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# The impact of elevated $CO_2$, increased nitrogen availability and biodiversity on plant tissue quality and decomposition

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**Abstract**

**Elevated $CO_2$, increased nitrogen (N) deposition and increasing species richness can increase net primary productivity (NPP). However, unless there are comparable changes in decomposition, increases in productivity will most likely be unsustainable. Without comparable increases in decomposition nutrients would accumulate in dead organic matter leading to nutrient limitations that could eventually prohibit additional increases in productivity. To address this issue, we measured aboveground plant and litter quality and belowground root quality, as well as decomposition of aboveground litter for one and 2-year periods using *in situ* litterbags in response to a three-way factorial manipulation of $CO_2$ (ambient vs. 560 ppm), N deposition (ambient vs. the addition of 4 g N $m^{-2}$ $yr^{-1}$) and plant species richness (one, four, nine and 16 species) in experimental grassland plots.**

**Litter chemistry responded to the $CO_2$, N and plant diversity treatments, but decomposition was much less responsive. Elevated $CO_2$ induced decreases in % N and % lignin in plant tissues. N addition led to increases in % N and decreases in % lignin. Increasing plant diversity led to decreases in % N and % lignin and an increase in % cellulose. In contrast to the litter chemistry changes, elevated $CO_2$ had a much lower impact on decomposition and resulted in only a 2.5% decrease in carbon (C) loss. Detectable responses were not observed either to N addition or to species richness.**

**These results suggest that global change factors such as biodiversity loss, elevated $CO_2$ and N deposition lead to significant changes in tissue quality; however, the response of decomposition is modest. Thus, the observed increases in productivity at higher diversity levels and with elevated $CO_2$ and N fertilization are not matched by an increase in decomposition rates. This lack of coupled responses between production and decomposition is likely to result in an accumulation of nutrients in the litter pool which will dampen the response of NPP to these factors over time.**

*Keywords:* biodiversity, decomposition, elevated $CO_2$, FACE, litter chemistry, litter quality, nitrogen fertilization, plant chemistry, plant quality

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## Introduction

Carbon (C) and nitrogen (N) content and other aspects of the chemistry of plant tissues (including both the C : N ratio, but also the specific chemistry of C- and N-containing compounds), significantly influence both decomposition and herbivory, two processes that form the basis for positive and negative feedbacks in ecosystem processes (Meentemeyer, 1978; Melillo *et al*., 1982). Plant tissue quality (used herein to describe both the stoichiometry and specific chemistry of C- and N-containing compounds), however, can change in response to resource availability, such as N fertilization (Koricheva *et al*., 1998; Fisk & Fahey, 2001) and elevated $CO_2$ (Franck *et al*., 1997; Gahrooee, 1998; Koricheva *et al*., 1998). In addition, plant tissue quality is sensitive to plant species composition and diversity (Hector *et al*.,

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2000; Knops *et al*., 2001). Yet, few experiments have examined the interactions among $CO_2$, N and plant diversity on ecosystem functioning, despite the increasing evidence that such global change factors can interact with one another (Reich *et al*., 2001a; Henry *et al*., 2005).

The impact of resource availability on tissue stoichiometry has been well studied for N additions. Many studies have reported an increase in plant tissue N concentrations as a result of N fertilization (DiTommaso & Aarssen, 1989; Wedin & Tilman, 1996). Elevated $CO_2$, in general, decreases plant tissue N concentrations (Koricheva *et al*., 1998; Lutze *et al*., 2000; Norby *et al*., 2001; Cotrufo *et al*., 2005). Research on the impact of plant species diversity on tissue quality has been rare, but in the few related studies, diversity and N concentrations were found to be negatively correlated (Tilman *et al*., 1997; Reich *et al*., 2001a, 2006; Novotny *et al*., 2007). In contrast to tissue % N, the impact of resource availability on tissue C allocation, or more specifically the relative proportions of soluble carbohydrates, cellulose and lignin in plant tissues, has received much less attention. Elevated $CO_2$ often leads to an increase in lignin (Koricheva *et al*., 1998; Penuelas & Estiarte, 1998; Norby *et al*., 2001), with some exceptions (Cotrufo *et al*., 2005). N fertilization impacts are not consistent among studies (Ohnmeiss & Baldwin, 1994; Feller, 1996; Hatcher *et al*., 1997a, b; John & Turkington, 1997). Only a few studies, however, have examined both plant N and C content responses to changes in more than one factor, most often elevated $CO_2$ and N supply. In these studies, responses appear to be additive and not synergistic (Lutze *et al*., 2000; Booker & Maier, 2001; King *et al*., 2001; Henry *et al*., 2005).

Outside of the elevated $CO_2$ literature, litter % N has been shown to be a consistent predictor of decomposition rates in many studies (Melillo *et al*., 1982; Berg & Matzner, 1997), including at the site of the present study (Hobbie, 2005). Hence, decreased tissue N concentrations under elevated $CO_2$ have been predicted to negatively affect decomposition, but observed decomposition rates under experimentally elevated $CO_2$ vary greatly, with some studies reporting a positive effect (Franck *et al*., 1997), some a negative effect (Boerner & Rebbeck, 1995; Scherzer *et al*., 1998) and some no effect at all (Torbert *et al*., 1995; Couteaux *et al*., 1999; Lutze *et al*., 2000). A meta-analysis by Norby *et al*. (2001) found a pattern of lower tissue N in leaf litter produced under elevated $CO_2$, but did not find consistent changes in decomposition rates. Interactions among global change factors on decomposition have received much less attention. Chiefly focusing on increased N addition and elevated $CO_2$, these studies have found subtle (Henry *et al*., 2005) or no interactions (Franck *et al*., 1997; Lutze *et al*., 2000; King *et al*., 2001).

