

Grassland species effects on soil CO₂ flux track the effects of elevated CO₂ and nitrogen

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Summary

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- Understanding and predicting the impacts of elevated atmospheric CO₂, elevated nitrogen deposition, and decreases in plant diversity require better understanding of the way in which plant species influence soil CO₂ flux.
- In experimental grassland plots where atmospheric CO₂, nitrogen supply, and species composition and diversity were manipulated, species effects on soil CO₂ flux during 19 sampling periods over 2 yr were determined for 16 grassland species.
- The average effect of a species on soil CO₂ flux was correlated with biomass of the species grown in monoculture, suggesting that effects of species on soil CO₂ 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 CO₂ on soil CO₂ flux and during these times deeper-rooted species contribute to soil CO₂ flux more than average.
- Although differences in responses to elevated CO₂ 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 CO₂, grasslands, N fertilization, plant diversity, Soil CO₂ flux.

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Introduction

Increasing atmospheric CO₂ 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 CO₂ 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 CO₂ 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 CO₂ flux and search for ways to reduce the complexity that may exist among species in their effects on soil CO₂ flux.

Soil CO₂ flux varies over the year and temporal patterns of soil CO₂ flux are important to models of ecosystem C balance

(Raich & Potter, 1995). Factors that affect soil CO₂ flux with similar temporal patterns are more likely to affect soil CO₂ flux through the same mechanism or interact with abiotic factors in the same way. Quantifying the relationships over time between soil CO₂ flux and the effects of resource supplies or species on soil CO₂ 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 CO₂ flux and the response of soil CO₂ flux to changes in atmospheric CO₂ 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 (C₃ grass, C₄ grass, C₃ forb, and C₃ legume), but also vary in ecologically important traits that do not necessarily correspond to the most commonly used functional classifications (Craine *et al.*, 2001). In this paper, we utilize a multiple regression technique that determines the effects of species on soil CO₂ flux, adjusted for the presence of other species in a given plot while

controlling for plot species diversity, CO₂ treatment and N treatment. A subset of this data was analyzed in Craine *et al.* (2001), focusing on the effects of CO₂, N, and diversity, with no analyses of species composition. We examine not only the average effects of species and treatments on soil CO₂ 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 CO₂ flux. We also examined the relationships among effects of the different treatments and species and their relationships to other measures such as average soil CO₂ flux, soil temperature, and soil moisture.

Materials and Methods

BioCON design and sampling

We addressed the effects of species on soil CO₂ flux in the BioCON (Biodiversity, CO₂ 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 (C₃ grass, C₄ grass, forb, legume). In the experiment, 296 plots were evenly divided among the four CO₂ × 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 CO₂ and N combinations (48 plots total). Three additional 4-species plots were included such that there were 5 replicates at each CO₂ and N combination of 2-functional group plots. The remaining 12 plots provided three additional replicates at each CO₂ 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 NH₄NO₃ 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 CO₂ and three

to elevated CO₂ mole fractions during daylight hours (550 μmol mol⁻¹) using Free Air CO₂ Enrichment (FACE) technology (Lewin *et al.*, 1994). CO₂ 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 CO₂ 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 CO₂ 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 m before the first measurement of soil CO₂ 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 CO₂ flux that spanned a 60-s period. At the beginning of each measurement, CO₂ in the chamber was scrubbed down to below ambient CO₂ concentration with soda lime. Soil CO₂ flux was then calculated at the ambient CO₂ concentration using linear regression (see Craine *et al.*, 1998 for details on soil CO₂ flux measurement protocols). With each measurement of soil CO₂ 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 CO₂ flux of all plots for that period. Soil CO₂ flux is reported in units of μmol m⁻² s⁻¹. For reference, 1 μmol CO₂ m⁻² s⁻¹ is equivalent to *c.* 1.04 g C m⁻² day.

We made additional measurements of soil CO₂ 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 CO₂ 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

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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 CO₂ 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 CO₂ 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 CO₂ flux and total degree C days for each year and the differences between years.

