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Elevated CO_2 and plant species diversity interact to slow root decomposition

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ABSTRACT

Changes in plant species diversity can result in synergistic increases in decomposition rates, while elevated atmospheric CO_2 can slow the decomposition rates; yet it remains unclear how diversity and changes in atmospheric CO_2 may interact to alter root decomposition. To investigate how elevated CO_2 interacts with changes in root-litter diversity to alter decomposition rates, we conducted a 120-day laboratory incubation. Roots from three species (*Trifolium repens*, *Lespedeza cuneata*, and *Festuca pratense*) grown under ambient or elevated CO_2 were incubated individually or in combination in soils that were exposed to ambient or elevated CO_2 for five years. Our experiment resulted in two main findings: (1) Roots from *T. repens* and *L. cuneata*, both nitrogen (N) fixers, grown under elevated CO_2 treatments had significantly slower decomposition rates than similar roots grown under ambient CO_2 treatments; but the decomposition rate of *F. pratense* roots (a non-N-fixing species) was similar regardless of CO_2 treatment. (2) Roots of the three species grown under ambient CO_2 and decomposed in combination with each other had faster decomposition rates than when they were decomposed as single species. However, roots of the three species grown under elevated CO_2 had similar decomposition rates when they were incubated alone or in combination with other species. These data suggest that if elevated CO_2 reduces the root decomposition rate of even a few species in the community, it may slow root decomposition of the entire plant community.

1. Introduction

Human activities have increased atmospheric carbon dioxide CO_2 concentrations (Houghton and Ding, 2001), which can decrease litter quality (Cotrufo et al., 1998; Knops et al., 2007). A decrease in litter quality may alter the way that ecosystems process nutrients and change the efflux of CO_2 from the soil to the atmosphere (Dukes and Hungate, 2002). Litter quality, defined by the percent of nitrogen (N), carbon (C), lignin, or other structural properties in plant tissues, is the dominant controller of decomposition processes when environmental controls are held constant (Meentemeyer, 1978; Silver and Miya, 2001). The effect of increasing atmospheric CO_2 on aboveground litter quality and decomposition rates has been well studied and reviewed and is relatively minor in most ecosystems (de Graaff et al., 2006a,b; Luo et al., 2006; Norby et al., 2001; Six et al., 2001). These studies have not included root litter, which represents up to 70% of net primary production in terrestrial ecosystems. Living roots turnover rapidly and therefore root C is returned to the soil on relatively short time scales, making it an important component of the terrestrial C cycle (Vogt et al., 1986). In addition, elevated atmospheric CO_2 can stimulate the allocation of C to roots (Iversen, 2010), making root contributions to soil C an even more important part of the terrestrial C cycle in a CO_2-enriched world. Studies that evaluated the response of root tissue chemistry and decomposition to elevated atmospheric CO_2 have principally focused on the response of a single species' roots (Ball and Drake, 1998; Gorissen et al., 1995, King et al., 2001; van Groenigen et al., 2005). Yet, in most natural ecosystems roots of multiple species, with varying litter quality, mingle belowground, making it important to understand how CO_2-induced changes in root-litter quality of single species within a community affect decomposition of the mix of roots within that community.

The decomposition rate of litter, either aboveground or belowground, from different species decomposing together, hereafter 'mixed litter', is not always simply derived from the weighted

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average of the sum of each litter type. Instead, non-additive patterns in the abundance and activity of decomposers are frequently reported when litter of different species decomposes together (Anderson and Hetherington 1999; Blair et al., 1990; Briones and Ineson 1996; Wardle et al., 1997). Indeed, Gartner and Cardon (2004) reported that non-additive patterns of mass loss were observed in 67% of studies reviewed, and non-additive synergistic patterns in aboveground litter decomposition rates, where mass loss exceeded expected decay by ~20%, were common. To the best of our knowledge, only one reported study to date has evaluated the impact of mixing different species' roots on decomposition rates, revealing that mixing root litter may also cause non-additive increases in root-litter decomposition (Robinson et al., 1999).

