daylight hours (550 µmol mol^{-1}) using Free Air CO_2 Enrichment (FACE) technology (Lewin *et al.*, 1994). CO_2 treatment began in April, 1998 and the first N treatment began in May, 1998. Plots were irrigated during the 1997 growing season to ensure good establishment, but were not irrigated in subsequent years. Plots were weeded at least twice a year during 1998 and 1999 to remove undesired species. Precipitation during May 1–September 30, 1998 was 38.8 cm, and 56.2 cm during the same period in 1999. This range of dates approximates the average growing season for most species in this study.

Soil CO_2 flux measurements were made during the 1998 and 1999 growing seasons, measured approx. every 3 wk between May 26 and Sept 30, 1998 (7 sampling dates) and approx. every 2 wk between April 13 and October 13, 1999 (12 sampling dates). Soil CO_2 flux was measured with the Li-Cor 6200 gas exchange system (Li-Cor, Lincoln, NE, USA) fitted with the Li-Cor 6400–09 soil respiration chamber. Measurements occurred at a permanent location in each plot. At this location on April 30, 1998, *c.* 1 mo before the first measurement of soil CO_2 flux, all aboveground vegetation was clipped from a 10-cm × 10 cm location in the plot. In the recently clipped area, a plastic collar, 10 cm across and 5 cm high was inserted *c.* 2.5 cm into the soil. Any subsequent aboveground materials were removed at each subsequent sampling. Any moss that had accumulated during each year was scraped from the surface approximately midseason.

Each measurement consisted of 6, 5-s integrations of soil CO_2 flux that spanned a 60-s period. At the beginning of each measurement, CO_2 in the chamber was scrubbed down to below ambient CO_2 concentration with soda lime. Soil CO_2 flux was then calculated at the ambient CO_2 concentration using linear regression (see Craine *et al.*, 1998 for details on soil CO_2 flux measurement protocols). With each measurement of soil CO_2 flux, we also measured the temperature of the soil at 10 cm depth. All measurements were made over the course of 2 d at each sampling between the hours of 08:30 and 18:00. A total of 10 measurements (< 0.2% of all measurements) had missing data points due to various factors and were replaced with the average soil CO_2 flux of all plots for that period. Soil CO_2 flux is reported in units of µmol m^{-2} s^{-1}. For reference, 1 µmol CO_2 m^{-2} s^{-1} is equivalent to *c.* 1.04 g C m^{-2} day.

We made additional measurements of soil CO_2 flux in June and August of both years on the long-term collars and on temporary collars placed in a location where aboveground vegetation had recently been clipped (< 2 h). Adjusted for the time since the aboveground vegetation was clipped, the long-term collars underestimated soil CO_2 flux compared with recently clipped area by 18%. There were no differences among the 4 harvests in this value nor was this value affected by treatments (results not shown).

For each plot, percent soil moisture was measured 0–20 cm for 9 of the sampling periods over the two growing seasons using time-domain reflectometry (TDR) (Topp & Davis, 1985). The values for TDR that we use have not been calibrated for the BioCON experiment, but are generally well-correlated ($r = 0.8-0.9$) with gravimetric soil moisture on similar soils at Cedar Creek (K. Wrage, unpublished). Measurements of soil moisture in a plot were generally made within a few hours of the soil CO_2 flux measurement. We report the average soil moisture for 16-species plots.

Statistical Analyses

All statistical analyses were performed in JMP 3.2.2 (SAS Institute, Cary, NC, USA). We used the time-weighted average of soil CO_2 flux and soil temperature over the sampling periods of May 26 to September 30 (day numbers 145–272) for each year to determine total soil CO_2 flux and total degree C days for each year and the differences between years.

Patterns of soil CO_2 flux over the 19 samplings were analyzed with a multivariate repeated measures linear regression model. This model included CO_2 treatment, the identity of the ring nested within the CO_2 treatment, N treatment, and 16 different species treatments – 16 variables that represented whether or not each of the 16 species was seeded into a plot or not (otherwise know as species presence). Species richness of 0.1 m^2 clipped aboveground biomass samples was 1.0, 3.8, 8.0 and 13.6 species on average for plots planted with 1, 4, 9 and 16 species (Reich *et al.*, 2001). Therefore, it is not unreasonable to expect that a given species was present in plots where seeded.

From the results of the regression model, quantitative estimates of the treatment effects are expressed to represent the change in soil CO_2 flux that results when that treatment (CO_2, N, or the seeds of a given species) is applied to a plot. Treatment effects that are reported for the between-plot portion of the model represent the influence of that treatment on soil CO_2 flux averaged across all dates, controlling for CO_2 and N treatments and the presence or absence of all other species. Therefore, a significant species effect for the between-plot portion of the model represents the influence of a species on soil CO_2 flux averaged across all dates, independent of treatments and the presence or absence of other species. The within-plot portion of the model provides estimates of the treatment effects at each sampling period. A significant within-plot portion of the model for a treatment signifies that the treatment significantly increased or decreased soil CO_2 flux above or below the average effect for that species for at least one date during the 19 sampling periods.