Given both the paucity and mixed nature of the results of studies that have examined global change impacts on plant tissue quality and decomposition, we conducted a long-term field manipulation of global change factors on grassland ecosystem function. Specifically, we determined tissue quality and decomposition in a direct three-way multifactorial manipulation of elevated $CO_2$ (ambient and 560 ppm), N fertilization (ambient and 4 g N $m^{-2}$ $yr^{-1}$) and species diversity (one, four, nine and 16 plant species). We examined four possible ecosystem responses to these manipulations. First, we examined if elevated $CO_2$, N fertilization and variation in species diversity led to changes in plant tissue quality both in terms of N content and C allocation. Second, we examined if there were changes in decomposition rates. Third, we examined whether any changes in decomposition rates were caused by tissue quality changes or by changes in the microclimate resulting from changes in plant community composition and structure due to N addition, elevated $CO_2$ and variation in species diversity. Fourth, we examined if the manipulation of these factors led to additive or complex changes (i.e. significant interactions among the factors) in decomposition rates.

## Methods

This study was part of a free-air $CO_2$ enrichment (Biocon) experiment at the Cedar Creek Natural History Area in east-central Minnesota, USA. Cedar Creek has a mid-continental climate with hot, humid summers and cold winters. The average date of the last spring freeze is May 9 and the average first fall freeze is September 27. Soils were formed from a sandy glacial outwash and are low in organic matter, N, clay and water-holding capacity (Grigal *et al*., 1974). The average soil N accumulation was approximately 1.2 g $m^{-2}$ $yr^{-1}$ from 1983 to 1995, with atmospheric wet N deposition contributing 0.4–0.5 g $m^{-2}$ $yr^{-1}$ (Knops & Tilman, 2000).

The Biocon study incorporates three treatments: elevated vs. ambient $CO_2$ (three 20 m diameter rings for each), elevated (4 g N $m^{-2}$ $yr^{-1}$) vs. ambient N and four biodiversity treatments planted with one, four, nine or 16 herbaceous plant species. The design consisted of a split plot arrangement of treatments in a completely randomized design with the $CO_2$ treatment as a whole-plot factor, which was replicated three times among the six rings. The subplot factors of species identity and N treatment were randomly assigned and replicated in individual plots among the six rings (Reich *et al*., 2001a, 2000). In total there were 371 plots, including 12 bare soil plots. For each of the four combinations of $CO_2$ and N, there were 32 randomly assigned replicates for the plots planted to one species, 15 for those planted to four

 

species, 15 for nine species and 12 for 15 species. In addition, 63 plots of four plant species were added to increase the replication to allow testing of functional group diversity (Reich *et al*., 2004). We used four native or naturalized plant species which commonly occur at Cedar Creek Natural History Area for each functional group. Functional groups and species were C4 grasses *Andropogon gerardii*, *Bouteloua gracilis*, *Schizachyrium scoparium*, *Sorgastrum nutans*; C3 grasses *Agropyron repens*, *Bromus inermis*, *Koeleria cristata*, *Poa pratensis*; forbs *Achillea millefolium*, *Anemone cylindrica*, *Asclepias tuberosa*, *Solidago rigida*; and legumes *Amorpha canescens*, *Lespedeza capitata*, *Lupinus perennis*, *Petalostemum villosum*. $CO_2$ was applied to maintain an atmospheric concentration of 560 ppm during daylight hours in the growing season (April–October), while N fertilizer was applied three times during the growing season. A total of 371 plots, each 4 $m^2$, were planted in 1997, and the elevated $CO_2$ and N treatments began in April 1998.

### *Tissue and litter collection*

Aboveground leaf samples were collected in mid-August 1999 by clipping a 10 cm × 100 cm strip at the soil surface, and sorted into live plant by species and senesced litter (Reich *et al*., 2001a, 2000). Aboveground tissues were combined by plot and a composite sample for each plot was analyzed for tissue quality. Roots were collected at 0−20 cm depth using three 5 cm wide cores in the same area used for aboveground biomass clipping. Aboveground fully abscised leaf samples were collected from all plots ($N = 359$ plots), except the bare soil plots in August–October of 1999, depending on when each plant species shed its leaves.

### *Litterbags*

We used a common substrate of *B. inermis* litter in all plots, including the zero-diversity plots, to assess the impact on decomposition of microclimate differences caused either directly by the treatments, or indirectly by changes in plant composition and biomass, in each plot.

We combined litter from the selected plant species in accordance with their relative abundance for both the litter quality analysis and for the material subsequently placed in the litterbags. We used the August aboveground biomass to determine the relative abundance of each plant species within each plot. It was not feasible to use all plant species because we placed approximately 1 g of plant material in each bag, and we found that the three most abundant plant species comprised approximately 90–100% of the August clipped plant biomass. We constructed three identical sets for each plot, based on the relative abundance of the three most abundant plant species for each individual plot. One set was used for the initial litter quality, one set was incubated *in situ* for 1 year, and one set was incubated *in situ* for 2 years. In total there were 366 plots, with seven zero-species plots, resulting in 359 plots with litter; however, one bag of the 2001 harvest was damaged by small rodents and excluded from the analysis. These sets of *in situ* litter allowed us to examine the treatment effects on decomposition within each plot.

All litterbags were 5 cm × 10 cm polyester cloth bags (50 μm pore size, Ankom Tech., Fairport, NY, USA) and were filled with approximately 1 g of 2–4-cm-long plant material. Litterbags were placed and staked on the soil surface in the center of each plot starting in November 1999 and collected after 1 year in October 2000, and after 2 years in October 2001.