Non-additive synergistic effects of mixing litter on decomposition rates have been linked to the chemical properties of each litter type in the mixture (e.g., Meier and Bowman, 2008). As such, it is the composition and diversity of chemical litter traits that alter soil C cycling, rather than plant species diversity *per se* (Meier and Bowman, 2008). The positive correlation between decomposition rates and high litter chemical diversity may be explained by priming, which is defined here as the stimulation of decomposition caused by the addition of labile substrates, such as litter of high quality (Dalenberg and Jager, 1989; Jenkinson et al., 1985). If elevated atmospheric CO_2 decreases the chemical composition of one or more of the species in the mix, it could slow overall root decomposition rates in soils. To better predict how root decomposition will change under elevated atmospheric CO_2, we need to better understand how a CO_2-induced change in root-litter decomposition of individual species may affect root decomposition rates of coexisting species.

Here, we examined the interactions among root tissue diversity, elevated CO_2, root decomposition, and N-mineralization rates to test three hypotheses: (1) individual species' roots grown under elevated atmospheric CO_2 will decompose more slowly and soil amended with these roots will have lower N-mineralization rates than soils amended with roots grown under ambient conditions; (2) when species with high N concentrations are mixed with species of low N concentration, root decomposition and N mineralization will be greater than predicted from individual decomposition rates; and (3) elevated atmospheric CO_2 will reduce root decomposition and soil N-mineralization rates of soils amended with several plant species by increasing the recalcitrance of the most labile tissue in the mix.

Using a long-term climate change experiment, we collected soil and roots from three different species: *Trifolium repens*, *Lespedeza cuneata*, and *Festuca pratense*. The plants and soil had been exposed to elevated atmospheric CO_2 for five years and the three species chosen were among the most dominant species across experimental treatments (Engel et al., 2009; Kardol et al., 2010a,b). To test the interactions between exposure to elevated atmospheric CO_2 and root species decomposition, we incubated the roots of the three species grown under elevated *versus* ambient CO_2, individually and in combination for 120 days. A depleted ^{13}C label that was applied to the elevated CO_2 plots during the experiment enabled us to partition soil respiration between native SOM decomposition and the decomposition of the added root substrate. Roots produced under elevated and ambient atmospheric CO_2 were incubated in soil exposed to ambient or elevated CO_2. By incubating roots produced under ambient atmospheric CO_2 in a soil exposed to elevated atmospheric CO_2 and *vice versa*, we were able to assess whether differences in the rate of decomposition or N mineralization were controlled by root tissue quality or by changes in the native soil microbial activity.

2. Materials and methods

2.1. Sample collection and initial analysis

We collected soil and root samples from the Old Field Community Climate and Atmosphere Manipulation (OCCAM) experiment located at the Oak Ridge National Environmental Research Park, Oak Ridge, Tennessee (25°54′ N; 84°21′ W). The site is an abandoned agricultural field and the soil is classified as Captina silt loam with moderate-to-medium granular structure and medium internal drainage (Edwards and Norby, 1999). Whole soil N (1.62 g N kg^{-1}) and C (18.3 g C kg^{-1}), determined prior to the start of the experiment, were not affected by the climate change treatments and did not change significantly over time (Garten et al., 2009). In 2002, twelve experimental plots were established and planted with seven old-field plant species: *Plantago lanceolata* L., a herbaceous forb; *Andropogon virginicus* L., a cespitose C_4 bunchgrass; *F. pratense* L. syn F. elatior L., a C_3 bunchgrass; *Dactylis glomerata* L., a C_3 bunchgrass; *Trifolium pratense* L., a herbaceous legume; *Solidago canadensis*, a herbaceous forb; and *L. cuneata*, a N_2-fixing sub-shrub (see Engel et al., 2009; Kardol et al., 2010a,b). The experimental design is described in detail elsewhere (Dermody et al., 2007; Garten et al., 2008; Wan et al., 2007). Briefly, in 2003, CO_2, temperature, and soil moisture treatments were applied through the use of open-top chambers and rain-out shelters, which were installed on top of 12 plots (4 m in diameter). Whole plots received treatments of ambient or elevated (ambient +300 ppm) atmospheric CO_2 in combination with ambient or elevated (ambient +3 °C) air temperature in a randomized, complete-block design ($n = 3$). An open-sided shelter was assembled over each open-top chamber to exclude precipitation. Each split plot within each whole plot was randomly assigned a soil moisture treatment ('wet' or 'dry') created by differential irrigation (2 mm/25 mm per week). The CO_2 applied to the elevated atmospheric CO_2 treatments was depleted in ^{13}C (−51‰) compared to ambient atmospheric CO_2 treatments resulting in distinct soil and tissue ^{13}C signatures (Garten et al., 2009).