Treatment effects can be used to predict soil CO_2 flux for any particular treatment combination, either averaged across all dates or for a particular date. The intercept that we report is interpreted as the soil CO_2 flux of a plot with no planted species at ambient levels of CO_2 and N. Adding the coefficient for a given species to the intercept produces the predicted soil CO_2 flux of a monoculture at ambient CO_2 and N for that species. This model structure considers treatment effects on soil CO_2 flux as strictly additive and therefore coefficients for treatment effects can be used to estimate soil CO_2 flux for plots of various treatments and species compositions and diversities. For example, adding the coefficients of species effects for four given species produces the predicted soil CO_2 flux for a plot that contains those four species. For a plot at elevated N (or CO_2), the coefficient for the N effect (or CO_2 effect) is added to the soil CO_2 flux predicted by the intercept and the species composition of the plot. Average soil CO_2 flux of a given diversity treatment can be derived by adding estimates for the intercept and the average of the coefficients for 16 species effects multiplied by the number of species. Comparing the results of a model that includes diversity explicitly as a categorical term and the results of a model that includes diversity implicitly through species effects, the regression technique that utilizes species effects captures the basic diversity effect relatively well, within 5% at each diversity level (results not shown).

No pairwise interactions between CO_2 and any individual species were found to be significant in alternative models, nor between N and any individual species. Likewise, no pairwise interactions between species were significant. Therefore, no pairwise interactions involving CO_2, N, and any individual species were included in the final regression model.

In order to see if the effects of species on soil CO_2 flux were robust with respect to the type of model that we ran, we also tested alternative models that examined the soil CO_2 flux of monocultures only, and species effects at both the 4- and 9-species diversity levels. The estimates of the species effects among models were correlated (results not shown) and not specific to any of the four models. In another model that included interactions between species presence/absence and the diversity of each plot, the amount of variation explained by interactions between species presence/absence and plot diversity was relatively small. Therefore we assume species effects could be considered additive (results not shown).

In order to examine the temporal relationship among soil CO_2 flux, soil temperature, and the 18 treatment effects (CO_2, N, and the 16 species effects), we ran a single principal components analysis (PCA) with the values of these 20 parameters from each of the 19 sampling periods. Parameters that have high coefficients on a given axis (i.e. load strongly on the axis) can be thought of as covarying across time. For example, if two parameters both load strongly on an axis (both have large positive or negative coefficients), the conditions that lead to a large, positive value for one parameter also lead to a large, positive value for the other. Two parameters with coefficients of different signs on an axis indicates that the two parameters are inversely related such that at times when one is more positive, the other is more negative. As data on soil moisture were taken only occasionally throughout the two growing seasons at the same time as soil CO_2 flux, and were not included in

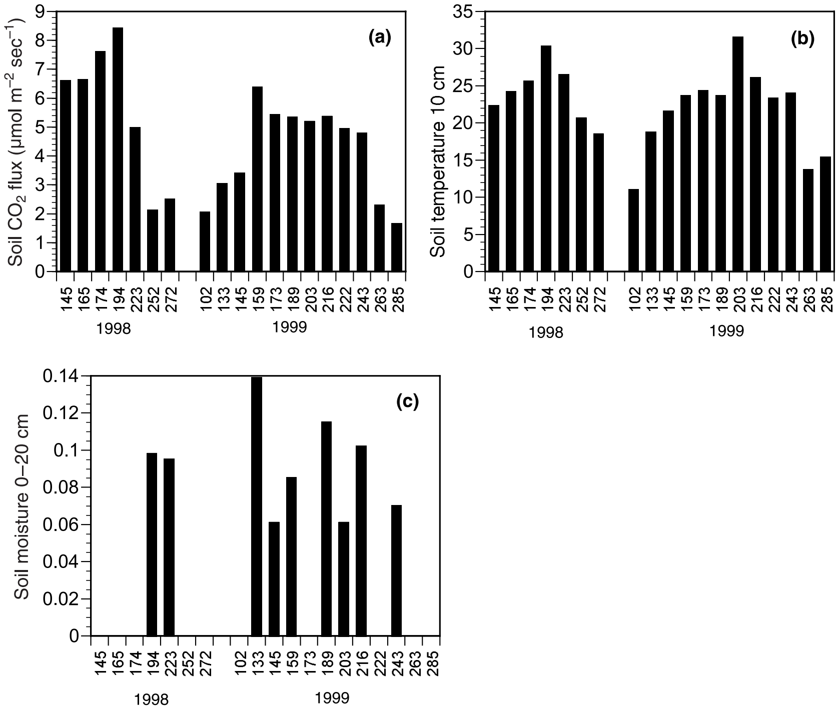

Fig. 1 (a) Soil CO_2 flux, (b) soil temperature at 10 cm (°C), and (c) soil moisture 0–20 cm (% moisture) averaged for all plots at each sampling period.

the PCA, but we examine the correlations between soil moisture and given effects and PCA axes.

Results

Total soil CO_2 flux and soil temperature were similar between years with soil CO_2 flux 17% greater in 1998 than 1999, and soils an average of 1.6°C warmer in 1998 than 1999 (Fig. 1, Table 1). The repeated measures model of soil CO_2 flux explained 66% of the total variation in the data. Both the CO_2 and N treatments were significant with similar patterns (Fig. 2a,b) as have previously been described from analyses using a subset of the data analyzed here (Craine *et al.*, 2001). On average, elevated CO_2 and N increased soil CO_2 flux by 0.57 and 0.34 μmol m^{-2} s^{-1}, respectively (16%, 9% increases) (Table 2).