Because of mineral soil contamination of litterbag samples, we ash corrected all weights. Soils contain on average 0.85% C (Harmon & Lajtha, 1999).

### *Plant quality analysis*

All samples were dried to constant dry weight at 55 °C and ground through a 20 mesh with a Wiley mill. C and N were analyzed with a Costech ECS 4010 element analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA). Biomass fractions were determined by sequential extraction following standard forage chemistry methods (Van Soest, 1982). The first extraction (neutral detergent) removes cell contents including soluble carbohydrates, lipids, pectin, starch, soluble protein and nonprotein N. The second fraction (acid detergent) removes hemicellulose and protein bound to cell walls. The third digestion (72% $H_2SO_4$) hydrolyzes and removes cellulose, leaving lignin and related recalcitrant materials as the fourth and final fraction. Although we refer to these fractions by their predominant biochemical components (i.e. soluble carbohydrates, cellulose, hemicellulose and lignin), these fractions are somewhat heterogeneous and not well defined from a strictly biochemical point of view (Van Soest, 1982). Extractions were performed on 0.5 g plant samples placed in acid-resistant heat sealed pouches, which were sequentially extracted in an Ankom fiber analyzer (Ankom Tech.), with samples dried and reweighed after each step.

### *Statistics*

All statistics were performed with SPSS 14 for Windows. The $CO_2$ treatment is nested within ring (1 df), and was tested against the random effect of ring nested within $CO_2$ (4 df). The main effect of species (15 df), N (1 df) and interactions between $CO_2$, N and species diversity

were tested against the residual error (Reich *et al*., 2001b). Because the $CO_2$ treatment was tested against the random effect of ring nested within $CO_2$, it has a much lower power to detect significant differences. Therefore, we used a $P$ value of 0.1 as the critical level to detect significant differences for the $CO_2$ treatment and a $P$ value of 0.05 to detect significant differences in all other treatments and interactions.

The soluble, hemicellulose, cellulose and lignin fractions add up to 100% and a decrease in fraction corresponds with an increase in the other fractions. Consequently, tissue quality measurements are all highly correlated. We used a type III general linear model (GLM) multivariate analysis of variance to examine the overall impact of species diversity, $CO_2$ and N treatment on tissue quality. Subsequently, we used a type III univariate GLM to determine which quality factor caused the overall significance. We also used the GLM procedure to estimate the marginal means for each factor (i.e. species, $CO_2$ and N levels).

We only present C mass loss throughout the paper because mass loss showed the same identical patterns as C loss. Initial % C and % N of the common substrate, *B. inermis*, was determined from 10 replicate samples. The initial % C and % N of each litter sample was determined on a composite sample for each individual plot (see 'Litterbags'). Percent C loss was calculated as (((initial % C × initial litter sample weight)−(final % C × final litter sample weight))/(initial % C × initial litter sample weight)) × 100. Percent N loss was calculated similarly.

Lastly, we used plant tissue N concentration throughout the manuscript for N quality. We also calculated C : N ratio of plant and litter tissues, but the C : N ratio showed the exact same significance of % N, except its correlation with other factors, or $P$ value, was slightly lower.

## Results

### *Tissue quality*

All three treatments (i.e. $CO_2$, N and species diversity) significantly affected aboveground biomass, belowground biomass and litter quality. However, there was no interaction among any of the treatments (Table 1). Some tissue quality metrics were highly correlated: % N was negatively correlated with % hemicellulose and cellulose, and positively correlated with % soluble (except in the root tissues) and % lignin (Table 2).

Elevated $CO_2$ decreased % N in aboveground biomass and litter (Table 3, Fig. 1a). Elevated $CO_2$ also decreased the % lignin in the roots and litter (Table 3, Fig. 1a), increased the % soluble (control 30.9%, SE 0.9%, elevated $CO_2$ 33.9%, SE 0.9%) and decreased the % hemicellulose (control 28.2%, SE 0.6%, elevated $CO_2$ 26.9%, SE 0.6%) in the belowground biomass (Table 3). The N treatment increased the % N in all three tissue types and decreased the % lignin in the aboveground biomass (Table 3, Fig. 1b). Increasing species diversity treatment decreased the % N and lignin in all three tissues, and increased the % cellulose in both the aboveground biomass and the litter (Table 3, Fig. 1c).

### *Decomposition*

We found a significant effect of diversity on *B. inermis*, the common substrate litter, when all plots were

**Table 1** Tissue quality: multivariate analysis of aboveground biomass, root and litter with as quality aspects % N, % soluble, % hemicellulose, % cellulose and % lignin and as independent factors $CO_2$ treatment, N treatment and plant diversity

| | Biomass | | Roots | | Litter | |
|---|---|---|---|---|---|---|
| Treatment (df) | *F* | *P* | *F* | *P* | *F* | *P* |
| $CO_2$ (1, 4) | 1.6 | 0.040 | 1.6 | 0.058 | 2.1 | 0.004 |
| N (1, 338) | 4.8 | 0.000 | 4.7 | 0.001 | 3.1 | 0.010 |
| Species diversity (3, 338) | 9.4 | 0.000 | 3.1 | 0.000 | 10.0 | 0.000 |
| $CO_2$ × N (1, 338) | 0.7 | 0.621 | 0.5 | 0.701 | 0.3 | 0.922 |
| $CO_2$ × species (3, 338) | 0.6 | 0.850 | 0.6 | 0.835 | 0.4 | 0.978 |
| N × species (3, 338) | 0.9 | 0.531 | 0.8 | 0.610 | 0.8 | 0.641 |
| $CO_2$ × N × species (3, 338) | 0.6 | 0.901 | 0.6 | 0.879 | 0.4 | 0.989 |

The $CO_2$ is nested within ring. Given are the $F$ value of the Pillai's trace and the $P$ value. (Pillai's trace and Roy's largest root gave the same results, except for the root $CO_2$ where the Roy's largest root gave an $F$ value of 4.4 and a $P$ value of 0.002.) Pillai's trace is more robust to violations of assumptions, whereas Roy's largest root has the greatest power (Scheiner, 2001). All the litter data, the % N, the soluble and lignin for aboveground biomass and the % N for roots are Ln transformed to improve normality.
N, nitrogen.