Patterns of soil CO₂ flux over the 19 samplings were analyzed with a multivariate repeated measures linear regression model. This model included CO₂ treatment, the identity of the ring nested within the CO₂ 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 known as species presence). Species richness of 0.1 m² 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 CO₂ flux that results when that treatment (CO₂, 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 CO₂ flux averaged across all dates, controlling for CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ flux of a plot with no planted species at ambient levels of CO₂ and N. Adding the coefficient for a given species to the intercept produces the predicted soil

CO₂ flux of a monoculture at ambient CO₂ and N for that species. This model structure considers treatment effects on soil CO₂ flux as strictly additive and therefore coefficients for treatment effects can be used to estimate soil CO₂ 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 CO₂ flux for a plot that contains those four species. For a plot at elevated N (or CO₂), the coefficient for the N effect (or CO₂ effect) is added to the soil CO₂ flux predicted by the intercept and the species composition of the plot. Average soil CO₂ 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 CO₂ 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 CO₂, N, and any individual species were included in the final regression model.

In order to see if the effects of species on soil CO₂ flux were robust with respect to the type of model that we ran, we also tested alternative models that examined the soil CO₂ 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 CO₂ flux, soil temperature, and the 18 treatment effects (CO₂, 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 CO₂ flux, and were not included in

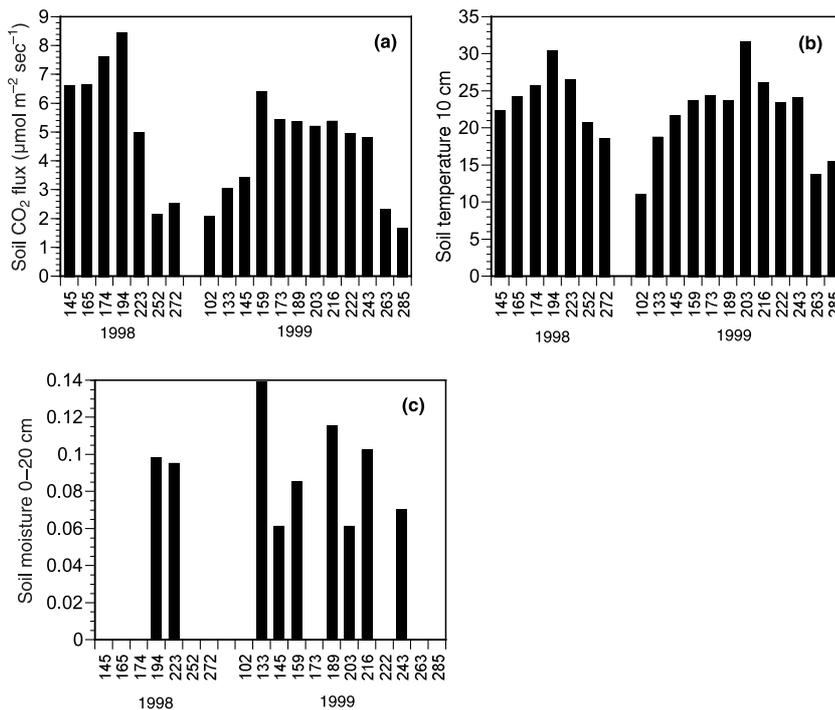


Fig. 1 (a) Soil CO₂ flux, (b) soil temperature at 10 cm (°C), and (c) soil moisture 0–20 cm (% moisture) averaged for all plots at each sampling period.

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

Results

Total soil CO₂ flux and soil temperature were similar between years with soil CO₂ 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 CO₂ flux explained 66% of the total variation in the data. Both the CO₂ 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 CO₂ and N increased soil CO₂ 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 CO₂ 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 CO₂ flux (*Bromus inermis* (C₃ grass), *Poa pratensis* (C₃ grass), *A. millefolium* (C₃ forb), *Solidago rigida* (C₃ forb), and *Lupinus perennis* (legume)). Three species had negative effects on soil CO₂ flux (*Schizachyrium scoparium* (C₄ grass), *Asclepias tuberosa* (C₃ forb), and *A. canescens* (legume)). Eight species had no significant effect on soil CO₂ flux. There was little correspondence among species of a functional group in their effects on soil CO₂ 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 strongly 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 CO₂ flux (*A. canescens* (legume), *A. cylindrica* (forb), *A. tuberosa* (forb), *L. capitata* (legume), *K. cristata* (C₃ grass) and *P. 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. *B. 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 *S. nutans* had greater effects on soil CO₂ flux in 1998 than in 1999 (Figs 4d, 5d; Table 1), while *A. gerardii*, *S. scoparium*, and *P. villosum* had greater effects on soil CO₂ flux in 1999 than in 1998 (Figs 5a,c; 7d; Table 2).

