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Xiaoan Zuo
Chinese Academy of Sciences, zuoxa@lzb.ac.cn

Johannes Knops
University of Nebraska - Lincoln, jknops@unl.edu

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Effects of elevated CO_2, increased nitrogen deposition, and plant diversity on aboveground litter and root decomposition

Xiaoan Zuo[1,2] and Johannes M. H. Knops[2,]†

[1]Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou 730000 China
[2]School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, Nebraska 62588 USA

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Abstract. Global change-induced litter decomposition strongly affects the carbon (C) and nitrogen (N) dynamics in grassland ecosystems. However, few studies show the interactive effects of global change factors on litter and root decomposition. We conducted a four-year grassland field experiment to examine the quality and decomposition of litter and root in a three-factorial experiment with elevated CO_2, increased N deposition, and plant species richness. We found that elevated CO_2 decreased the litter N content and root lignin content. N addition increased the root N content and decreased the litter lignin content. Increasing plant richness decreased the N and lignin contents in litter and root. In contrast to the quality changes, elevated CO_2 had no effect on decomposition of litter and root. N addition increased the C loss of the litter by 4.8%, but did not affect C and N loss in root. Increasing plant richness affected the C and N loss in litter and root. ANCOVAs showed that tissue quality and root biomass affected the C and N loss in litter and root, and soil C and N affected the N loss of litter and root. However, changes in tissue quality, biomass, and soil as covariates did not significantly change the effects of CO_2, N, and plant richness on decomposition. The structural equation model showed that elevated CO_2 indirectly decreased litter N loss and increased root N loss, while N addition indirectly increased the C and N loss in litter and root, via their effects on tissue quality. Increasing plant richness increased litter C and N loss, but indirectly decreased root C and N loss. N deposition can accelerate litter and root decomposition, thus modifying the limitation of elevated CO_2 on soil N availability. Biodiversity loss greatly alters litter and root decomposition, potentially driving any changes in C and N cycling. Our study clearly demonstrates a relative certainty of a predicted increase in the C loss and N release in litter and root decomposition with increased N deposition, whereas the effects of elevated CO_2 and plant diversity changes on decomposition strongly differ between litter and root in grassland ecosystems.

Key words: biodiversity; decomposition rate; direct or indirect effect; global change; nitrogen addition; tissue quality.

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† **E-mail:** jknops2@unl.edu

Introduction

Litter decomposition may accelerate or decelerate global climate changes, because changes in litter quantity and quality can change both the cycling rates and the pools of carbon (C) and nutrients within an ecosystem (Dijkstra et al. 2004, Sierra et al. 2011, van Groenigen et al. 2014). Litter decomposition in terrestrial ecosystems is mainly driven by the litter chemical components and climatic factors (Coûteaux et al. 1995, Garcia-Palacios et al. 2013*a*, 2017). Global climate changes can strongly affect litter decomposition due to the changes in physical decomposition environment induced by temperature or precipitation and the indirect roles through their effects on productivity and litter quality (Zhang et al. 2008, Boyero et al. 2011). Global changes, such as elevated atmospheric CO_2, increased nitrogen (N) deposition, and decreased plant species richness, occur simultaneously and have different effects on litter decomposition, thus contributing to the uncertainty about these effects and their interactions (Chung et al. 2007, Vivanco and Austin 2011, Mueller et al. 2013). Thus, there is an increasing need for studies to examine whether these global change factors affect litter decomposition and whether litter quality changes drive the C and N release in litter decomposition.

Many studies have shown that elevated CO_2 stimulates net primary productivity (NPP) and increases litter mass (Reich et al. 2001*a*, Cotrufo et al. 2005). Such increase in litter mass can affect C and N release from litter decomposition (Kemp et al. 1994). There is abundant evidence that elevated CO_2 increases the C/N ratios and lignin concentrations and thereby decreases litter quality (Cotrufo et al. 1994, 1998*a*, De Angelis et al. 2000), which in turn slows litter decomposition (De Angelis et al. 2000, Weatherly et al. 2003). Increased NPP induced by elevated CO_2 coupled with a lower litter decomposition rate may increase C-storage in the litter and soil pools (van Groenigen et al. 2014). However, this decrease in litter decomposition can also result in a negative feedback because the decrease in nutrient mineralization leads to the increased N limitation of NPP (Cotrufo and Ineson 1996). In addition, global changes can also induce the microclimate and soil biota changes (Adair et al. 2011, Garcia-Palacios et al. 2013*b*). Changes in NPP and litter cover caused by elevated CO_2 may alter soil moisture and temperature, which can alter the microbial activity and thereby decomposition rates (Hall et al. 2006). Thus, to develop an understanding of why ecosystems differ in their responses to elevated CO_2, there is an increased need to examine how the litter decomposition and nutrient release were determined by the changes in litter quality, quantity, microclimate, and soil biota caused by elevated CO_2.

Increased N deposition has also the major effect on litter decomposition through changes in tissue quality, root:shoot ratio, and soil microbe (Chung et al. 2007, Li et al. 2017*a*). Nitrogen addition can increase litter N concentration, thus accelerating litter decomposition by improving substrate quality (Liu et al. 2010, Yang et al. 2011). Nitrogen addition often leads to increased NPP and standing plant biomass, with a higher shoot: root ratio, which is likely to lead to changes in microclimates that can influence microbial functioning and thereby litter decomposition (Seith et al. 1996). Plant species differences may also be key in determining N addition's effects within ecosystems. For instance, C_4 plant litter has lower N concentrations compared to C_3 plant litter, which leads to the differences in N immobilization or mineralization (Koukoura 1998) and thereby responds differently to N addition. Soil N availability from N addition also causes changes in not only the decomposition environment (i.e., soil nutrient level and decomposer microbes) and the quality of decomposing litter (Liu et al. 2010), but also the response of NPP to a rising CO_2 effect (Reich and Hobbie 2013). Thus, similar to elevated CO_2, there is an increased need for studies to examine N-induced changes in litter quantity, quality, microclimate, and soil biota that determine changes in decomposition and thereby ecosystem changes.

No consistent pattern of plant species richness effect on litter decomposition has been found. Increasing plant richness can increase litter decomposition rates because of increased microbial community activity and microclimate change caused by increased NPP (Zak et al. 2003, Siegenthaler et al. 2010). However, increasing plant richness interacted with elevated CO_2 can show a negative effect on root decomposition rates (de Graaff et al. 2011). Increasing plant richness decreased the N and lignin contents and increased the cellulose content in litter (Knops et al. 2007), thereby affecting the mass loss and nutrient release of the litter. In addition, plant species richness also can be a significant determinant of microbial communities occurring in soils responding to elevated CO_2 and N deposition because the higher NPP resulting from the higher plant species richness can potentially alter the availability of organic C and N substances in litter which structures soil microbial communities (Chung et al. 2007). Thus, to better understand why plant richness shows such different impacts on the decomposition rates, we need to examine the mechanisms by which changes in plant richness affect litter decomposition rates.

Most studies have shown the inconsistent results between aboveground litter and root decomposition, including the faster (Moretto et al. 2001), slower (Vivanco and Austin 2006), and no differences (Cusack et al. 2009). Nitrogen release in the decomposition between litter and root is quite different. The initial tissue N concentration primarily drives N release in litter decomposition. In contrast, the N release of root increased linearly with decomposition (Parton et al. 2007). Rapid decomposition of fine root and leaf litter can be associated with the high hemicellulose and low lignin concentrations, respectively (Hobbie et al. 2010). Elevated CO_2 can also affect the soil microbial activity through NPP changes (Chung et al. 2007), which further constrains the root decomposition processes (Silver and Miya 2001) due to the greater relative importance of microbial decomposers within the soil. However, because the roots are hidden belowground, it is more difficult to accurately estimate root decomposition rates (Birouste et al. 2012). In total, because the majority of the plant biomass is belowground in grassland ecosystems (Lamb 2008), developing a better understanding of the root decomposition under global change scenarios is of importance for understanding the biogeochemical cycling changes induced by global changes (Chen et al. 2017*a*).