In June of 2007, we randomly collected 30 soil samples (1 cm diameter core; 0–10 cm) from each of the irrigated (wet) and ambient temperature subplots exposed to both ambient and elevated atmospheric CO_2. The individual soil cores taken from a subplot were composited, sieved to 2 mm, and visible roots >2 mm were removed. Soils were processed and stored at 6 °C for approximately 4 weeks. Roots (0–10 cm) from each of the three species (i.e. *F. pratense*, *T. repens*, and *L. cuneata*) were harvested from the same plots as the soil, using a shovel to collect intact plants to a depth of 15 cm. Collected roots were washed, sorted for fine roots (<2 mm) and stored at 6 °C. Fine roots were dried at 70 °C and ground to 2.5 mm. Subsamples of the roots and of the root-mix were ground to a fine homogeneous powder prior to elemental and isotopic analysis. Carbon and N concentrations were measured with a LECO CN-2000 elemental analyzer (LECO incorporated, St. Joseph, MI). The instrument was calibrated using LECO standards traceable to National Institute of Standards and Technology (NIST), Gaithersburg, MD. Samples were analyzed for stable C-isotope ratios ($^{13}C/^{12}C$) using an Integra-CN, continuous flow, isotope ratio mass spectrometer (SerCon Ltd, Crewe, United Kingdom). Leaf lignin concentrations were quantified with the acid-fiber method (using an ANKOM-200; Ankom Technology, Macedon NY), oak was used as a standard.

2.2. Incubation experimental design

Soils and roots were incubated for 120 days at 20 °C (replicates were field plots; $n = 3$). A 0.6 g subsample of root material

from each species, *F. pratense*, *T. repens*, and *L. cuneata*, was mixed with approximately 60 g (DW) of soil in 120 mL specimen cups. In addition, a 0.6 g root-mix of the species (0.2 g *F. pratense* + 0.2 g *T. repens* + 0.2 g *L. cuneata*) was added to 60 g of soil.

We used a reciprocal transplant design to tease apart if changes in decomposition were due to a CO_2-induced shift in the soil microbial community (Castro et al., 2010; Kardol et al., 2010b), or due to a change in tissue quality. We added roots previously exposed to ambient levels of atmospheric CO_2 to soils previously exposed to both ambient and elevated atmospheric CO_2, and we added 'elevated CO_2 roots' to both 'ambient and elevated CO_2 soils'. As a result, all combinations of root material produced under ambient and elevated CO_2 and soil exposed to ambient and elevated CO_2 were used in the incubation study (Table 1). Triplicates of unamended soils previously exposed to ambient and elevated CO_2 (control soils) were incubated along with the root tissue amended soils, to account for CO_2 evolution from the pre-existing soil C. Soil water-holding capacity of the soils was determined by calculating the difference in weight of soils at its saturation point and its oven-dry weight (100 °C). For the incubation, water was added to obtain 60% saturation. Specimen cups were placed in 1 L mason jars and 5 mL of water was added to the bottom of the jar to maintain humidity. A septum in the jar lid allowed gas samples to be removed from the headspace with a 10 mL syringe. CO_2 samples were collected in 12 mL Vacutainers where they were stored until samples were analyzed (Labco limited, Buckinghamshire, UK) and stored until samples were analyzed with a continuous flow, isotope mass spectrometer (PDZ Europa TGII trace gas analyzer and Geo 20–20 isotope ratio mass spectrometer, Cheshire UK) at the Stable Isotope Facility in Davis, CA.