The first three axes of the PCA of soil CO₂ 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 CO₂ 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 CO₂ 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
<i>Agropyron repens</i> (C ₃)	9.22	29.01	-19.79
<i>Bromus inermis</i> (C ₃)	50.80	43.48	7.33
<i>Koeleria cristata</i> (C ₃)	13.65	22.04	-8.39
<i>Poa pratensis</i> (C ₃)	93.99	4.62	89.36
<i>Andropogon gerardii</i> (C ₄)	5.57	40.89	-35.32
<i>Bouteloua gracilis</i> (C ₄)	-16.03	-24.70	8.67
<i>Schizachyrium scoparium</i> (C ₄)	-61.29	-14.51	-46.77
<i>Sorghastrum nutans</i> (C ₄)	34.38	0.28	34.10
<i>Achillea millefolium</i> (F)	97.04	74.14	22.90
<i>Anemone cylindrica</i> (F)	-7.64	-14.95	7.31
<i>Asclepias tuberosa</i> (F)	-42.72	-28.14	-14.58
<i>Solidago rigida</i> (F)	40.18	33.11	7.07
<i>Amorpha canescens</i> (L)	-56.67	-32.58	-24.09
<i>Lespedeza capitata</i> (L)	-27.76	-19.70	-8.06
<i>Lupinus perennis</i> (L)	81.92	58.55	23.37
<i>Petalostemum villosum</i> (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 CO₂ flux (g C m⁻²) during the same period and estimated soil temperature (degree C days). Included for each species is standard functional classification: C₃ grass (C₃); C₄ grass (C₄); forb (F); legume (L).

Soil CO₂ 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 CO₂ flux, and soil temperature were all correlated. The conditions that led to high soil CO₂ flux and high soil temperature also led to a strong effect of N on soil CO₂ 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 CO₂ flux and soil temperature lead to a greater N effect, but also lead to larger positive and negative species-specific effects on soil CO₂ 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 2 yr (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 CO₂ flux between the 2 yr. Yet, contrary to this potential interpretation of Axis 2, the correlation between the difference in integrated

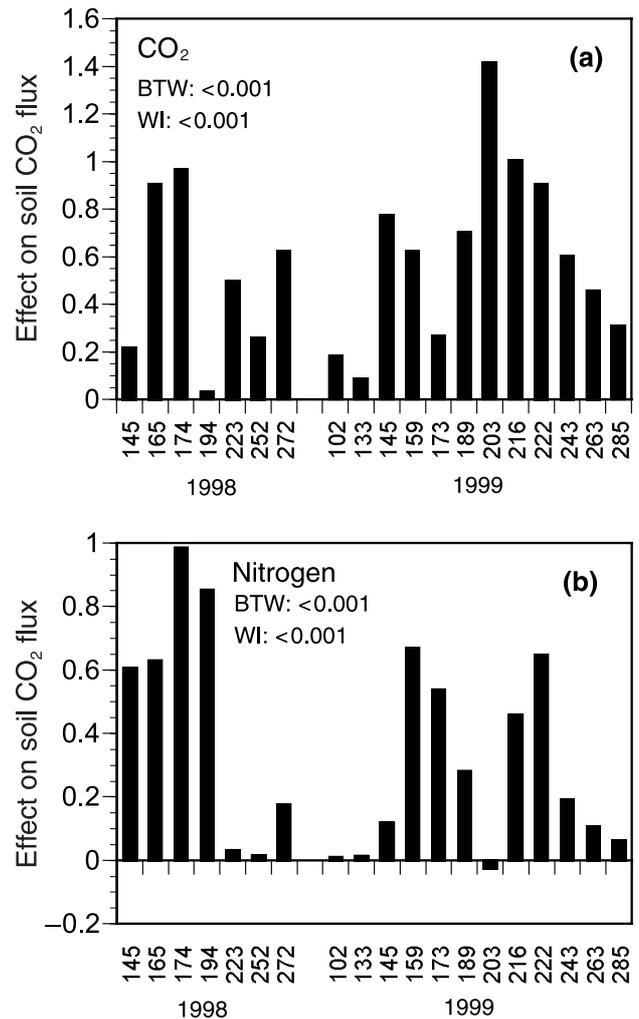


Fig. 2 Effects of (a) elevated CO₂ and (b) N fertilization on soil CO₂ flux, independent of all other treatments. Also shown are the significance of the main effect for the between- and within-plot portions of the regression model.