 

**Table 2** Correlations among tissue quality measures in aboveground biomass (A), belowground biomass (B) and (C) litter

| | % C | % N | % soluble | % hemicellulose | % cellulose |
|---|---|---|---|---|---|
| *(A) Aboveground biomass* | | | | | |
| % N | 0.069$^{ns}$ | | | | |
| % soluble | −0.258*** | 0.277*** | | | |
| % hemicellulose | 0.074$^{ns}$ | −0.333*** | −0.698*** | | |
| % cellulose | 0.343*** | −0.336*** | −0.800*** | 0.379*** | |
| % lignin | 0.181** | 0.413*** | 0.268*** | −0.770*** | −0.116* |
| *(B) Belowground biomass* | | | | | |
| % N | 0.209*** | | | | |
| % soluble | −0.425*** | 0.064$^{ns}$ | | | |
| % hemicellulose | 0.356*** | −0.129* | −0.914*** | | |
| % cellulose | 0.325*** | −0.034$^{ns}$ | −0.807*** | 0.596*** | |
| % lignin | 0.393*** | 0.176** | −0.514*** | 0.288*** | 0.315*** |
| *(C) Litter* | | | | | |
| % N | 0.061$^{ns}$ | | | | |
| % soluble | −0.327*** | 0.519*** | | | |
| % hemicellulose | 0.211*** | −0.505*** | −0.880*** | | |
| % cellulose | 0.239*** | −0.656*** | −0.854*** | 0.708*** | |
| % lignin | 0.100$^{ns}$ | 0.558*** | 0.496*** | −0.724*** | −0.497*** |

Sample size is 359 for each analysis.
$^{ns}P > 0.05$, $*P < 0.05$, $**P < 0.01$, $***P < 0.0001$.
The C : N ratio, which would show the opposite of the % N, is not shown.
C, carbon; N, nitrogen.

**Table 3** Tissue quality: ANOVAs of aboveground biomass (A), belowground biomass (B) and litter (C) with as quality aspects % N, % soluble, % hemicellulose, % cellulose and % lignin and as independent factors $CO_2$ treatment, N treatment and plant diversity

| | % N | | % Soluble | | % hemicellulose | | % cellulose | | % lignin | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment (df) | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| *(A) Aboveground biomass* | | | | | | | | | | |
| $CO_2$ (1, 4) | 13.2 | 0.022 | 0.1 | 0.721 | 0.9 | 0.396 | 0.2 | 0.647 | 0.2 | 0.678 |
| N (1, 347) | 4.2 | 0.041 | 0.2 | 0.682 | 2.0 | 0.161 | 0.2 | 0.661 | 7.3 | 0.007 |
| Species diversity (4, 347) | 6.9 | 0.000 | 0.8 | 0.513 | 0.8 | 0.511 | 11.0 | 0.000 | 4.0 | 0.008 |
| *(B) Belowground biomass* | | | | | | | | | | |
| $CO_2$ (1, 4) | 3.0 | 0.160 | 7.3 | 0.054 | 6.2 | 0.068 | 1.0 | 0.384 | 8.6 | 0.042 |
| N (1, 347) | 7.5 | 0.007 | 0.5 | 0.480 | 0.2 | 0.669 | 2.9 | 0.090 | 2.1 | 0.152 |
| Species diversity (4, 347) | 3.0 | 0.029 | 1.5 | 0.210 | 0.7 | 0.392 | 0.6 | 0.640 | 9.9 | 0.000 |
| *(C) Litter* | | | | | | | | | | |
| $CO_2$ (1, 4) | 5.0 | 0.089 | 0.0 | 0.873 | 0.0 | 0.889 | 1.8 | 0.256 | 5.3 | 0.082 |
| N (1, 347) | 4.6 | 0.033 | 0.0 | 0.931 | 0.7 | 0.387 | 0.2 | 0.679 | 1.1 | 0.293 |
| Species diversity (4, 347) | 3.3 | 0.020 | 0.7 | 0.574 | 2.1 | 0.105 | 6.6 | 0.000 | 10.0 | 0.000 |

Given are the *F* and *P* values from a GLM with as factors $CO_2$ nested within ring, N and plant species diversity. Percent N was Ln transformed to improve normality.
N, nitrogen; GLM, general linear model.

**Fig. 1** Tissues quality responses to manipulated global change factors. (a) Responses to elevated $CO_2$. (b) Responses to increased nitrogen (N) deposition. (c) Responses to manipulated levels of plant species richness. Note that there were no significant interactions among treatments (Table 1). Shown are the means adjusted for the other treatments ± standard error for each treatment effects on % N and lignin for the $CO_2$ and N treatments and % N, cellulose and lignin for the diversity treatment. Different letters denote $P < 0.05$, Bonferroni corrected for multiple comparisons (see Table 3).