Soil CO_2 evolution was measured on Day 1, 2, 3, 5, 8, 15, 30, 60, 90 and 120 of the incubation. Following gas sampling, the caps were removed and the jars were flushed with air for 30 min. Additional mason jars (six for each treatment) with soils and roots were included to allow destructive sampling for N-mineralization measurements ($n = 3$; at each time point). Soil N mineralization was measured at days 0, 30, 60 and 120, by extracting 10 g of soil with 50 mL 2 M KCL. The solution was shaken for 45 min, extracted and analyzed for NO_3^- and NH_4^+ using a Lachat Automated Ion Analyzer.

Table 1
Reciprocal transplant decomposition experimental design: We added roots previously exposed to ambient levels of atmospheric CO_2 to soils previously exposed to both ambient and elevated atmospheric CO_2, and we added 'elevated CO_2 roots' to both 'ambient and elevated CO_2 soils'.

Soil	Roots incorporated in soil	
CO_2 treatment	CO_2 treatment	Species
Ambient	Ambient	*T. repens*
		F. pratense
		L. cuneata
		Litter mix
	Elevated	*T. repens*
		F. pratense
		L. cuneata
		Litter mix
Elevated	Ambient	*T. repens*
		F. pratense
		L. cuneata
		litter mix
	Elevated	*T. repens*
		F. pratense
		L. cuneata
		Litter mix

2.3. CO_2 and ^{13}C-isotope analyses

The concentration of CO_2 and its PDB-^{13}C signature were determined at the University of California-Davis Stable Isotope Facility using a continuous flow, isotope mass spectrometer (PDZ Europa TGII trace gas analyzer and Geo 20–20 isotope ratio mass spectrometer, Cheshire UK). Carbon mineralization data are expressed on a soil oven-dry (100 °C) basis.

Results of the C-isotope analyses are expressed in δ units (‰). The $\delta^{13}C$ values were determined in relation to Vienna–Pee Dee Belemnite as follows:

$$\delta^{13}C = \left(\left(R_{sample}/R_{standard}\right) - 1\right) \quad (1)$$

To calculate the amount of CO_2-C derived from plant material produced under ambient and elevated CO_2 and incubated in soil formerly exposed to elevated and ambient CO_2 the following mass balance was used (Denef et al., 2002)

$$Q_t \times \delta^{13}C_t = Q_p \times \delta^{13}C_p + Q_s \times \delta^{13}C_s + Q_b \times \delta^{13}C_b \quad (2)$$

where Q_t is the total amount of CO_2-C, $\delta^{13}C_t$ is its isotopic composition, Q_p the amount of CO_2-C derived C from the plant material, $\delta^{13}C_p$ the isotopic composition of plant material, Q_s the amount of CO_2-C derived from the unlabeled soil, $\delta^{13}C_s$ the isotopic composition of the unlabeled soil, Q_b the CO_2-C amount in the control blank jar and $\delta^{13}C_b$ the isotopic composition of CO_2 in the control blank jar. The CO_2-C derived from the plant materials (Q_p) during the incubation was quantified by subtracting soil respiration ($Q_s + Q_b$) from the respiration of soil with incorporated plant materials (Q_t).

2.4. Statistical analysis

Addition of roots from *F. pratense*, *T. repens*, *L. cuneata*, and the mix of species produced under ambient or elevated CO_2 to soil exposed to ambient or elevated CO_2 resulted in three different treatments (Table 1). A three-way comparison was made using the Univariate GLM in SPSS Statistics 17.0. An ANOVA was conducted with blocks (i.e. the field plots) as random effects and treatments (i.e. CO_2 and species treatments) as fixed effects. Statistical tests were performed on cumulative respired CO_2 at the last date of the incubation. Means were compared by the Tukey test, after confirmation that the analysis of variance was significant. The level of significance was $P \leq 0.05$.