soil CO₂ flux between the 2 yr 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 CO₂ 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 CO₂ flux and effects on soil CO₂ flux that are associated with soil moisture. The CO₂ 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 CO₂ effect was greater than average, the effects of species such as *A. repens* and *L. capitata* on soil CO₂

Parameter	Between-plot			Within-plot	
	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
<i>A. repens</i> (C ₃)	0.15 ± 0.07	2.6	ns	1.9	< 0.001
<i>B. inermis</i> (C ₃)	0.30 ± 0.05	11.5	< 0.001	2.1	< 0.05
<i>K. cristata</i> (C ₃)	0.12 ± 0.06	1.5	ns	1.5	ns
<i>P. pratensis</i> (C ₃)	0.27 ± 0.05	9.3	< 0.01	3.4	< 0.001
<i>A. gerardii</i> (C ₄)	0.13 ± 0.09	2.4	ns	2.3	< 0.01
<i>B. gracilis</i> (C ₄)	-0.16 ± 0.06	3.1	ns	1.9	< 0.05
<i>S. scoparium</i> (C ₄)	-0.23 ± 0.05	6.2	< 0.05	2.0	< 0.01
<i>S. nutans</i> (C ₄)	0.13 ± 0.08	2.1	ns	2.1	< 0.01
<i>A. millefolium</i> (F)	0.60 ± 0.07	45.2	< 0.001	3.7	< 0.001
<i>A. cylindrica</i> (F)	-0.06 ± 0.07	0.5	ns	1.3	ns
<i>A. tuberosa</i> (F)	-0.24 ± 0.06	7.3	< 0.01	0.7	ns
<i>S. rigida</i> (F)	0.23 ± 0.04	6.9	< 0.01	1.7	< 0.05
<i>A. canescens</i> (L)	-0.29 ± 0.06	10.1	< 0.01	1.1	ns
<i>L. capitata</i> (L)	-0.13 ± 0.05	2.2	ns	0.9	ns
<i>L. perennis</i> (L)	0.48 ± 0.05	27.7	< 0.001	5.7	< 0.001
<i>P. villosum</i> (L)	-0.09 ± 0.10	1.0	ns	1.3	ns

Table 2 Results of the repeated measures model of soil CO₂ 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

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The parameter estimates for the six rings were not included. Included for each species is standard functional classification: C₃ grass (C₃); C₄ grass (C₄); forb (F); legume (L). ns, not significant.

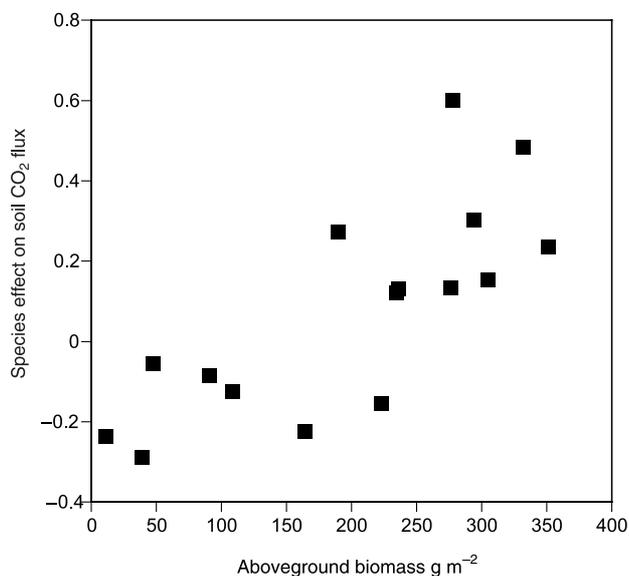


Fig. 3 Relationship between aboveground biomass (g m^{-2}) for a species in monoculture averaged across all treatments, and the species effect on soil CO₂ flux ($\mu\text{mol m}^{-2} \text{s}^{-1}$) from the between-plot portion of the regression model.

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 CO₂ effect was less than average, species such as *A.*

repens and *L. capitata* contributed to soil CO₂ 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.).

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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 CO₂ 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 CO₂ on soil CO₂ flux and during these times deeper rooted species contribute to soil CO₂ flux more than average. Warm, wet conditions that do not favour greater soil CO₂ flux under elevated CO₂ are conditions when more shallowly rooted species contribute more to soil CO₂ flux.