Eight species had significant average effects on soil CO_2 flux across the 19 sampling periods (i.e. significant between-plot effects). Species effects ranged from −0.29 μmol m^{-2} s^{-1} for *Amorpha canescens* to +0.60 μmol m^{-2} s^{-1} for *Achillea millefolium* (Table 2). Five species had positive effects on soil CO_2 flux (*Bromus inermis* (C_3 grass), *Poa pratensis* (C_3 grass), *A. millefolium* (C_3 forb), *Solidago rigida* (C_3 forb), and *Lupinus perennis* (legume)). Three species had negative effects on soil CO_2 flux (*Schizachyrium scoparium* (C_4 grass), *Asclepias tuberosa* (C_3 forb), and *A. canescens* (legume)). Eight species had no significant effect on soil CO_2 flux. There was little correspondence among species of a functional group in their effects on soil CO_2 flux (Table 2). The species effects were correlated with total plant biomass of these species in monoculture, averaged across four harvests in June and August of 1998 and 1999 ($r = 0.71$, $P < 0.01$, see Reich *et al.* 2001 for details on biomass harvests). The species effects were more stongly correlated with aboveground biomass (Fig. 3) than belowground biomass ($r = 0.77$, $P < 0.001$ vs $r = 0.62$, $P < 0.05$).

There was no significant variation in the effects of six species (i.e. within-plot variation) on soil CO_2 flux (*A. canescens* (legume), *Anenome cylindrica* (forb), *A. tuberosa* (forb), *Lespedeza capitata* (legume), *Koeleria cristata* (C_3 grass) and *Petalostemum villosum* (legume)), and significant variation in the temporal pattern of effects for 10 species (Table 2). The significant within-plot variation for the 10 species generally represented seasonal patterns of effects that were higher in the mid-season than early or late in the growing season. These seasonal patterns were both positive (e.g. *A. millefolium*) and negative (e.g. *Bouteloua gracilis*) (Figs 4, 5, 6, 7). The effects of some species were also greater in one year than the other. For example, both *P. pratensis* and *Sorghastrum nutans* had greater effects on soil CO_2 flux in 1998 than in 1999 (Figs 4d, 5d; Table 1), while *Andropogon gerardii*, *S. scoparium*, and *P. villosum* had greater effects on soil CO_2 flux in 1999 than in 1998 (Figs 5a,c; 7d; Table 2).

The first three axes of the PCA of soil CO_2 flux, soil temperature and the treatment effects were determined to be the most explanatory and biologically interpretable and considered here in this section. The first three axes explained 68% of the variation in the 20 parameters explained by the PCA; Axis 4 only explained 9% of the explainable variation (5.5% expected) (Table 3). In general, Axis 1 appears to represent variance associated with parameters that have seasonal patterns as well as the greater flux in 1998 than 1999 (Fig. 8a).

Table 1 Integrated effects on total soil CO_2 flux (g C m^{-2}) during the period from May 26–September 30 during 1998 and 1999 and the difference in integrated effect on total soil CO_2 flux between the two years

Parameter	1998	1999	Difference
Soil CO_2 flux	727.65	624.24	103.42
Soil temperature	3143.96	2946.28	197.68
CO_2	59.35	93.48	−32.81
Nitrogen	55.57	42.65	12.42
Agropyron repens (C_3)	9.22	29.01	−19.79
Bromus inermis (C_3)	50.80	43.48	7.33
Koeleria cristata (C_3)	13.65	22.04	−8.39
Poa pratensis (C_3)	93.99	4.62	89.36
Andropogon gerardii (C_4)	5.57	40.89	−35.32
Bouteloua gracilis (C_4)	−16.03	−24.70	8.67
Schizachyrium scoparium (C_4)	−61.29	−14.51	−46.77
Sorghastrum nutans (C_4)	34.38	0.28	34.10
Achillea millefolium (F)	97.04	74.14	22.90
Anemone cylindrica (F)	−7.64	−14.95	7.31
Asclepias tuberosa (F)	−42.72	−28.14	−14.58
Solidago rigida (F)	40.18	33.11	7.07
Amorpha canescens (L)	−56.67	−32.58	−24.09
Lespedeza capitata (L)	−27.76	−19.70	−8.06
Lupinus perennis (L)	81.92	58.55	23.37
Petalostemum villosum (L)	−48.35	7.33	−55.68

Effects for each year are derived from the coefficients of the parameters at each time period. Also included are estimated total soil CO_2 flux (g C m^{-2}) during the same period and estimated soil temperature (degree C days). Included for each species is standard functional classification: C_3 grass (C_3); C_4 grass (C_4); forb (F); legume (L).