To assess if mixing root tissue of multiple species resulted in non-additive higher decomposition rates, we compared differences in C- and N-mineralization rates between the predicted mineralization rates based on mineralization of individual species (hereafter referred to as 'predicted') and observed mineralization rates of the mix of species (hereafter referred to as 'observed'). Predicted mineralization was calculated within a block using the following method (Blair et al., 1990; Wardle et al., 1997):

$$\text{Expected respiration} = (S1_1 + S2_1 + S3_1)/3 \quad (3)$$

where the predicted mineralization is calculated by summing the root tissue derived C and N mineralization of the individual species 1–3 in each block (i.e. $S1_1 + S2_1 + S3_1$) divided by the number of species in the mix. After confirming normality and homogeneity of variances, we compared the observed versus the predicted cumulative CO_2 respiration with a student's *t*-test across blocks. In addition, we performed a linear regression with the 'predicted' and 'observed' mineralization data across times and tested whether the regression lines were different from a 1:1 line. Differences between means were tested using least significant differences. The level of significance was $P \leq 0.05$.

3. Results

3.1. Elevated CO_2 impacts on root tissue quality

There was no direct effect of elevated atmospheric CO_2 on *T. repens*, *L. cuneata,* and *F. pratense* root tissue C:N, however, the C:N ratios among individual species differed significantly and reflected their N-fixing traits (Table 2). *T. repens* had the lowest C:N ratio among species, while *F. pratense* had the highest C:N ratio (Table 2). The lignin concentration of *L. cuneata* roots grown under ambient atmospheric CO_2 was 14%, whereas the concentration of lignin in roots grown under elevated atmospheric CO_2 roots was 16%. The lignin concentration of *F. pratense* roots grown under ambient atmospheric CO_2 was 16% while lignin concentrations in roots grown under elevated atmospheric CO_2 was 18%. Due to insufficient material, replicates were composited and no statistics were conducted on the lignin data.

3.2. Elevated CO_2 impacts on root tissue C and N mineralization

There were no differences in C respiration between the control soils (soils with no roots added) previously exposed to ambient atmospheric CO_2 *versus* exposed to elevated atmospheric CO_2 (Fig. 1). The $\delta^{13}C$ values of plants and soils varied significantly between ambient and elevated atmospheric CO_2 treatments, with roots grown under ambient atmospheric CO_2 averaging a $\delta^{13}C$ signature of $-26‰$ and roots grown under elevated atmospheric CO_2 averaging a $\delta^{13}C$ of $-49‰$. Total C in soils exposed to elevated CO_2 were, on average, $-3‰$ depleted in ^{13}C compared to soils exposed to ambient CO_2. The different isotopic signatures enabled us to partition between decomposition of the roots added to the incubation and decomposition of the pre-existing SOM. After 120 days, decomposition of root material varied significantly among the three plant species and across all treatments.

F. pratense roots had the slowest rate of decomposition while *T. repens* roots had the fastest rates of decomposition independent of treatment ($P \leq 0.001$; Fig. 2a–c). In general, roots of N-fixers (*L. cuneata* and *T. repens*) decomposed more slowly when grown under elevated CO_2 compared to roots grown under ambient atmospheric CO_2; while grass (*F. pratense*) roots grown under elevated or ambient atmospheric CO_2 decomposed at a similar rate. Decomposition of *L. cuneata* roots grown under ambient atmospheric CO_2 and incubated in soils previously exposed to both ambient atmospheric CO_2 and elevated atmospheric CO_2 exceeded the decomposition rates of *L. cuneata* roots grown under elevated atmospheric CO_2 grown in soil previously exposed to ambient atmospheric CO_2 ($P \leq 0.05$ and $P \leq 0.05$, respectively; Fig. 2a). Decomposition of *T. repens* roots grown under ambient CO_2 and incubated in elevated soil exceeded decomposition of elevated roots in ambient soil and elevated roots in elevated soil (E_r in E_s) ($P \leq 0.001$, in both cases). In addition, decomposition of ambient *T. repens* roots incubated in ambient soil was faster than decomposition of elevated roots in elevated soil ($P \leq 0.05$; Fig. 2b). We found a significant interaction between soil CO_2 and root CO_2 treatments for root decomposition in soils incubated with *T. repens* roots (Psoil × root ≤ 0.05). Decomposition of *F. pratense* roots was not affected by the atmospheric CO_2 treatments (Fig. 2c). When the root tissue from the three species was mixed, roots produced under ambient CO_2 incubated in soil previously exposed to elevated CO_2 decomposed at a greater rate than elevated roots incubated in ambient soil ($P \leq 0.01$; Fig. 2d).