Because of the lack of studies examining interactions among global change factors on tissue quality and decomposition, within a long-term three-factorial grassland field experiment of biodiversity, CO_2, and N in Minnesota (Reich et al. 2001*b*), we conducted a four-year field experiment to examine how the decomposition of aboveground litter and root responded to elevated CO_2, increased N deposition, and changes in plant richness. In this experiment, elevated CO_2, increased N deposition, and increasing plant richness affect productivity (Reich et al. 2001*a*), litter quality (Knops et al. 2007), soil microbe (Chung et al. 2007), and soil C and N dynamics (Dijkstra et al. 2005, Adair et al. 2009, Mueller et al. 2013). Therefore, we addressed the following questions. First, we determined whether elevated CO_2, N addition, and plant richness led to changes in plant tissue quality. Second, we examined whether above- and belowground plant tissue quality shows the same response to the experimental CO_2, N, and plant richness treatments. Third, we determined the degree to which the experimental treatments acted individually or interactively to alter decomposition. Fourth, we examined whether the decomposition of litter and root was caused by changes in tissue quality, productivity, or environment induced by global change factors. Specifically, we hypothesized that elevated CO_2, increased N addition, and plant richness may individually affect the litter and root quality. We also predicted in a second hypothesis that elevated CO_2, increased N addition, and plant richness have the divergent effects on litter and root decomposition through their effects on tissue quality.

Methods

This study was conducted at the Cedar Creek Ecosystem Science Reserve in east-central Minnesota, USA (45°24′ N, 93°12′ W; Reich et al. 2001*a*, *b*). Minnesota experiences a mid-continental climate with hot, humid summers, and cold winters. On average, annual rainfall is 800 mm with 60% of the precipitation occurring in the growing season from May to September. Mean annual temperature is 6.7°C, and mean monthly temperatures in the warmest and coldest months are −10° and 22°C (Adair et al. 2011). Nitrogen is the main limiting factor of NPP within these grasslands (Tilman 1987).

The experimental design was a randomized split-plot arrangement using a three-factorial combination of CO_2 (ambient or 560 μmol/mol), plant species number (1, 4, 9, and 16), and N level (control and 4 $g \cdot m^{-2} \cdot yr^{-1}$). CO_2 treatment is a whole plot factor and was replicated three times among six circular areas (20 m diameter rings), and the plot treatments of plant richness and N were randomly replicated in individual plots within the six rings (Reich et al. 2001*b*). In 1997, 359 plots (2 × 2 m) evenly distributed among six rings were sown with either 1, 4, 9, or 16 species that were randomly chosen from a pool of 16 species equally divided among the four functional groups of C_4 grasses, C_3 grasses, legumes, and forbs (Reich et al. 2001*a*, *b*). An additional 12 plots (two in each ring) were bare soil plots. For each of the four combinations of CO_2 and N, 32 plots were planted with one species, 15 plots planted with four species, 15 plots planted with nine species, and 12 plots planted with 16 species. The elevated CO_2 and N treatments began in April 1998. The CO_2 was implemented with Face technology (Reich et al. 2001*a*) during daylight hours in the growing season from April until October. N fertilizer was applied annually in three applications. Both aboveground biomass and root (0–20 cm) biomass were measured in June and August of each year (Reich et al. 2001*a*), and root production (0–20 cm) estimated from ingrowth cores collected in each plot across a growing season (Craine et al. 2002).

Collection of aboveground litter and belowground root

An experiment was conducted to assess effects on litter and root decomposition of microclimate differences caused either directly by treatments or indirectly by changes in plant functional group and species richness. We applied the following substrates of different species composition, including (1) *Bromus inermis* (C_3 grass, C: N = 58:1); (2) *Schizachyrium scoparium* (C_4 grass, C: N = 94:1); and (3) in situ litter and root from each plot. Standing litter samples of each plant species were collected from all 359 plots in October of 2003, when leaves were either abscised or dead. Root was taken at 0–20 cm depth using three 5 cm wide cores in same plot used for aboveground litter collection. As the common two substrates, litter and root of *B. inermis* and *S. scoparium* were separately collected in relatively monotypic stands within the same field in which the experiment was located in October of 2003. Root was washed to remove the soil and separated from soil debris by hand in laboratory. All litter and root were separately air-dried and oven-dried at 40°C in laboratory. Ten replicated samples were analyzed to determine the initial quality of litter and root of *B. inermis* and *S. scoparium*.

Litterbags and root containers

For each in situ litterbag, we used the most abundant plant species present in each plot which comprises 90–100% of the species present in each plot. Litter from the selected plant species was combined in litterbags in accordance with their relative abundance determined by the aboveground biomass in August in each plot. In total, we placed approximately 1 g (dry weight) litter in each bag. We set up four identical litter sets for each plot. One set was used to measure the initial litter quality, and three sets were incubated in situ for 1, 2, and 4 yr. We also set up separately three identical sets for the two common substrates of *B. inermis* and *S. scoparium* in each plot. All litterbags were 5 × 10 cm polyester cloth bags (50-mm pore size). Litterbags were placed and staked on the soil surface in the center of each plot starting in November 2004 and collected separately in October 2005, 2006, and 2008. We were not able to separate live from dead root for either the in situ or the *Bromus* or *Schizachyrium* root and used the total of the root samples collected in October of 2003. For each plot, we divided all root in four visually identical groups. One set of the in situ root was used to measure the initial root quality. The other three sets were placed in three Polyvinyl chloride (PVC) bars (20 × 2 × 2 cm), which were incubated in situ for 1, 2, and 4 yr. Each PVC bar had eight holes (Kochsiek et al. 2013), in which we placed two replicate root containers for *B. inermis* root, two replicate containers for *S. scoparium* root, and four replicate containers for the in situ root samples. In each container, which was 2 cm long with a 1.5 cm diameter, we placed approximately 0.1 g (dry weight) of root. Both ends of the container were covered with a 0.1-mm mesh plastic cloth. In total, eight containers were inserted in eight holes with 0.5 cm apart in one plastic bar (20 × 2 × 2 cm). This setup of a plastic PVC bar with individual containers allows us to relocate each individual sample while minimizing plot disturbance and minimizing the space used within each plot (Eisenbeis et al. 1999, Kochsiek et al. 2009, 2013).

Litter and root quality

All samples were dried to constant dry weight at 55°C, ball-milled, and then passed through a 20 mesh. Carbon and N were analyzed with a Costech ECS 4010 element analyzer (Costech Analytical Technologies, Valencia, California, USA). Biochemical component analyses relating to the quality of litter and root were determined by Ankom 200/220 Fiber Analyzer (Ankom Technology, Macedon, New York, USA), which is a standard forage chemistry method (Van Soest et al. 1991). This technique uses a sequential extraction to partition plant tissues into four fractions (Knops et al. 2007, Kochsiek et al. 2009, 2013). The first fraction includes the soluble carbohydrates, lipids, pectin, starch, soluble protein, and nonprotein N. The second fraction includes hemicellulose and cell wall-bound protein. The third fraction includes the cellulose, and the fourth fraction includes the lignin and related recalcitrant material. We refer to the dominant fractions here, soluble carbohydrates, cellulose, hemicellulose, and lignin; however, note these fractions are heterogeneous (Van Soest 1982).

After harvest, all samples incubated for 1, 2, and 4 yr were also dried, weighed, milled, and analyzed for C and N.