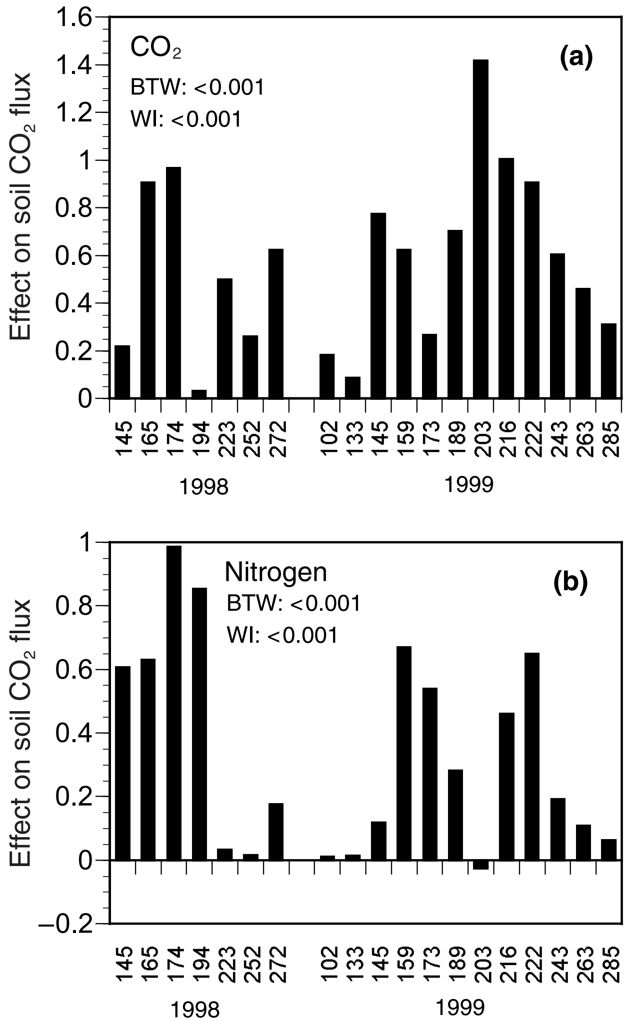

Fig. 2 Effects of (a) elevated CO_2 and (b) N fertilization on soil CO_2 flux, independent of all other treatments. Also shown are the significance of the main effect for the between- and within-plot portions of the regression model.

Soil CO_2 flux, soil temperature, and the N effect all loaded strongly positive on Axis 1 (Table 3). This implies that there was a general set of correlations over the two growing seasons such that the N effect, soil CO_2 flux, and soil temperature were all correlated. The conditions that led to high soil CO_2 flux and high soil temperature also led to a strong effect of N on soil CO_2 flux. *B. inermis*, *P. pratensis*, *S. nutans*, *A. millefolium*, *S. rigida*, and *L. perennis* loaded strongly positive on Axis 1, while *S. scoparium*, *A. tuberosa*, *A. canescens*, and *P. villosum* all loaded strongly negative (Table 3). This implies that not only do the conditions that lead to high soil CO_2 flux and soil temperature lead to a greater N effect, but also lead to larger positive and negative species-specific effects on soil CO_2 flux for the above-mentioned species.

Species effects explained most of the variation in Axis 2 (Table 3). *K. cristata*, *A. gerardii*, *S. scoparium*, *A. millefolium*, *S. rigida*, and *P. villosum* all loaded strongly positive, while *B. gracilis*, *S. nutans*, *A. cylindrica*, and *L. capitata* all loaded strongly negative. The differences in Axis 2 scores of dates between the two years (Fig. 8b) suggest that this axis may represent variance associated with differences in species effects that were more or less than the average differences in soil CO_2 flux between the two years. Yet, contrary to this potential interpretation of Axis 2, the correlation between the difference in integrated soil CO_2 flux between the two years for each species and their coefficients on Axis 2 was somewhat weak (r = −0.38, P = 0.12). Interpretation of this axis is made difficult by the patterns of effects on soil CO_2 flux for individual dates and species. Day number 194 of 1998 scored strongly positive on Axis 2, while all other dates for 1998 were negative. In addition, *P. pratensis* differed greatly between the years (Table 1), yet it does not load strongly onto Axis 2 (Table 3).

Axis 3 appears to represent patterns of soil CO_2 flux and effects on soil CO_2 flux that are associated with soil moisture. The CO_2 effect, soil temperature, *A. repens*, *L. capitata*, *B. inermis*, *A. gerardii*, *P. villosum* all loaded strongly and their coefficients were positive, while *P. pratensis* and *K. cristata* loaded strongly, but had negative coefficients (Table 3). This implies that when the CO_2 effect was greater than average, the effects of species such as *A. repens* and *L. capitata* on soil CO_2

Table 2 Results of the repeated measures model of soil CO_2 flux that includes the explanations of between-plot and within-plot variation. For the within-plot portion of the model, the statistics refer to the differences in treatments over the 19 periods, that is interactions between factors and period

	Between-plot			Within-plot	
Parameter	Estimate	F ratio	Prob > F	F ratio	Prob > F
Intercept	3.62 ± 0.37	< 0.001	< 0.001		
CO_2	0.57 ± 0.46	81.7	< 0.001	13.0	< 0.001
Ring[CO_2]		11.1	< 0.001	18.4	< 0.001
Nitrogen	0.34 ± 0.08	27.2	< 0.001	4.7	< 0.001
A. repens (C_3)	0.15 ± 0.07	2.6	ns	1.9	< 0.001
B. inermis (C_3)	0.30 ± 0.05	11.5	< 0.001	2.1	< 0.05
K. cristata (C_3)	0.12 ± 0.06	1.5	ns	1.5	ns
P. pratensis (C_3)	0.27 ± 0.05	9.3	< 0.01	3.4	< 0.001
A. gerardii (C_4)	0.13 ± 0.09	2.4	ns	2.3	< 0.01
B. gracilis (C_4)	−0.16 ± 0.06	3.1	ns	1.9	< 0.05
S. scoparium (C_4)	−0.23 ± 0.05	6.2	< 0.05	2.0	< 0.01
S. nutans (C_4)	0.13 ± 0.08	2.1	ns	2.1	< 0.01
A. millefolium (F)	0.60 ± 0.07	45.2	< 0.001	3.7	< 0.001
A. cylindrica (F)	−0.06 ± 0.07	0.5	ns	1.3	ns
A. tuberosa (F)	−0.24 ± 0.06	7.3	< 0.01	0.7	ns
S. rigida (F)	0.23 ± 0.04	6.9	< 0.01	1.7	< 0.05
A. canescens (L)	−0.29 ± 0.06	10.1	< 0.01	1.1	ns
L. capitata (L)	−0.13 ± 0.05	2.2	ns	0.9	ns
L. perennis (L)	0.48 ± 0.05	27.7	< 0.001	5.7	< 0.001
P. villosum (L)	−0.09 ± 0.10	1.0	ns	1.3	ns

The parameter estimates for the six rings were not included. Included for each species is standard functional classification: C_3 grass (C_3); C_4 grass (C_4); forb (F); legume (L). ns, not significant.