 

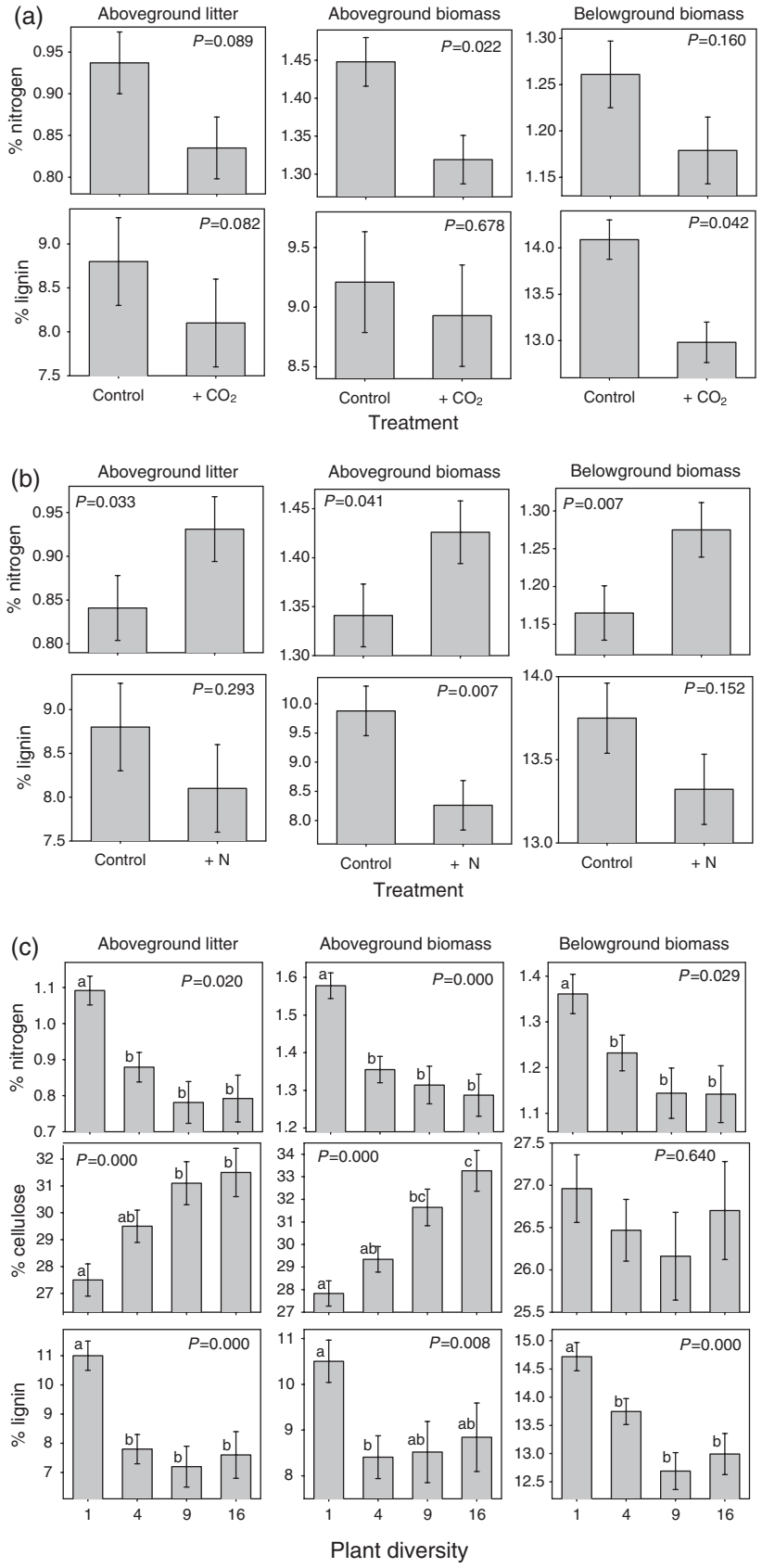

**Table 4** Decomposition: ANOVAs of carbon (C) and nitrogen (N) loss over a 1- and a 2-year period, with as independent factors $CO_2$ treatment, N treatment and plant diversity of (A) a common substrate (*Bromus inermis*) litter, (B) *Bromus* litter, with the bare ground plots excluded and (C) the *in situ* plot representative litter