Discussion

It is clear that there are different patterns among species in their effects on soil CO₂ flux. Effects of a species on soil CO₂ flux could be due to changing the allocation of C belowground or altering the mean residence time of C belowground. For

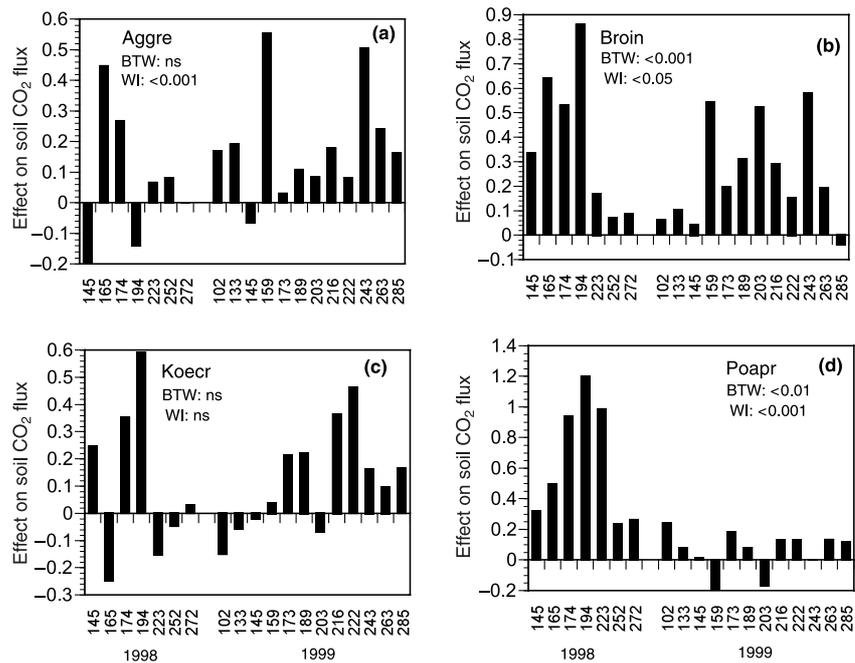


Fig. 4 Species effects on soil CO₂ flux ($\mu\text{mol m}^{-2} \text{s}^{-1}$) from the within-plot portion of the regression model for the C₃ grasses. Also shown are the significance of the main effects for the between- and within-plot portions of the regression model.

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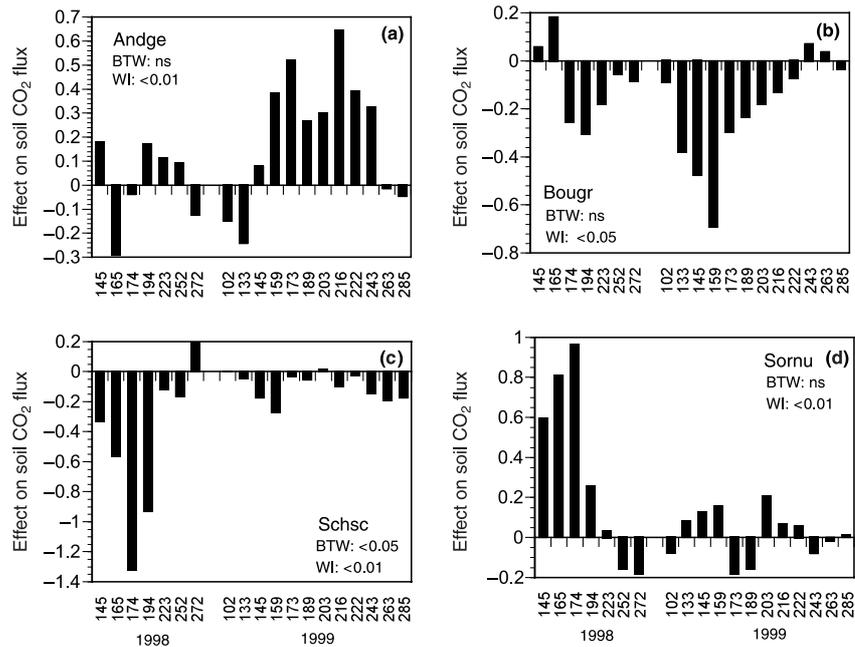


Fig. 5 Species effects on soil CO₂ flux ($\mu\text{mol m}^{-2} \text{s}^{-1}$) from the within-plot portion of the regression model for the C₄ grasses. Also shown are the significance of the main effects for the between- and within-plot portions of the regression model.