Data analysis

The CO_2 treatment was nested within ring (1 df) and was tested against the random effect of ring nested within CO_2 (4 df; Knops et al. 2007). The main effect of plant richness (15 df) and N (1 df) and interactions between CO_2, N, and plant richness were tested against the residual error (Reich et al. 2001*a*). A type III general linear model (GLM) multivariate analysis of variance was applied to examine the overall effects of CO_2, N, and plant richness treatments on the quality of litter and root, as well as to determine which quality fraction caused the overall significance (Knops et al. 2007). An ANOVA was conducted with the rings as random effects and the treatments of CO_2, N and plant richness as fixed effects.

We present C mass loss throughout the paper because the mass loss showed the same, identical patterns as C loss. Percent C loss was calculated as follows: 100 × ((initial C% × initial sample weight) − (final C% × final sample weight))/(initial C% × initial sample weight). Percent N loss was calculated similarly. To determine the main effects of CO_2, N, and plant richness treatments and their interactions on the decomposition of litter and root, we used four-way analysis of variance (ANOVA), as well the analysis of covariance (ANCOVA) with tissue quality and ecosystem parameters (soil C, soil N, aboveground biomass, belowground biomass, root productivity) as separated covariates. Further, we tested the correlations among the C or N loss of litter or root, soil C, soil N, aboveground biomass, belowground biomass, root productivity, tissue quality, CO_2, N, and plant richness. Based on the correlations, we constructed the best structural equation model (SEM) to investigate the direct and indirect effects of the combination of factors on C or N loss of litter or root (Spasojevic et al. 2014, White et al. 2014, Zuo et al. 2016).

Tukey tests were performed for multiple comparisons among different treatments. All statistical analyses were performed by SPSS 16 (SPSS Inc., Chicago, Illinois, USA) for Microsoft Windows. The structural equation modeling was performed using AMOS 20.0 software (IBM Corp., Armonk, New York, USA).

Results

Influence of CO_2, N, and plant species richness on litter and root quality

The litter quality and root quality were significantly affected by all the three treatments (i.e., CO_2, N, and plant richness), and there was no interaction among any of the treatments (Table 1). Further, ANOVA showed that the N or lignin content in plant tissue significantly responded to the global change factors (Appendix S1: Table S1). Elevated CO_2 induced a 9.0% decrease in N content in aboveground litter and a 7.4% decrease in root lignin content (Fig. 1). N addition induced a 9.4% increase in root N content and a 16.4% decrease in litter lignin content (Fig. 2). Higher plant richness induced a decrease by 14.0% and 16.0% of the N and lignin content in litter and also induced a decrease by 10.0% and 6.0% of the N and lignin content in root, respectively (Fig. 3).

Bare soil plots—decomposition in the absence of plants

Bare soil plots without vegetation can be used to examine the direct impact of CO_2 and N on decomposition using the two common substrates of *Bromus inermis* and *Schizachyrium scoparium*. Not surprisingly, species differed in C loss and the C loss increased with decomposition duration (Table 2, Fig. 4). However, we found no difference of C loss between litter and root (Table 2). Similar to C loss, species differed in N loss and the N loss increased during decomposition for *Bromus* and *Schizachyrium* root. *Schizachyrium* litter immobilized N in all three years, whereas

Table 1. Tissue quality changes caused by CO_2, N, and species richness treatments.

Treatment	Aboveground litter	Root
CO_2	2.60*	3.31**
N	4.80***	2.61*
Richness	6.53***	2.69***
CO_2 × N	0.56	0.77
CO_2 × Richness	0.58	0.71
N × Richness	0.69	0.70
CO_2 × N × Richness	0.48	0.64

Note: Presented are the *F* value of the Pillai's trace of a multivariate GLM of aboveground litter and belowground root with as litter quality included N (%), soluble (%), hemicellulose (%), cellulose (%), and lignin (%).

$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, all others are $P > 0.05$.

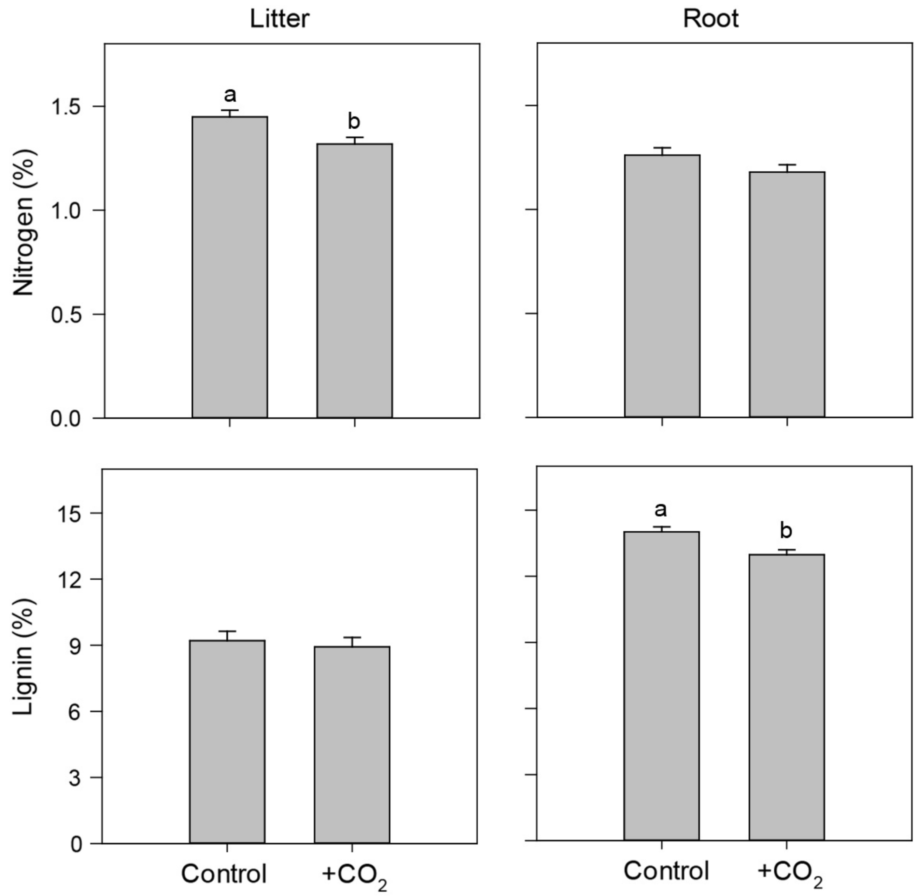

Fig. 1. Nitrogen (%) and lignin (%) of aboveground litter and belowground roots response to elevated CO_2. Shown are the means for CO_2 treatment, adjusted for the N and plant species richness treatments ± standard error. Different letters denote a significant difference at $P < 0.05$ level.

Bromus litter mineralized N in year 2 and year 4 (Fig. 4). Analyses of interactions between CO_2 and plant tissue showed that elevated CO_2 also increased the C loss of the root by 11%, but did not change litter C loss and had no effect on N loss (Table 2, Fig. 4). N addition had neither a significant impact on C loss nor on N loss. Thus, there was no direct effect of N addition, while elevated CO_2 only exerted a significant effect on root C loss.

Effect of CO_2, N, and plant species richness on root and litter decomposition

The four-way factor ANOVA showed that elevated CO_2 had no significant impact on litter or root C and N loss (Table 3). N addition significantly increased the C loss by 4.8% for the litter, but had no effect on litter N loss nor root C and N loss (Table 3, Fig. 5). N addition also had a significant interaction with species richness for litter N loss in which N addition increased N loss in one species and decreased N loss in four species ($P < 0.01$). Plant richness had the largest impact on C and N loss in litter and root (Table 3, Fig. 5). With the increasing plant richness, the litter C loss increased from 39.1% to 43.9% in year 1, 50.5% to 54.6% in year 2, and 70.1% to 72.1% in year 4, and the litter N loss increased from 3.2% to 22.2% in year 1, −0.5% to 25.7% in year 2, and 29.6% to 47.5% in year 4 (Fig. 5). However, the root C and N loss had the fluctuating changes with the increasing plant richness.