| | C loss | | | | N loss | | | |
|---|---|---|---|---|---|---|---|---|
| | 2000 | | 2001 | | 2000 | | 2001 | |
| Treatment (df) | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| *(A) Bromus litter* | | | | | | | | |
| $CO_2$ (1, 4) | 0.0 | 0.854 | 2.2 | 0.216 | 0.2 | 0.647 | 0.3 | 0.598 |
| N (1, 347) | 2.2 | 0.140 | 0.8 | 0.367 | 1.7 | 0.193 | 2.0 | 0.157 |
| Species diversity (4, 347) | 4.2 | 0.002 | 7.8 | 0.000 | 3.8 | 0.005 | 3.5 | 0.008 |
| $CO_2 \times$ N (1, 347) | 0.0 | 0.837 | 0.1 | 0.761 | 0.2 | 0.699 | 0.5 | 0.467 |
| $CO_2 \times$ species (4, 347) | 0.5 | 0.721 | 0.7 | 0.582 | 0.2 | 0.944 | 2.1 | 0.079 |
| N × Species (4, 347) | 1.2 | 0.314 | 0.9 | 0.481 | 0.3 | 0.863 | 0.5 | 0.724 |
| $CO_2 \times$ N × species (4, 347) | 0.6 | 0.649 | 2.6 | 0.036 | 0.4 | 0.795 | 0.9 | 0.476 |
| *(B) Bromus litter, no bare ground plots* | | | | | | | | |
| $CO_2$ (1, 4) | 0.0 | 0.963 | 1.9 | 0.241 | 0.6 | 0.496 | 0.1 | 0.723 |
| N (1, 339) | 0.0 | 0.927 | 1.8 | 0.184 | 2.1 | 0.145 | 1.6 | 0.205 |
| Species diversity (3, 339) | 0.3 | 0.845 | 1.4 | 0.229 | 1.5 | 0.217 | 2.4 | 0.068 |
| $CO_2 \times$ N (1, 339) | 1.1 | 0.303 | 3.6 | 0.059 | 0.2 | 0.667 | 4.3 | 0.040 |
| $CO_2 \times$ species (3, 339) | 0.6 | 0.586 | 0.6 | 0.585 | 0.2 | 0.871 | 2.1 | 0.096 |
| N × species (3, 339) | 0.5 | 0.716 | 1.2 | 0.326 | 0.4 | 0.731 | 0.6 | 0.606 |
| $CO_2 \times$ N × species (3, 339) | 0.7 | 0.572 | 2.9 | 0.035 | 0.6 | 0.624 | 0.8 | 0.497 |
| *(C) In situ, plot representative litter* | | | | | | | | |
| $CO_2$ (1, 4) | 3.6 | 0.129 | 7.2 | 0.055 | 0.0 | 0.839 | 0.2 | 0.652 |
| N (1, 339) | 2.7 | 0.104 | 0.6 | 0.452 | 0.0 | 0.949 | 0.4 | 0.538 |
| Species diversity (3, 339) | 13.9 | 0.000 | 11.4 | 0.000 | 0.5 | 0.661 | 1.0 | 0.387 |
| $CO_2 \times$ N (1, 339) | 0.0 | 0.957 | 0.1 | 0.793 | 0.4 | 0.507 | 0.0 | 0.829 |
| $CO_2 \times$ species (3, 339) | 2.3 | 0.081 | 0.7 | 0.535 | 0.4 | 0.744 | 1.1 | 0.332 |
| N × species (3, 339) | 0.2 | 0.878 | 0.0 | 0.987 | 0.2 | 0.925 | 0.1 | 0.978 |
| $CO_2 \times$ N × species (3, 339) | 0.3 | 0.830 | 0.2 | 0.895 | 0.3 | 0.835 | 0.1 | 0.951 |

Given are the *F* values from a GLM with as factors $CO_2$ nested within ring, N and plant species diversity. All data Ln transformed. GLM, general linear model.

included (Table 4a, Fig. 2), but no diversity effect when the zero-diversity plots were excluded from the analysis (Table 4b). Excluding the zero-diversity plots, in year 1 we found that there were no significant main effects of $CO_2$ or N on decomposition, nor a significant interaction (Table 4b). The N loss in the second year showed a significant $CO_2 \times$ N interaction (Table 4b) caused by a 3% lower N loss from the elevated $CO_2$ and N fertilized treatment; but this was not matched by a similar difference in C release (Fig. 3). Within the monocultures, there were also no significant differences among the functional groups (all $P > 0.2$).

Litter, representative of the plot and decomposed *in situ*, showed no significant change in N loss. The 1-year litter immobilized on average 20.6% of its original N amount and the 2-year litter immobilized on average 8.9% of its original N content. C did show an absolute 2.5% lower C loss rate under elevated $CO_2$ in both years (Fig. 4), but this was only significant over the 2 years (Table 4c). However, this decomposition change was relatively small compared with the overall C loss [i.e. 33.0% in the control and 31.1% in the elevated $CO_2$ treatment in the first year, and 54.8% in the control and 52.3% in the elevated $CO_2$ treatment over 2 years (Fig. 4)]. There was a significant increase in C loss rate with increasing species richness (Table 4c), which was driven by the monoculture plots, which on average lost about 5% less C as compared with all other diversity levels (Fig. 5).

## Discussion

### *Tissue quality*

We found significant and similar correlations between the components of tissue quality for above- and below-