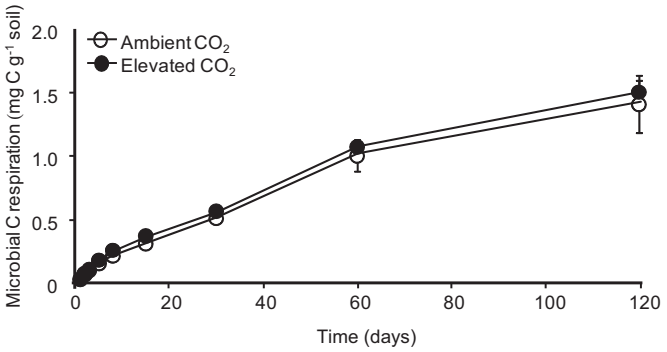

Fig. 1. Cumulative CO_2-C respiration over a 120-day incubation derived from soils previously exposed for 5 years to ambient or elevated atmospheric CO_2. Values are means ± SE ($n = 3$).

Root tissue quality impacts on potential N mineralization were similar to root tissue quality impacts on soil C mineralization, however, N mineralization was less influenced by the elevated atmospheric CO_2 treatment. Although, potential N mineralization in soil with *T. repens* roots grown under ambient CO_2 was greater than N mineralization in soil with *T. repens* roots grown under elevated CO_2 ($P \leq 0.05$; Fig. 3b). Soil potential N mineralization in ambient and elevated atmospheric CO_2 treatments was similar across the three species as well as when the species were mixed (Fig. 3a, c and d). There were no differences in potential N mineralization between soils previously exposed to ambient or elevated atmospheric CO_2 that did not have root material added ('control soils', data not shown). We did not find any significant interactions between soil CO_2 and root CO_2 treatments for soil N mineralization.

3.3. Interactions among elevated CO_2, root tissue diversity, and decomposition

We compared differences in C- and N-mineralization rates among treatments between the predicted and observed mineralization in two ways: (1) We performed a linear regression with the 'predicted' and 'observed' mineralization data across time and tested whether the regression lines were different from a 1:1 line (Fig. 4a), and (2) we compared the total C mineralization of the 'predicted' and 'mix' using a Student's *t*-test (Fig. 4b). Using the first method, we found that the observed root-derived soil C-efflux was significantly greater than the predicted root-derived soil C-efflux when roots grown under ambient atmospheric CO_2 were added to soil previously exposed to ambient atmospheric CO_2, as revealed by the diversion of the regression line compared to the 1:1 line ($P \leq 0.05$, Fig. 4a). Using the second method we found that when

Table 2
Impact of elevated CO_2 on root-litter carbon (C) and nitrogen (N) concentrations. Values are means ± SE ($n = 3$).

Species	CO_2 treatment	N (mg g^{-1})	C (mg g^{-1})	C:N
T. repens	Ambient CO_2	26.9 ± 0.54	413.2 ± 2.43	15.3 ± 0.33
	Elevated CO_2	26.8 ± 1.36	415.0 ± 4.57	15.4 ± 0.81
F. pratense	Ambient CO_2	15.4 ± 0.45	410.6 ± 3.36	26.6 ± 0.96
	Elevated CO_2	15.0 ± 0.95	403.8 ± 4.92	26.9 ± 2.15
L. cuneata	Ambient CO_2	7.66 ± 0.25	366.2 ± 20.9	47.8 ± 1.51
	Elevated CO_2	7.74 ± 0.06	399.2 ± 6.54	51.5 ± 3.86
Source of variation ANOVA				
T. repens				
CO_2		ns	ns	ns
F. pratense				
CO_2		ns	ns	ns
L. cuneata				
CO_2		ns	ns	ns
Species mixture				
CO_2		ns	ns	ns
Species		$P \leq 0.01$	ns	$P \leq 0.01$
CO_2 × species		ns	ns	ns