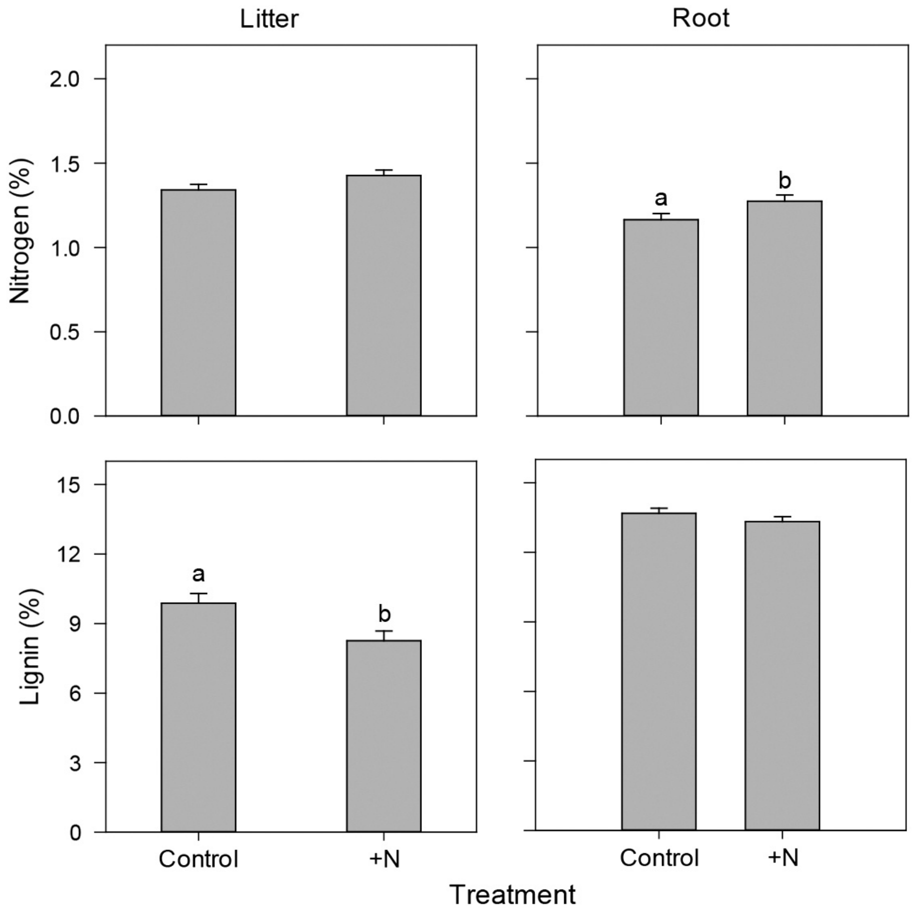

Fig. 2. Nitrogen (%) and lignin (%) of aboveground litter and belowground roots. Shown are the means for the nitrogen treatment, adjusted for the CO_2 and plant species richness treatments ± standard error. Different letters indicated the significant difference at $P < 0.05$ level.

Do CO_2, N, and plant species richness affect decomposition through tissue quality, vegetation, and soil?

The four-way factor ANCOVA with tissue percentage of N and percentage of lignin as covariates showed that both of tissue N and lignin had the significant impact on C and N loss in litter and root (Table 4). Thus, the tissue quality differences induced by global change factors had a strong impact on decomposition. However, the main treatments of CO_2, N, and plant richness did not change when the tissue quality covariates were included (Table 3 vs. Table 4).

In addition, the four-way factor ANCOVA with aboveground biomass, root biomass, root productivity, soil C, and soil N as covariates showed that changes in biomass or soil C and N pools also had the significant effects on decomposition (Table 5). Similar to the effects of CO_2, N, and plant richness on decomposition (Table 3), we found that the main effects of CO_2 were not significant and plant richness remained significant. N addition was significant not only for litter C loss, but also for litter N loss and root C loss (Table 5). Soil C and N had no significant effect on litter C loss, but significantly affected root C loss and both litter and root N loss (Table 5). Aboveground biomass had no significant effect on litter C or N loss, but significantly changed root C loss. Belowground root biomass had the largest effect on the C and N loss in litter and root.

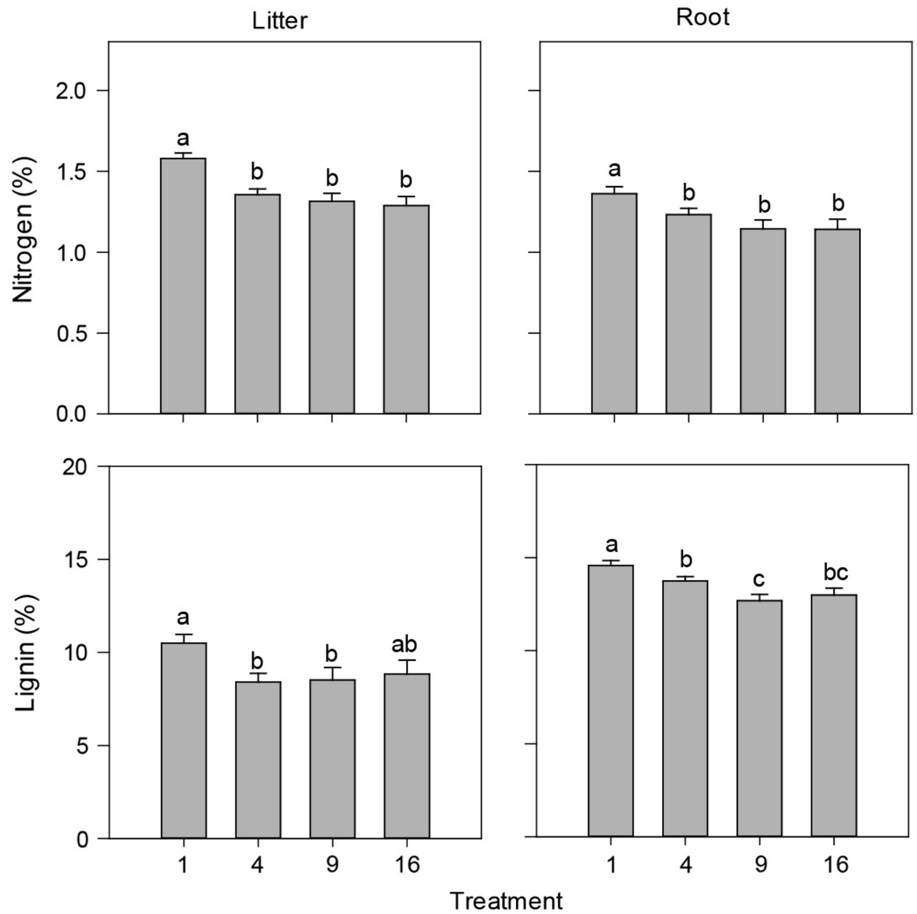

Fig. 3. Nitrogen (%) and lignin (%) of aboveground litter and belowground root response to plant species richness. Shown are the means for plant species richness treatment, adjusted for the CO_2 and N treatments ± standard error. Different letters denote a significant difference at $P < 0.05$ level.