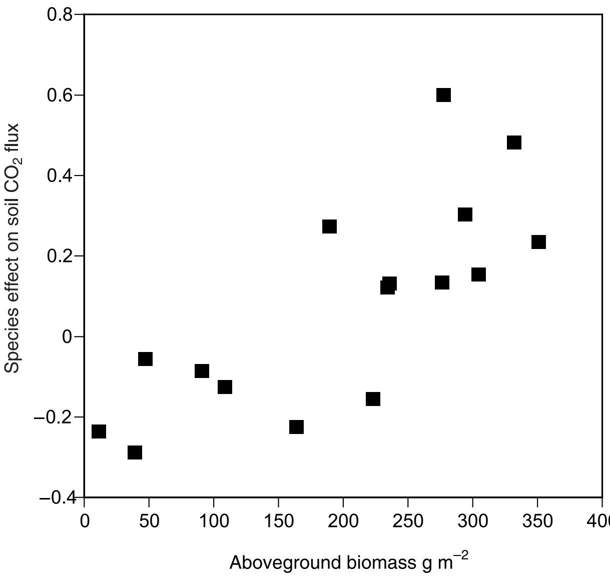

Fig. 3 Relationship between aboveground biomass (g m^{-2}) for a species in monoculture averaged across all treatments, and the species effect on soil CO_2 flux (μmol m^{-2} s^{-1}) from the between-plot portion of the regression model.

flux were more positive than their average effect, while the effects of species such as *P. pratensis* and *K. cristata* were more negative than their average effect. The converse also holds: when the CO_2 effect was less than average, species such as *A. repens* and *L. capitata* contributed to soil CO_2 flux less than their average, while species such as *P. pratensis* contributed more than their average. Species that loaded positively on Axis 3 are all deeply rooted species, while *P. pratensis* and *K. cristata* are very shallowly rooted species, with observed rooting depths for *P. pratensis* in other areas at Cedar Creek often no deeper than 10 cm (J. M. Craine, pers. comm.).

Because soil moisture was not measured for every sampling period, we examined its relationship to the other variables by examining its correlation with each of the three main PCA Axes. Soil moisture was negatively correlated with Axis 3 ($r = -0.46$, $P = 0.22$), though more strongly correlated with the CO_2 effect ($r = -0.59$, $P = 0.09$). Most of the residuals were associated with early season sampling periods that also had low soil temperatures (data not shown). In all, Axis 3 suggests that during dry, warm conditions there is a greater effect of CO_2 on soil CO_2 flux and during these times deeper rooted species contribute to soil CO_2 flux more than average. Warm, wet conditions that do not favour greater soil CO_2 flux under elevated CO_2 are conditions when more shallowly rooted species contribute more to soil CO_2 flux.

Discussion

It is clear that there are different patterns among species in their effects on soil CO_2 flux. Effects of a species on soil CO_2 flux could be due to changing the allocation of C belowground or altering the mean residence time of C belowground. For

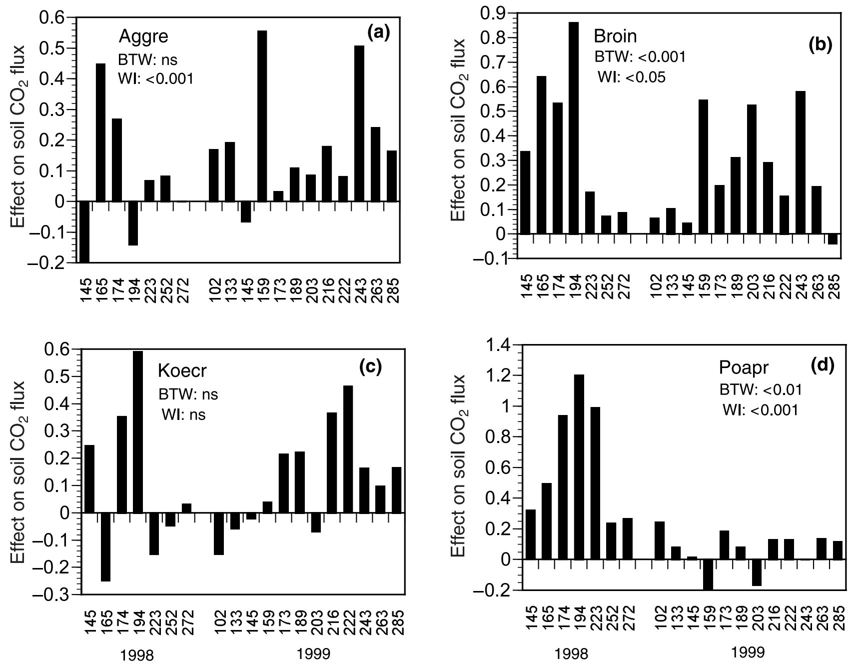

Fig. 4 Species effects on soil CO_2 flux (μmol m^{-2} s^{-1}) from the within-plot portion of the regression model for the C_3 grasses. Also shown are the significance of the main effects for the between- and within-plot portions of the regression model.