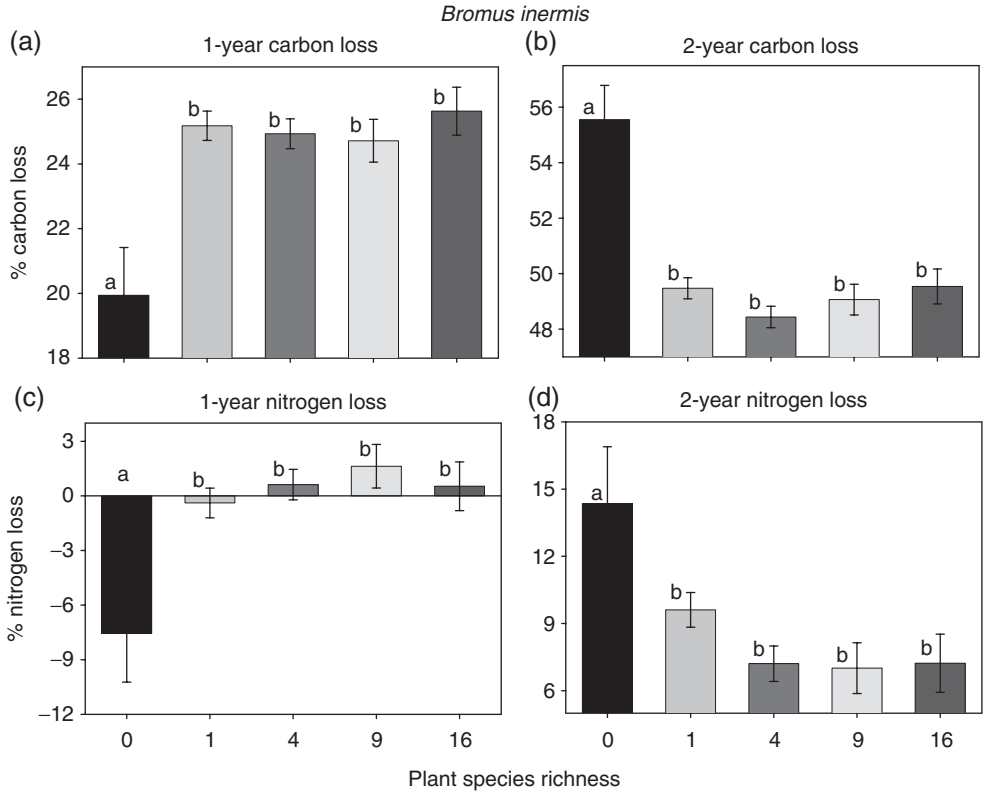


**Fig. 2** Percent carbon (C) loss (a, b) and % nitrogen (N) loss (c, d) of a common substrate litter (*Bromus inermis*) over 1- and 2-year periods vs. plant species richness. Shown are the means for each species richness treatment, adjusted for the $CO_2$ and N treatments ± standard error. Different letters denote $P > 0.05$ (see Table 4a).

ground biomass and litter, consistent with earlier studies (Norby *et al*., 2000; Hoorens *et al*., 2003). This consistency indicates that resource induced changes in plant tissue quality are also reflected in changes in litter quality, and that changes in green and senesced tissue chemistry changes correspondingly.

Within this study, we found no significant interactions among elevated $CO_2$, N fertilization and changes in plant species diversity on plant tissue quality. Thus, the impact of elevated $CO_2$, N fertilization and biodiversity on tissue quality are independent of one another within this study. Tissue N concentrations increased with N fertilization and decreased with elevated $CO_2$, but the latter was only significant for the belowground biomass. The observed shifts in tissue C chemistry due to elevated $CO_2$ and N fertilization were also consistent with prior studies (Ohnmeiss & Baldwin, 1994; Feller, 1996; Hatcher *et al*., 1997a, b; John & Turkington, 1997; Penuelas & Estiarte, 1998; Norby *et al*., 2001).

The species richness treatments had the strongest influence on the litter quality ($F$ value of 10.0 vs. 2.1 for $CO_2$ and 3.1 for N), and the litter quality showed a significant decrease in % N and lignin and an increase in cellulose with increasing plant diversity.

### *Decomposition*

Elevated $CO_2$, N fertilization and different levels of diversity can impact decomposition in two ways. First, resource changes can lead to changes in the vegetation structure and the standing biomass (Reich *et al*., 2001a), which in turn change the microclimate and communities of soil microbial decomposers communities (Epstein *et al*., 2002; Waldrop & Firestone, 2004). Second, resource changes can lead to changes in tissue quality.

Our common substrate showed no effect on its decomposition rate from either N fertilization, elevated $CO_2$ or any interactions among the treatments. The lack of a N fertilization effect is similar to other studies with a similar range of N additions (Knorr *et al*., 2005). The lack of a $CO_2$ response for decomposition of the common substrate contrasts with the $CO_2$ response of productivity which resulted in changes in aboveground biomass (Reich *et al*., 2001a). Decomposition rates were lower in bare soil plots and elevated in the second year, but we found no significant differences at any diversity level at which plants were present. This lack of a diversity effect is consistent with results from a common litter within a similar biodiversity experiment at

the same site of plots that differed twofold in biomass (Knops *et al*., 2001). Thus, there is no indication that changes in the vegetation structure and the standing biomass (Reich *et al*., 2001a) have any impact on decomposition.

The litter produced within a plot, which did show changes in N concentration and C fractions, also showed changes in decomposition, but these changes were relatively minor and smaller than litter and plant biomass changes (Reich *et al*., 2001a). Corresponding with the plant and litter tissue quality, we found no interactions among our treatments on decomposition. The C loss was 2.5% lower under elevated $CO_2$, both after 1 and 2 years, and increased 5% from the monoculture to the other diversity levels. Surprisingly, we found no significant effect of the N treatment, even though the N treatment led to higher tissue N concentrations. Thus, even though there are consistent differences in litter quality, litter quality only has a minor influence on C loss rates and no impact on N loss rates. Likewise, other studies have found that elevated $CO_2$ (Norby *et al*., 2001; Hoorens *et al*., 2003) and N fertilization (Knorr *et al*., 2005) can lead to plant tissue changes, but often do not lead to changes in decomposition.

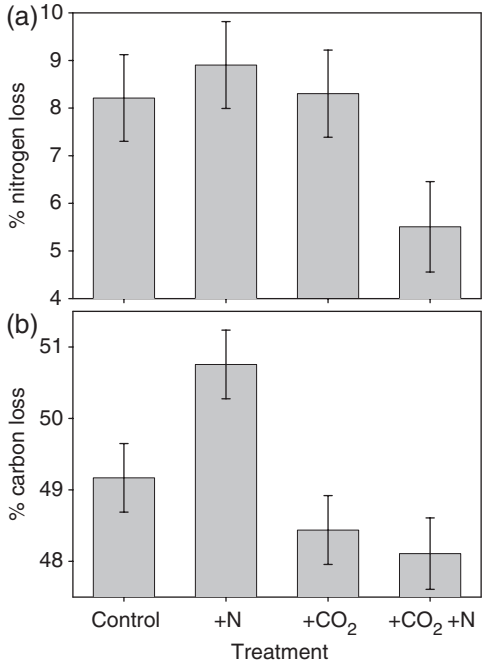


**Fig. 3** Percent nitrogen (N) loss (a) and % carbon (C) loss (b) of the common substrate litter (*Bromus inermis*) over a 2-year period. Shown are the means for each $CO_2 \times N$ treatment adjusted for the species richness treatments ±standard error (see Table 4b).