The direct or indirect effect of CO_2, N, and plant species richness on decomposition

There were significant associations of the decomposition of litter and root with the tissue quality, biomass, and soil induced by global change factors (Appendix S1: Table S2). However, the final best SEM showed that elevated CO_2, N additions, and plant richness changes did not affect the decomposition of litter and root through changes in biomass and soil. The SEMs including the tissue N or lignin content and global change factors were the best fitting to explain the variance of the C and N loss in litter and root (Fig. 6; Appendix S1: Table S3). Elevated CO_2 had the indirect effect on the loss of N in litter and root through its effect on the N content of litter and root ($P < 0.01$). Elevated CO_2 indirectly decreased the loss of N in litter, but increased the loss of N in root. N addition indirectly increased the loss of C and N in litter and root through its positive effect on the N content of litter and root ($P < 0.01$). Plant richness changes directly affected the loss of C and N in litter ($P < 0.001$) and also indirectly affected the loss of C or N in litter and root through its effect on the N or lignin content of litter and root ($P < 0.001$, Fig. 6). However, increasing plant richness increased the loss of C and N in litter, but indirectly decreased the loss of C and N in root. The elevated CO_2, N addition, and plant richness changes had the different indirect effects on the C or N loss in litter or root via their effects on tissue quality.

Table 2. Carbon and nitrogen loss in common substrate litter placed in plots without vegetation.

Treatment	Carbon loss	Nitrogen loss
CO_2	3.76	7.63
N	0.02	0.17
Decomposition duration	71.43***	4.39*
Plant tissue	0.02	140.83***
Common substrate species	47.71***	65.30***
$CO_2 \times$ N	0.93	3.13
$CO_2 \times$ Year	0.49	0.65
$CO_2 \times$ Plant tissue	4.10 *	0.24
$CO_2 \times$ Substrate species	0.21	0.50
N × Decomposition duration	0.96	1.02
N × Plant tissue	0.21	0.25
N × Substrate species	0.05	0.02
Decomposition duration × Plant tissue	0.74	1.60
Decomposition duration × Substrate species	0.74	3.94*
Plant tissue × Substrate species	0.74	10.71**

Notes: Shown are the F and P values from a five-factor GLM with as factors decomposition duration (after 1, 2, and 4 yr), CO_2 (elevated and control), nitrogen addition (elevated and control), plant tissue (above-, and belowground), and common substrate species (*Bromus inermis* and *Schizachyrium scoparium*). CO_2 was nested within ring, and only the two-way interactions are included.

$^{*}P < 0.05$, $^{***}P < 0.001$, all other F values are $P > 0.05$.

Discussion

Tissue quality

Our study clearly illustrates that elevated CO_2, N addition, and increasing plant species richness cause the changes in both aboveground litter tissue quality and belowground root tissue quality. However, we found no significant interactions among elevated CO_2, N addition and increasing plant richness, which is consistent with a number of other studies (Cotrufo et al. 1998*b*, Knops et al. 2007). Thus, contrary to what is often assumed, there is increasing evidence that global change factors individually affect plant litter and root quality, but do not interact in changing tissue quality.

We found that elevated CO_2 had divergent effects on aboveground litter vs. root quality. Elevated CO_2 decreased the aboveground litter quality as indicated by a lower N content and increased the root quality as indicated by a decrease in the lignin content. These changes in litter N and lignin chemistry caused by elevated CO_2 are consistent with the previous studies (Cotrufo et al. 1998*a*, Norby et al. 2001, Stiling and Cornelissen 2007, Cha et al. 2017). Within the experiment, a fixed number of specific species were assigned in each plot and only relative changes within the 4, 9, and 16 species can be evaluated. So, both the aboveground and belowground tissue quality changes were entirely driven by within plant species tissue quality changes under CO_2 treatments. Within this experiment, the productivity increases with elevated CO_2 and N has become the increasing limitation for the productivity at elevated CO_2 level (Reich and Hobbie 2013). Thus, the likely mechanisms driving tissue quality changes caused by elevated CO_2 are the stoichiometry of C and N.

Elevated CO_2 increased the above- and belowground biomass or productivity in our experiment. Since the lignin is a structural biomass component, such productivity increase might lead to a relative decrease in lignin and a concurrent increase in other biomass fractions. Within our experiment in the typical grassland, a larger part of productivity is belowground (Reich et al. 2001*a*). Hence, the increase in productivity caused by elevated CO_2 might have a stronger effect on belowground biomass fractions. However, note that we were not able to separate dead and live root. Thus, the N content of belowground combined live and dead root is likely much higher than the N content of dead root only (Kunkle et al. 2009), and this might have masked a CO_2 treatment impact on root N content. The lignin is a structural component of plant tissues, and the retranslocation does not occur. Thus, the lignin of the combined live and dead root fraction likely matches the lignin content of the dead root much more than the N content.

As compared to the elevated CO_2, N addition increased the quality of both aboveground litter and root, while the different components of tissue quality changed aboveground vs. belowground. N addition decreased the litter lignin content and increased the root N content. Similar changes in lignin and N content responding to N addition were frequently observed in N addition experiments (Hobbie 2000, Henry et al. 2005, Liu et al. 2010). However, the effect of N addition on root N might be inflated because we analyzed both live and dead root together. There might be the N retranslation during root senescence, and the N content of dead root may be lower (Kunkle et al. 2009). Also, all 16 plant species in this experiment were perennials forb and grasses, in which the

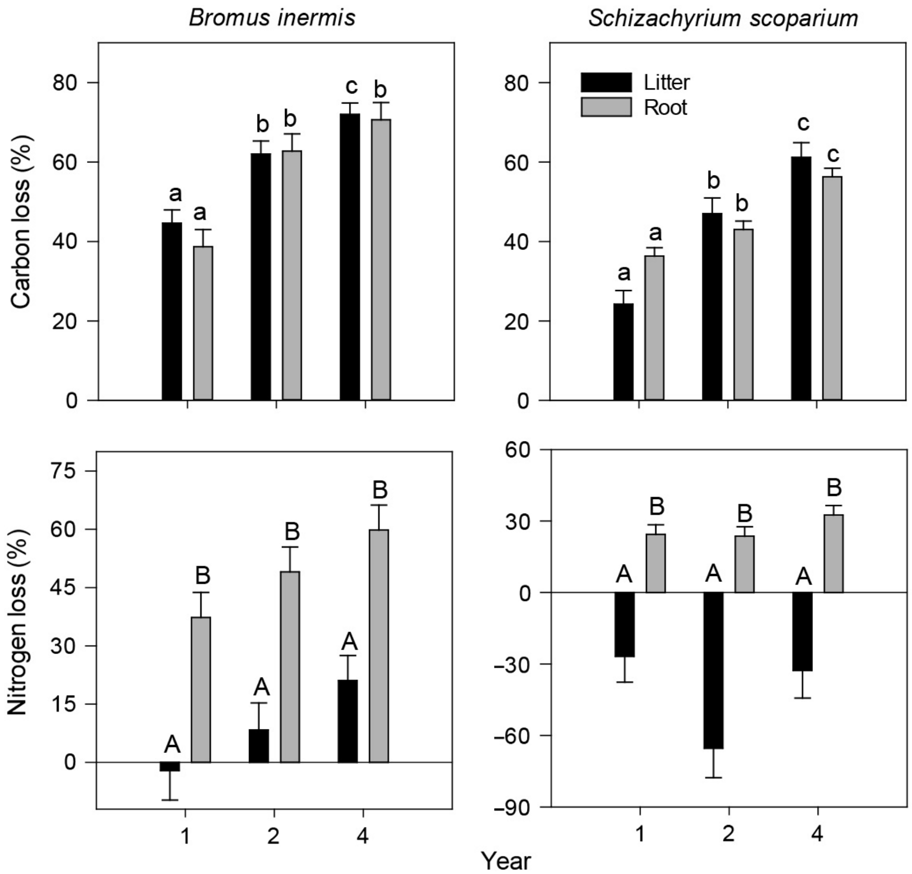

Fig. 4. Loss of C and N in aboveground litter and belowground root in bare ground plots for *Bromus inermis* and *Schizachyrium scoparium* using the means for year treatment, adjusted for the CO_2 and N treatments ± standard error. Different lowercases from the same variable in different years denote a significant difference at $P < 0.05$ level. Capital letters at the same treatment denote a significant difference between litter and root at $P < 0.05$ level.