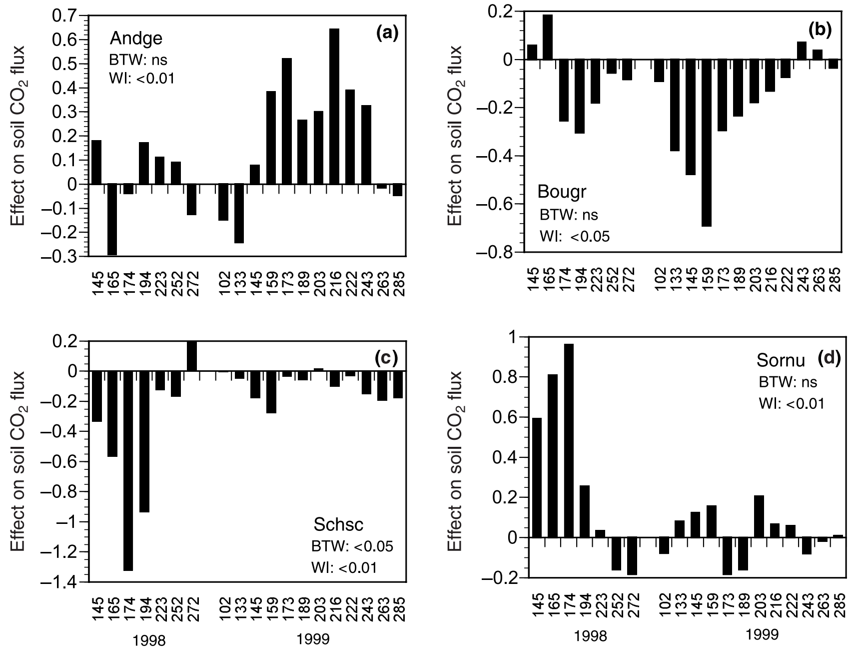

Fig. 5 Species effects on soil CO_2 flux (μmol m^{-2} s^{-1}) from the within-plot portion of the regression model for the C_4 grasses. Also shown are the significance of the main effects for the between- and within-plot portions of the regression model.

example, plants could alter total belowground C allocation by having inherently different photosynthetic rates or inherently different relative belowground allocation rates. Similarly, the effects species have on belowground C allocation could be indirect, for example by affecting N or water availability. These effects would alter the rates of below-ground C allocation of other species, too. Alternatively, species could alter soil CO_2 flux by affecting the mean residence time of C belowground, for example by altering the rates of decomposition (Hungate *et al.*, 1997).

Although more work is necessary to understand the specific mechanisms by which species alter soil CO_2 flux, the correlation between aboveground biomass in monocultures of a species and the effect of the species on soil CO_2 flux suggests species affect soil CO_2 flux by having greater or smaller rates of total C acquisition, mainly associated with differences in productivity. Plots that were seeded with poorly growing species would have less overall C gain and therefore less soil CO_2 flux.

Based on the species composition of regional native tallgrass prairie and oak savanna, we would expect that many of

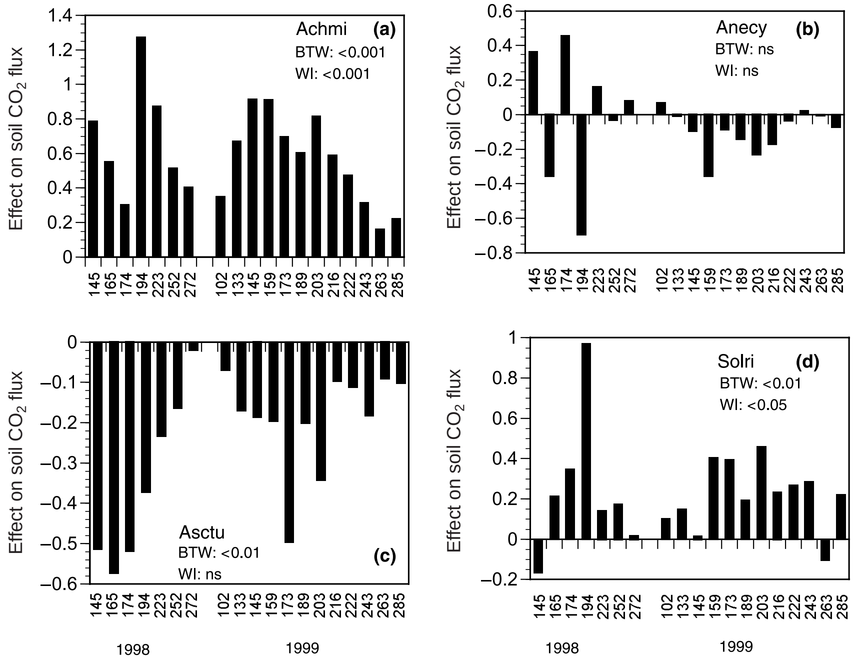

Fig. 6 Species effects on soil CO_2 flux (μmol m^{-2} s^{-1}) from the within-plot portion of the regression model for the C_3 forbs. Also shown are the significance of the main effects for the between- and within-plot portions of the regression model.