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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 CO₂ 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 CO₂ flux, the correlation between aboveground biomass in monocultures of a species and the effect of the species on soil CO₂ flux suggests species affect soil CO₂ 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 CO₂ flux.

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

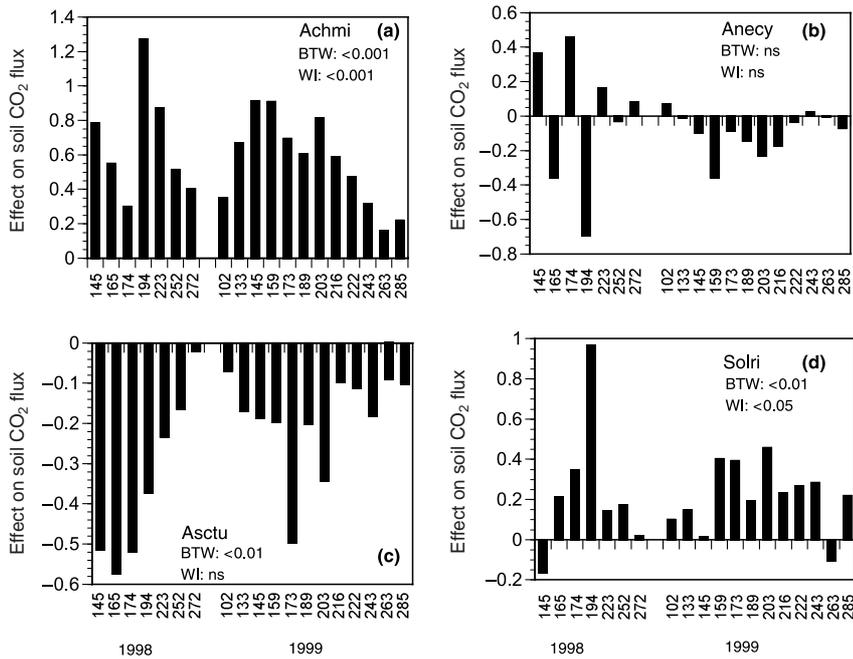


Fig. 6 Species effects on soil CO₂ flux ($\mu\text{mol m}^{-2} \text{s}^{-1}$) from the within-plot portion of the regression model for the C₃ forbs. Also shown are the significance of the main effects for the between- and within-plot portions of the regression model.

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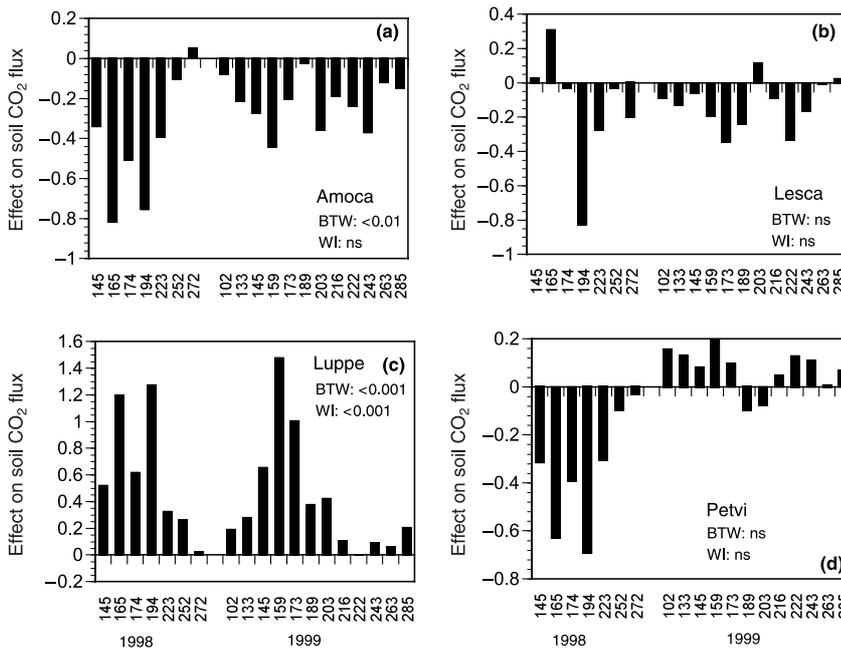


Fig. 7 Species effects on soil CO₂ flux ($\mu\text{mol m}^{-2} \text{s}^{-1}$) from the within-plot portion of the regression model for the legumes. Also shown are the significance of the main effects for the between- and within-plot portions of the regression model.