Table 3. Aboveground litter and belowground root carbon and nitrogen loss of in situ aboveground litter and belowground roots.

Source	Carbon loss		Nitrogen loss	
	Litter	Root	Litter	Root
CO_2	1.32	0.09	1.01	0.01
N	10.26**	0.6	0.03	0.97
Plant richness	3.83*	6.35***	20.70***	7.63***
Year	441.81***	214.17***	65.74***	125.25***
CO_2 × N	0.64	2.78	0.18	0.02
CO_2 × Richness	0.09	0.69	0.58	1.13
CO_2 × Year	0.59	0.45	0.5	1.18
N × Richness	2.22	1.89	3.41 *	0.44
N × Year	0.57	0.03	0.88	0.17
Richness × Year	1.12	0.30	1.12	0.80

Notes: Presented are the *F* values from a GLM with as factors CO_2 nested within ring (elevated and control), nitrogen addition (elevated and control), and plant species richness (1, 4, 9, and 16 species) over 1, 2, and 4 yr of decomposition duration.
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, all other $P > 0.05$.

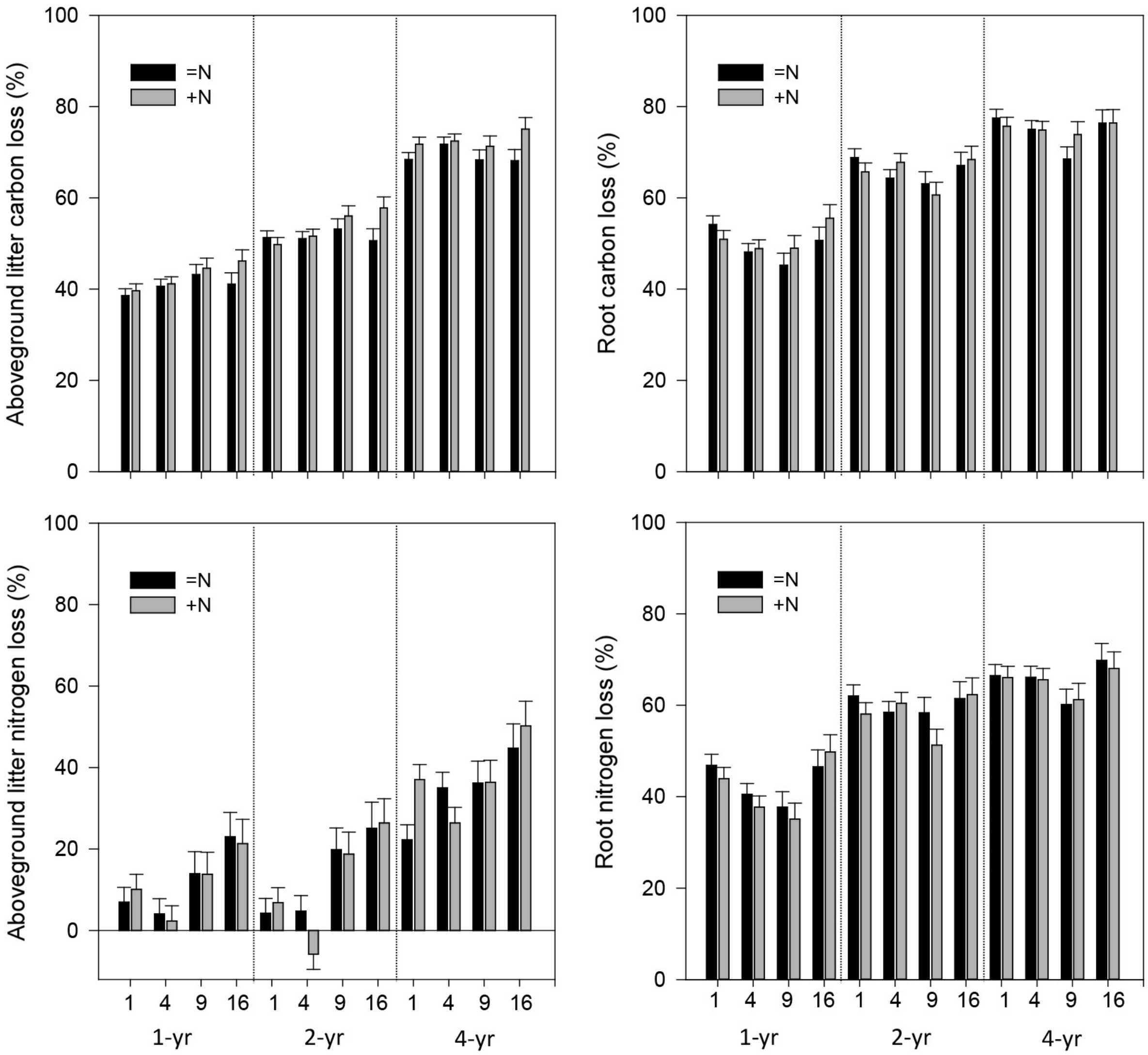

Fig. 5. Loss of C and N in aboveground litter and root for in situ substrates using the means for N, plant species richness, and year adjusted for the CO_2 treatments ± standard error.

aboveground tissues senesced and retranslocated the N into root. We sampled the root in late fall after the aboveground tissues senesced, and thus, the N retranslocation might have further increased the N content of the belowground tissues even more and further increased the N treatment effects. The tissue quality changes were mainly caused by changes within species tissue. This may be explained by the fact that N addition induces an increase in fast-growing species with high tissue N content, short-lived leaves and with a high specific leaf and root area which is associated with lower tissue lignin.

Species richness effects on tissue quality were stronger than either the elevated CO_2 or the N addition. Single species plots had the higher tissue N and lignin contents both above- and belowground, which supports the finding that the tissue N content decreases with increasing species richness (Chen et al. 2017*a*, *b*). Plot level tissue quality changes were mainly dependent on the species tissue quality in pant community.

Table 4. Litter quality influence on aboveground litter and belowground root carbon and nitrogen loss of the in situ aboveground litter and belowground roots.

Source	Carbon loss		Nitrogen loss	
	Litter	Root	Litter	Root
Tissue N	4.85*	36.47***	11.14***	37.99***
Lignin	19.33***	35.49***	54.56***	30.49***
CO_2	2.61	0.10	0.34	0.00
N	7.85**	0.02	1.11	3.26
Plant richness	1.51	5.37**	29.04***	7.72***
Year	458.79***	223.77***	71.12***	131.06***
CO_2 × N	1.93	2.48	0.60	0.02
CO_2 × Richness	0.15	0.98	0.42	3.17*
CO_2 × Year	0.21	0.62	0.22	0.96
N × Richness	1.34	0.73	4.02**	0.32
N × Year	0.37	0.19	0.62	0.15
Richness × Year	1.19	0.27	1.18	0.69

Notes: Presented are the F values from a ANCOVA with as factors CO_2 nested within ring (elevated and control), nitrogen addition (elevated and control), and plant species richness (1, 4, 9, and 16 species) over 1, 2, and 4 yr of decomposition duration. As covariates included are plant tissue, N, cellulose, and lignin (all as % of tissue dry weight).

$^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, all other $P > 0.05$.

Productivity within our experiment strongly increases with plant species richness (Reich et al. 2001*a*), and thus, the plant demand for N also increases with diversity and this is likely driving the tissue quality changes along the species richness treatments. Do note that these tissue quality changes were mainly between the one species plots and all other richness level, which is in contrast to the productivity which increases to much higher species richness levels.

Table 5. Plant and soil influence on aboveground litter and belowground root carbon and nitrogen loss of in situ aboveground litter and belowground roots.