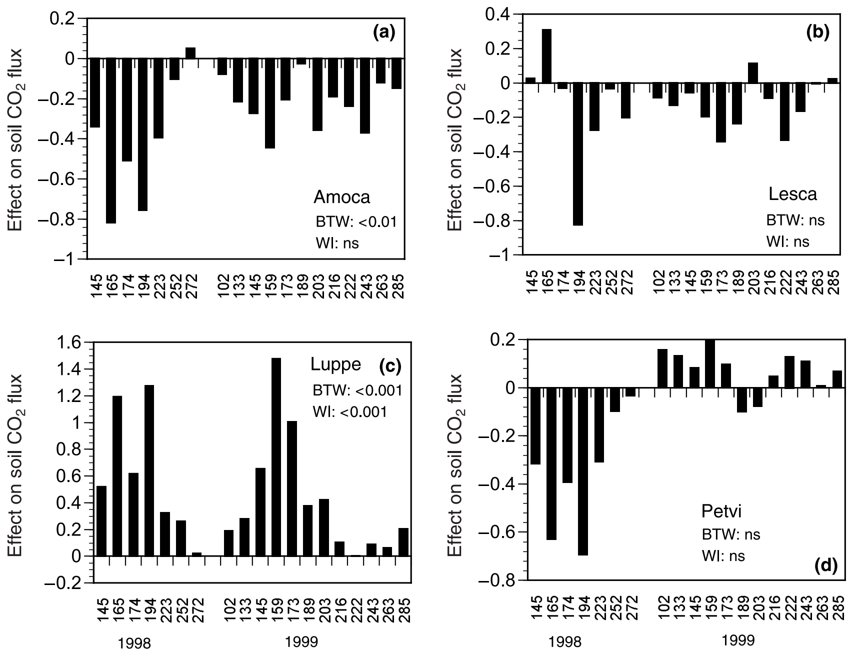

Fig. 7 Species effects on soil CO_2 flux (μmol m^{-2} s^{-1}) from the within-plot portion of the regression model for the legumes. Also shown are the significance of the main effects for the between- and within-plot portions of the regression model.

the species that had negative effects on soil CO_2 flux (e.g. *S. scoparium* or *A. canescens*) will have a greater effect on belowground processes with time as these species continue to grow and accumulate biomass. Many more of the differences among species are probably associated with cumulative effects on their environment (e.g. litter accumulation) and/or between season differences in stand development.

Numerous variables affected soil CO_2 flux, including both experimental treatments (N, CO_2, species diversity and species identity) and uncontrolled driving variables such as temperature and soil moisture. As a first step in identifying patterns within this complex set of variables, we used PCA and complimentary analyses of correlations with the PCA axes to examine how these variables covaried over 2 yr of sampling. Axis 1 explained the largest fraction of the variation in the data set, with strong loadings by soil CO_2 flux, soil temperature, the N effect and a set of species that show both positive and negative unimodal patterns in their effects on soil

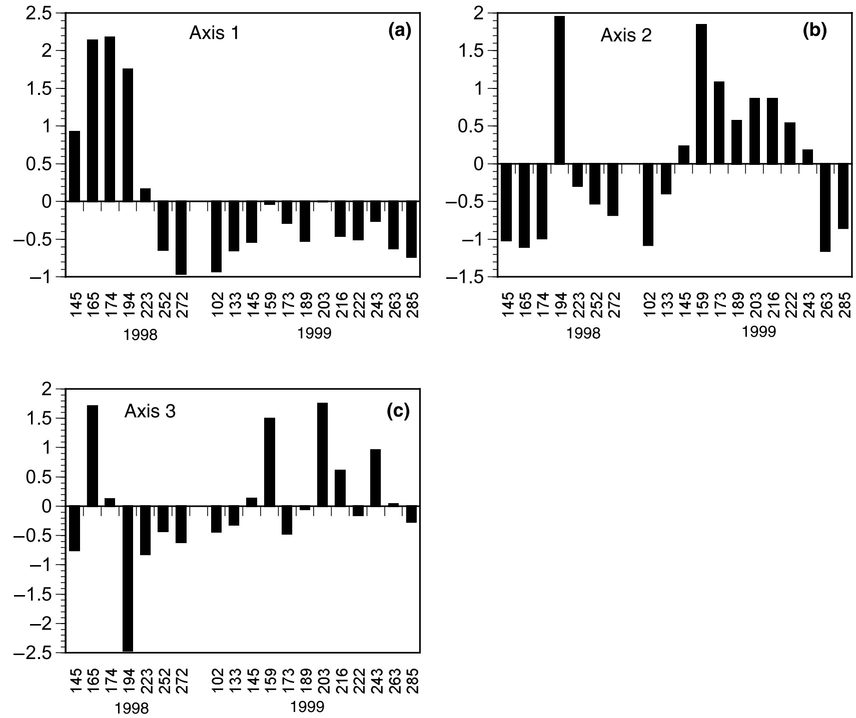

Fig. 8 Principal components analysis (PCA) axes scores averaged for all plots for each sampling period.

Table 3 Results of the principal components analysis of average soil CO_2 flux at each sampling period, average soil temperature of bare ground plots per period, the CO_2 treatment effect, the nitrogen treatment effect, and the coefficients of the effects of each of the 16 species in BioCON

Eigenvectors	Axis 1	Axis 2	Axis 3
Soil CO_2 flux	0.33	0.01	0.07
Soil temperature	0.27	0.14	0.18
CO_2	0.04	−0.09	0.47
Nitrogen	0.28	−0.03	−0.02
A. repens (C_3)	−0.01	−0.14	0.45
B. inermis (C_3)	0.30	−0.02	0.17
K. cristata (C_3)	0.15	0.22	−0.20
P. pratensis (C_3)	0.21	−0.12	−0.43
A. gerardii (C_4)	0.06	0.36	0.22
B. gracilis (C_4)	−0.07	−0.32	−0.10
S. scoparium (C_4)	−0.27	0.20	0.13
S. nutans (C_4)	0.23	−0.37	0.05
A. millefolium (F)	0.21	0.27	−0.04
A. cylindrica (F)	−0.14	−0.28	−0.16
A. tuberosa (F)	−0.26	0.21	−0.02
S. rigida (F)	0.25	0.25	0.03
A. canescens (L)	−0.31	0.14	−0.09
L. capitata (L)	−0.12	−0.40	0.32
L. perennis (L)	0.27	0.05	0.12
P. villosum (L)	−0.26	0.22	0.22