### *Ecosystem feedbacks through tissue quality changes and decomposition*

This study supports the view (Finzi & Schlesinger, 2002; Weatherly *et al*., 2003; Allard *et al*., 2004; Henry *et al*., 2005) that species compositional changes are much more important in determining feedbacks through N cycling, as compared with any direct or indirect change of species tissue quality change caused by either $CO_2$ or N fertilization. We found only minor changes of litter decomposition and no interactions between N fertilization and elevated $CO_2$. These and similar results (Cotrufo *et al*., 1994; Gahrooee, 1998; Norby *et al*., 2001; Finzi & Schlesinger, 2002; Hoorens *et al*., 2003; de Graaff *et al*., 2004) are increasing indications that there are none or only minor qualitative changes in litter decomposition due to elevated $CO_2$ (Zak *et al*., 2003; de Graaff *et al*., 2006). Therefore, feedbacks of tissue quality due to elevated $CO_2$ on N cycling seem unlikely. This study also shows that N fertilization under elevated $CO_2$ does not necessarily lead to increased decomposition rates, as we found no interactions for tissue quality

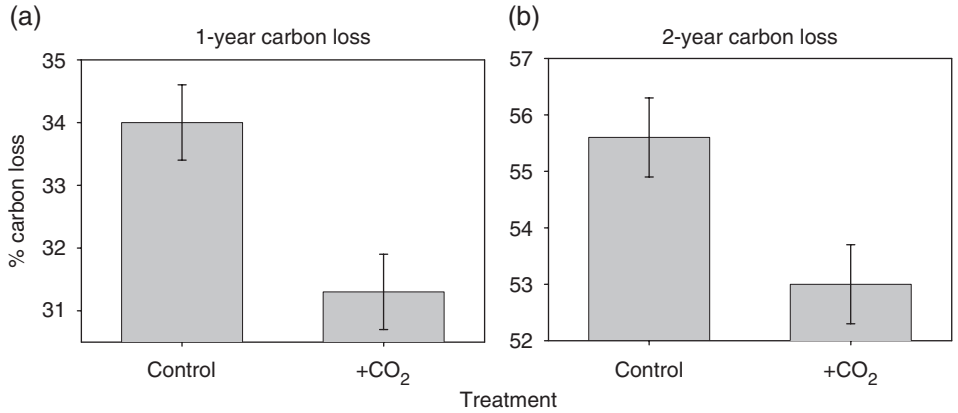


**Fig. 4** *In situ* % carbon (C) loss of a plot representative aboveground litter sample over a 1- (a) and 2-year (b) period. Shown are the means for each $CO_2$ treatment, adjusted for the nitrogen (N) and species richness treatments ± standard error (see Table 4c).

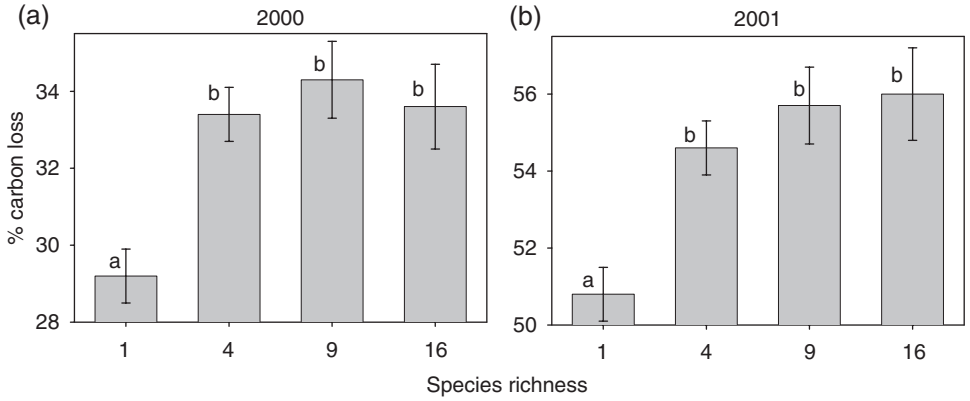


**Fig. 5** *In situ* % carbon (C) loss of a plot representative aboveground litter sample over a 1- (a) and 2-year (b) period. Shown are the means for the each species richness treatment, adjusted for the $CO_2$ and nitrogen (N) treatments ± standard error. Different letters denote $P > 0.05$ (see Table 4c).

or for decomposition, and N fertilization did not result in increased decomposition rates.

Productivity and biomass accumulation within this experiment, however, did increase in response to $CO_2$ and N as has been shown in the majority of elevated $CO_2$ studies (Luo *et al*., 2006) and showed a larger response in species rich plots (Reich *et al*., 2001a). Thus, there is an increase in litter inputs both with single global change factor responses and with a significant additive interaction among these factors. Quantitatively, this can lead to a negative feedback because the increased litter pool immobilizes 21% of its initial N content over 1 year and 9% over 2 years of decomposition, as this study shows. This may, over time with annual additions of different litter amounts, lead to a decrease in N availability and a negative feedback on productivity if a global change factor leads to increased productivity. Such a feedback has the potential to diminish any direct impact of global change on ecosystem productivity. Recent productivity data from this experiment shows that the initial increased productivity with elevated $CO_2$ has decreased (Reich *et al*., 2006).

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