Source	Carbon loss		Nitrogen loss	
	Litter	Root	Litter	Root
Aboveground biomass	1.05	4.46*	2.07	1.78
Belowground biomass	4.56*	37.30***	42.12***	44.87***
Root productivity	5.43*	0.11	2.43	0.20
Soil C	0.54	4.74*	7.52**	7.86**
Soil N	1.30	5.68*	7.40**	7.99**
N	11.75**	7.46**	6.46*	1.78
Richness	3.03*	6.86***	32.89***	11.14***
Year	284.19***	170.91***	53.68***	107.05***
CO_2 × N	0.85	5.24*	0.26	1.05
CO_2 × Richness	0.08	0.91	1.38	1.67
CO_2 × Year	0.61	0.67	0.53	0.82
N × Richness	1.9	3.27*	2.47	0.95
N × Year	0.72	0.37	1.18	0.21
Richness × Year	0.83	0.34	1.19	0.95

Notes: Presented are the F values from a ANCOVA with as factors CO_2 nested within ring (elevated and control), nitrogen addition (elevated and control), and plant species richness (1, 4, 9, and 16 species) over 1, 2, and 4 yr of decomposition duration. As covariates included are soil organic C and N from 2002, 2002, and 2007 at 0–20 cm soil depth (both % of total soil dry weight), aboveground and belowground standing plant biomass, and belowground root productivity in from 2005, 2006, and 2008 (all three in g/m^2).

$^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, all other $P > 0.05$.

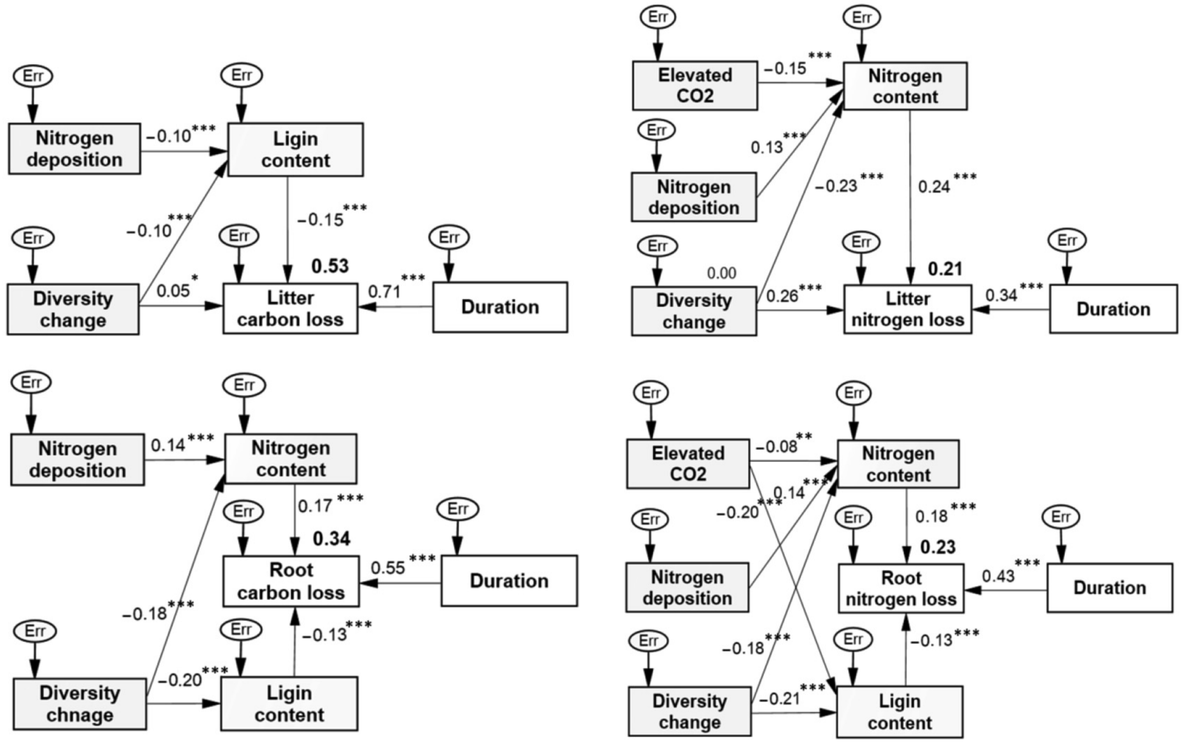

Fig. 6. Structural equation model showing the all pathways of elevated CO_2, nitrogen addition, and plant species on loss of C and N in litter and root. Single-headed arrows indicate paths. The exogenous unobserved variables Err account for the unexplained error. Standardized regression weights (along path) and total variance explained as a result of all predictors pointing to that variable (top right corner of rectangle). *, **, and *** indicate statistically significant paths at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Litter and root decomposition

Human activities have increased atmospheric CO_2 concentrations which can change litter quality and further alter litter decomposition and nutrient cycling process in ecosystems (Coûteaux et al. 1995, Henry et al. 2005, Iversen et al. 2012, Garcia-Palacios et al. 2017). Our results showed that elevated CO_2 did not directly change either litter or root decomposition, which was similar to a number of other studies (Johnson et al. 2000, Knops et al. 2007, de Graaff et al. 2011). However, elevated CO_2 indirectly affected the N loss of litter and root through its effect on the litter and root quality. Changes of N and lignin contents in litter and root induced by elevated CO_2 can better explain the observed patterns of N loss in litter and root. The reduction in the initial litter N content caused by the high CO_2 treatment leads to a decrease in the N loss in litter decomposition. However, the reduction induced by elevated CO_2 in the initial lignin content is more than in the initial root N, thus favoring the N release in root decomposition (Fig. 6; Appendix S1: Table S3). This can partly mitigate the limitation of elevated CO_2 on the soil N availability, since elevated CO_2 increases plant productivity and litter production in grasslands (Reich et al. 2001*b*) and restraints the N release in litter decomposition.

Not surprising, N addition indirectly increased the C loss and N release during the decomposition of litter and root through its effect on the initial N and lignin contents. The increased N deposition can greatly modify the limitation of elevated CO_2 on the available soil N and litter cycling. Our study supplies an evidence that the N availability is one of most important divers in litter decomposition and nutrient release (Liu et al. 2010, Zhu et al. 2016, Maaroufi et al. 2017). N addition induced the improvement of litter quality with a reduction in the initial lignin content and an increase in initial N content, leads to an increase in the C or N loss in litter decomposition. An increase in initial root N content induced by N addition can also favor the C and N loss in root decomposition. These results are also agreed with the previous studies that litter decomposition is often strongly controlled by initial N and lignin contents (Cotrufo et al. 1994, Knops et al. 2007, Parton et al. 2007, Liu et al. 2010), thereby supporting that the increasing litter nutrient contents as a consequence of N addition can accelerate the decomposition rate and N release (Liu et al. 2010, Vivanco and Austin 2011, Li et al. 2017*a*, *b*). In addition, the previous studies within the same experiment have found that N addition also induced the changes in soil microbial composition and functioning (Chung et al. 2007, He et al. 2010), thus potentially affecting the litter or root decomposition. However, the decreased microbial biomass carbon (Li et al. 2013) and increased the microbial N (Craine et al. 2007) caused by N addition may limit the litter decomposition, because the high N availability restraints the microbes to acquire N from the organic matter. Thus, the complicated and inconsistent effect of tissue quality and soil microbe induced by N addition on decomposition are likely to co-occur, thereby declining the explanation variance of decomposition caused by N addition.