Included for each species is standard functional classification: C_3 grass (C_3); C_4 grass (C_4); forb (F); legume (L). Each value represents the correlation between the parameter and the principal components analysis (PCA) axis. Axes 1, 2, and 3 explain 40.2, 16.7, and 11.2% of the variation, respectively (5.5% expected).

CO_2 flux over the growing season. Axis 1 most likely represents factors that are associated with differences in ecosystem C gain and therefore are associated with total C allocation belowground (Craine *et al.*, 1998). Since a large proportion of the C that is allocated belowground is quickly respired (Craine *et al.*, 1998), these differences in total C allocation are reflected in seasonal patterns of soil CO_2 flux.

The correspondence between the differential effects on soil CO_2 flux of deep and shallowly rooted species, the effect of elevated CO_2 on soil CO_2 flux, and soil moisture implies that soil CO_2 flux responses to moderate droughts may depend on the rooting depth of species in an ecosystem. In the future, more extensive measurements of soil moisture and root biomass throughout the soil profile should allow us to better understand the response of species to moderate drying of the soil and different species' effects on soil CO_2 flux.

Analyses of species effects in mixed communities on response variables should not only help us understand the functioning of individual species in ecosystems, but also community-level analyses, such as diversity-productivity relationships. For example, is the positive diversity-soil CO_2 flux relationship (J. M. Craine, unpublished) due to a diverse plot being more likely to have a species that is associated with high soil CO_2 flux overall, for example high Axis 1 species, or is it due to unquantified interactions among species (Tilman *et al.*, 1996)? Moreover, the models that includes species identity explicitly and the model that includes plot diversity (J. M. Craine, unpublished) have similar explanatory power

($r^2 = 0.77$ vs 0.70, respectively). As such, it is possible that only a few species determine the positive relationship between diversity and soil CO_2 flux, although the identity of those species might vary with time.

Acknowledgements

We thank the many individuals who assisted in the creation and sampling of this experiment, especially K. Wrage, J. Goth, W. Bengston, and A. Simpson. M. Tjoelker, L. Johnson, B. Lindgren, and D. Aeppli provided valuable statistical advice. Two anonymous reviewers provided helpful comments. JMC was supported by a NASA Earth Systems Fellowship, a NSF graduate fellowship, and a NSF dissertation improvement grant. Additional support was provided by NSF grant 9411972 and the U.S. Department of Energy.

References

Caldwell MM, Dawson TE, Richards JH. 1998. Hydraulic lift: Consequences of water efflux from the roots of plants. *Oecologia* **113**: 151–161.

Craine JM, Tilman DG, Wedin DA, Reich PB, Tjoelker MJ, Knops JMH. 2001. The relationship between plant functional strategies and growth in a low-nitrogen habitat. *Ecology* **Submitted 000**: 000–000.

Craine JM, Wedin DA, Chapin FS. 1998. Predominance of ecophysiological controls on soil CO_2 flux in a Minnesota grassland. *Plant and Soil* **207**: 77–86.

Hobbie SE. 1992. Effects of plant species on nutrient cycling. *Trends in Ecology and Evolution* 7: 336–339.

Hungate BA, Lund CP, Pearson HL, Chapin FSI. 1997. Elevated CO_2 and nutrient addition alter soil N cycling and N trace gas fluxes with early season wet-up in a California annual grassland. *Biogeochemistry Dordrecht* **37**: 89–109.

Lewin KF, Hendrey GR, Nagy J, LaMorte R. 1994. Design and application of free-air carbon dioxide enrichment facility. *Agricultural and Forest Meteorology* **70**: 15–29.

Raich JW, Nadelhoffer KJ. 1989. Belowground carbon allocation in forest ecosystems: Global trends. *Ecology* **70**: 1346–1354.

Raich JW, Potter CS. 1995. Global patterns of carbon dioxide emissions from soils. *Global Biogeochemical Cycles* **9**: 23–36.

Reich PB, Grigal DF, Aber JD, Gower ST. 1997. Nitrogen mineralization and productivity in 50 hardwood and conifer stands on diverse soils. *Ecology (Washington D C)* **78**: 335–347.

Reich PB, Knops J, Tilman D, Craine J, Ellsworth D, Tjoelker M, Lee T, Wedin D, Naeem S, Bahauddin D, Hendrey G, Jose S, Wrage K, Goth J, Bengston W. 2001. Plant diversity enhances ecosystem responses to elevated CO_2 and nitogen enrichment. *Nature* (In press): 000–000.

Tilman D. 1987. Secondary Succession and the Pattern of Plant Dominance Along Experimental Nitrogen Gradients. *Ecological Monographs* **57**: 189–214.

Tilman D, Wedin D, Knops J. 1996. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* **379**: 718–720.

Topp GC, Davis JL. 1985. Measurement of soil water content using time-domain reflectometry (TDR): a field evaluation. *Soil Science Society of America* **49**: 19–24.