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the species that had negative effects on soil CO₂ flux (e.g. *S. scoparium* or *A. canescens*) will have a greater effect on below-ground 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 CO₂ flux, including both experimental treatments (N, CO₂, 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 CO₂ flux, soil temperature, the N effect and a set of species that show both positive and negative unimodal patterns in their effects on soil

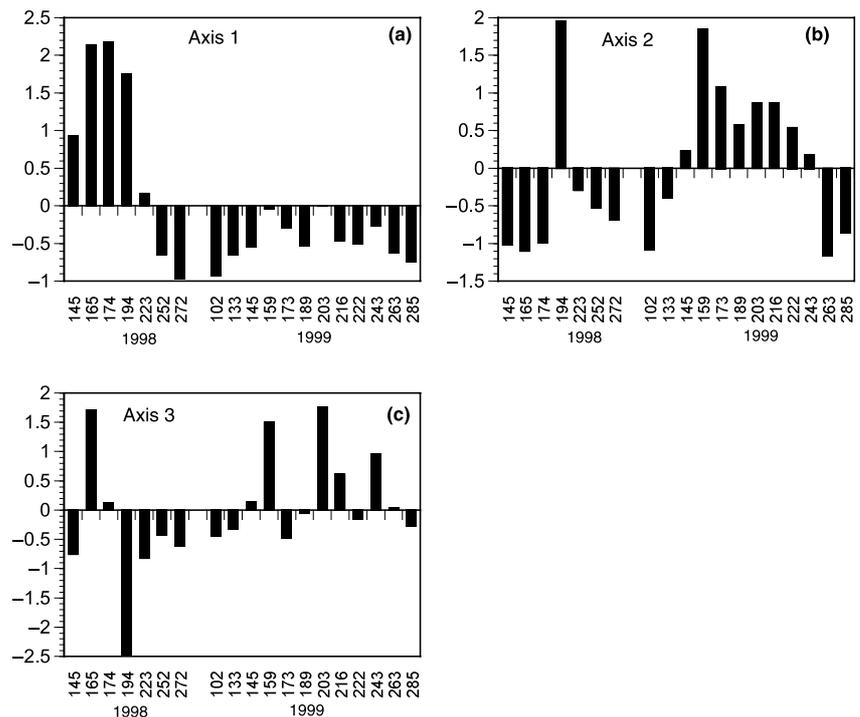


Fig. 8 Principal components analysis (PCA) axes scores averaged for all plots for each sampling period.

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Table 3 Results of the principal components analysis of average soil CO₂ flux at each sampling period, average soil temperature of bare ground plots per period, the CO₂ 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
<i>A. repens</i> (C ₃)	-0.01	-0.14	0.45
<i>B. inermis</i> (C ₃)	0.30	-0.02	0.17
<i>K. cristata</i> (C ₃)	0.15	0.22	-0.20
<i>P. pratensis</i> (C ₃)	0.21	-0.12	-0.43
<i>A. gerardii</i> (C ₄)	0.06	0.36	0.22
<i>B. gracilis</i> (C ₄)	-0.07	-0.32	-0.10
<i>S. scoparium</i> (C ₄)	-0.27	0.20	0.13
<i>S. nutans</i> (C ₄)	0.23	-0.37	0.05
<i>A. millefolium</i> (F)	0.21	0.27	-0.04
<i>A. cylindrica</i> (F)	-0.14	-0.28	-0.16
<i>A. tuberosa</i> (F)	-0.26	0.21	-0.02
<i>S. rigida</i> (F)	0.25	0.25	0.03
<i>A. canescens</i> (L)	-0.31	0.14	-0.09
<i>L. capitata</i> (L)	-0.12	-0.40	0.32
<i>L. perennis</i> (L)	0.27	0.05	0.12
<i>P. villosum</i> (L)	-0.26	0.22	0.22

Included for each species is standard functional classification: C₃ grass (C₃); C₄ grass (C₄); 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).

CO₂ 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 CO₂ flux.

The correspondence between the differential effects on soil CO₂ flux of deep and shallowly rooted species, the effect of elevated CO₂ on soil CO₂ flux, and soil moisture implies that soil CO₂ 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 CO₂ 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 CO₂ flux relationship (Craine *et al.*, 2001) due to a diverse plot being more likely to have a species that is associated with high soil CO₂ 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 (Craine *et al.*, 2001) 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 CO₂ flux, although the identity of those species might vary with time.

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