Plant richness changes strongly affected the C and N loss in both litter and root decomposition, and this result did not change when we included either litter quality, soil C or N, or plant and root biomass as covariant. Thus, as compared to elevated CO_2 and N addition, plant richness changes are the more important in driving litter and root decomposition (Szanser et al. 2011, Chen et al. 2017*a*, *b*), which is in contrast to several other studies that plant diversity has the much smaller effects on decomposition (Hector et al. 2000, Knops et al. 2007). However, our results suggest that the root decomposition and N release do not mirror those of aboveground litter, indicating that no consistent effects of plant richness on decomposition occur between litter and root. Our results are agreed with the previous study that increasing plant richness may increase the litter decomposition rates (Szanser et al. 2011, Jones and Swan 2016, Li et al. 2017*a*, *b*). This may be explained by the fact that the initial lower lignin content caused by increasing plant richness can accelerate the litter decomposition rate. In addition, the species-rich plant communities have the higher herbivory rates of arthropods (Ebeling et al. 2014) and the higher activity of microbial community (Chung et al. 2007), which further enhances the litter decomposition, because the higher plant biomass resulting from the higher plant richness can host the decomposing arthropods and structures soil microbial communities. In contrast to the increasing aboveground litter decomposition, our results also support the finding that a decrease in root N content with increasing richness may slow the root decomposition (Chen et al. 2017*a*, *b*). This can be interpreted by the fact that although the increasing plant richness decreases the initial N and lignin contents of root, the effect of N content change on root decomposition is the higher than the lignin contents, which leads the net effect of increasing plant richness on the root decomposition and N release is negative. Thus, our study fully supports that both of the N and lignin contents are often identified as the important factors in mediating the effect of plant diversity changes on the decomposition (Hobbie 2000, Zhao et al. 2014, Chen et al. 2017*a*, *b*).

Our results from the ANCOVAs are agreed with other studies that changes in vegetation and environmental factors induced by elevated CO_2, N additions, and changes in plant species richness can indirectly influence litter decomposition (Cornwell et al. 2008, Huttunen et al. 2009, Siegenthaler et al. 2010, He et al. 2012, Chen et al. 2017*a*, *b*). However, the final best SEM showed that global change factors did not affect the decomposition of litter and root via changes in changes in vegetation and soil. This may be explained by the fact that elevated CO_2, N addition, and plant richness changes can affect the decomposition more indirectly through tissue quality, microbial communities, and microclimate (Adair et al. 2011, Allison et al. 2013, Garcia-Palacios et al. 2013*b*). In our study, the previous studies showed that the productivity or biomass within this experiment increased in response to elevated CO_2, N addition, and increased plant richness (Reich et al. 2001*a*). It is plausible that the heterotrophic microbial communities would experience the greater substrate availability, potentially increasing soil respiration, microbial activity which further accelerate litter and root decomposition (Chung et al. 2007). Alternatively, the higher productivity can enhance soil C and N accumulation through the high litter production (Fornara and Tilman 2008), which further affect the soil respiration and water content. Thus, changes in soil microclimate linked to plant and root biomass may also affect the litter and root decomposition in grasslands (Adair et al. 2011).

In conclusion, our study clearly demonstrates that global change factors such as elevated CO_2, N deposition, and biodiversity loss do lead to the significant changes in litter and root quality. Both of the N and lignin contents may mediate the effect of global change factors on the decomposition and nutrient release in litter and root. Global change factors have the different influencing consequence on the C or N loss between litter and root decomposition through their effects on tissue quality. Elevated CO_2 is an important constraint on the available soil N, while N addition modifies the limitation of elevated CO_2 on soil N availability. As compared the indirect effects of elevated CO_2 and N addition, the biodiversity loss tends to drive any potential alterations in C and N cycling in grassland ecosystems. Climate change-induced alterations in species compositions are likely much more important in determining feedbacks through decomposition and N cycling. However, the root decomposition and nutrient release do not mirror those of aboveground litter among different plant richness treatment. This study provides the insight into the influencing mechanism of global change factors on the decomposition and nutrient release in litter and root, which is helpful to improve our understanding of the effect of global change factors on biogeochemical cycles in grassland ecosystems.

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Supporting Information

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Ecosphere

Effects of elevated CO_2, increased nitrogen deposition and plant diversity on aboveground litter and root decomposition

Xiaoan Zuo and Johannes M. H. Knops

Appendix S1

Table S1. Specific litter quality changes caused by CO_2, N and plant species richness treatments Presented are the F values from ANOVA's for the quality of aboveground litter and belowground root with as quality aspects; N (%), soluble (%), hemicellulose (%), cellulose (%) and lignin (%) and as independent factors CO_2, N and plant species richness treatments. * denotes *P*<0.05, ** *P*<0.01, *** *P*<0.001, all others are p>0.05

Source	N (%)		Soluble (%)		Hemicellulose (%)		Cellulose (%)		Lignin (%)	
	Aboveground litter	Root	Aboveground litter	Root	Aboveground litter	Root	Aboveground litter	Root	Aboveground litter	Root
CO_2	13.11*	2.27	0.15	7.45	0.90	6.32	0.25	0.57	0.20	10.21*
N	3.63	4.71*	0.17	0.26	1.97	0.24	0.19	1.92	7.28**	1.41
Richness	11.68***	4.55**	0.77	1.00	0.77	0.61	10.97***	0.25	4.03**	8.01**

Given are the F values from a GLM with as factors CO_2 nested within ring, N and plant species richness. N, nitrogen; GLM, general linear model. * *P*< 0.05; ** *P*< 0.01; *** *P* < 0.001; Measured, actual measured value; Expected, calculated expected value.

Table S2. Correlation coefficients of the C or N loss, CO_2, N, plant species richness, soil C, soil N, aboveground biomass, belowground biomass, root productivity and tissue quality.

	CO_2	N	Plant richness	Year	%C-loss	%N-loss	Soil-Carbon	Soil-Nitrogen	Aboveground total biomass	Total root biomass	% Nitrogen	% cellulose	% lignin
CO_2	1												
N	0.006	1											
Plant richness	0	0.003	1	0									
Year	0	0	0	1									
%C-loss	-0.061	0.051	0.069*	0.709**	1								
%N-loss	-0.062*	0.001	0.203**	0.337**	0.569**	1							
Soil-Carbon	0.001	0.052	0.307**	0.307**	0.195**	0.097**	1						
Soil-Nitrogen	0.008	0.029	0.249**	0.111**	0.049	0.049	0.865**	1					
Aboveground biomass	0.114**	0.101**	.459**	-0.213**	-0.138**	0.038	0.143**	0.160**	1				
Root biomass	0.130**	0.255**	.512**	-0.035	-0.006	-.095**	0.238**	0.173**	0.374**	1			
% Nitrogen	-0.153**	0.126**	-.232**	0	-0.106**	0.178**	-0.164**	-0.108**	-0.204**	-0.382**	1		
% cellulose	0.026	-0.011	0.293**	0	0.019	0.036	0.079**	0.093**	0.208**	0.383**	-0.411**	1	
% lignin	-0.044	-0.105**	-0.102**	0	-0.149**	0.272**	-0.145**	-.093**	0.045	-0.450**	0.484**	-0.184**	1

Table S3. Direct, indirect and total effects of CO_2, N and plant species richness on of the C or N loss in litter or roots based on standardized values of statistically significant SEM paths (*P*< 0.05).

		Predictor and effect		
	Pathway	CO_2	N	Richness
C loss in litter	Direct	NS	NS	0.053
	Indirect	NS	0.015	0.015
	Total	NS	0.015	0.068
N loss in litter	Direct	NS	NS	0.257
	Indirect	-0.037	0.03	-0.056
	Total	-0.037	0.03	0.201
C loss in roots	Direct	NS	NS	NS
	Indirect	NS	0.025	-0.004
	Total	NS	0.025	-0.004
N loss in roots	Direct	NS	NS	NS
	Indirect	0.01	0.026	-0.005
	Total	0.01	0.026	-0.005

C, carbon; N, nitrogen. NS, non-significant relationships.