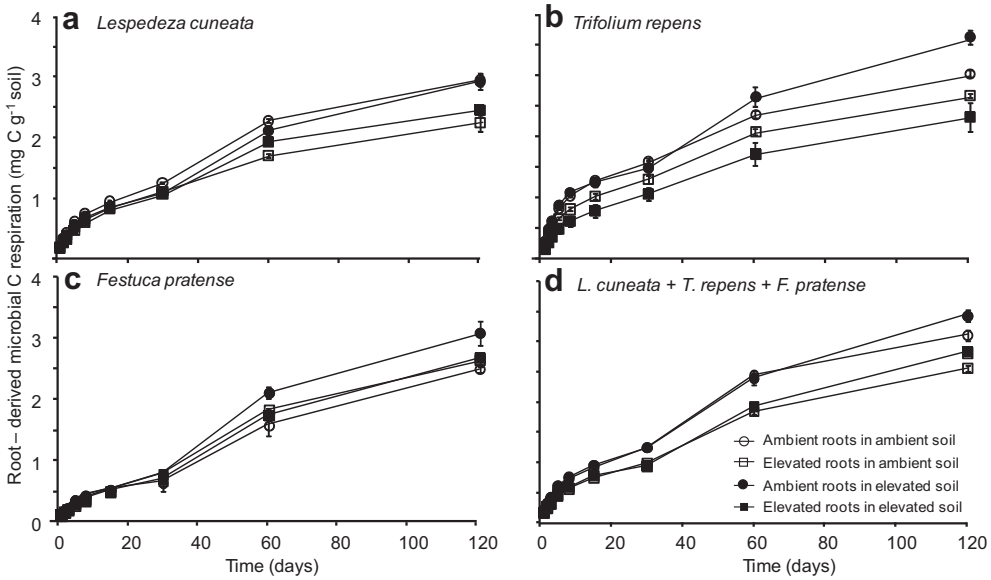

Fig. 2. Cumulative CO_2-C respiration over a 120-day decomposition incubation of soils previously exposed to ambient or elevated atmospheric CO_2 and amended with roots grown under ambient or elevated atmospheric CO_2. The reciprocal transplant design enabled us to tease apart the impact of soil origin versus the impact of substrate quality on CO_2-C respiration. Roots grown under ambient or elevated atmospheric CO_2 conditions were incubated in soils developed under ambient or elevated atmospheric CO_2 conditions. Roots of three species were incubated individually and in a mixture: (a) CO_2-C derived from decomposition of *Lespedeza cuneata* roots; (b) CO_2-C derived from decomposition of *Trifolium repens* roots; (c) CO_2-C derived from decomposition of *Festuca pratense* roots; (d) CO_2-C derived from decomposition of a mixture of *L. cuneata*, *T. repens* and *F. pratense* roots. Values are means ± SE ($n = 3$).

we compared root-derived C decomposition from roots grown under ambient atmospheric CO_2 *versus* roots grown under elevated atmospheric CO_2, mixing root species significantly increased total C respiration of the roots previously exposed to ambient atmospheric CO_2 ($P \leq 0.05$), but not of the roots previously exposed to elevated atmospheric CO_2 ($P = 0.169$; Fig. 4b). Mixing root tissue did not significantly alter N-mineralization rates within any of the treatments.

4. Discussion

Increasing plant species diversity often results in a non-additive increase in litter decomposition rates (Gartner and Cardon, 2004), while elevated atmospheric CO_2 may slow down decomposition rates of coexisting plant species. Yet, it remains uncertain how elevated CO_2 and plant species diversity may interact to alter decomposition rates. Our experiment resulted in three main

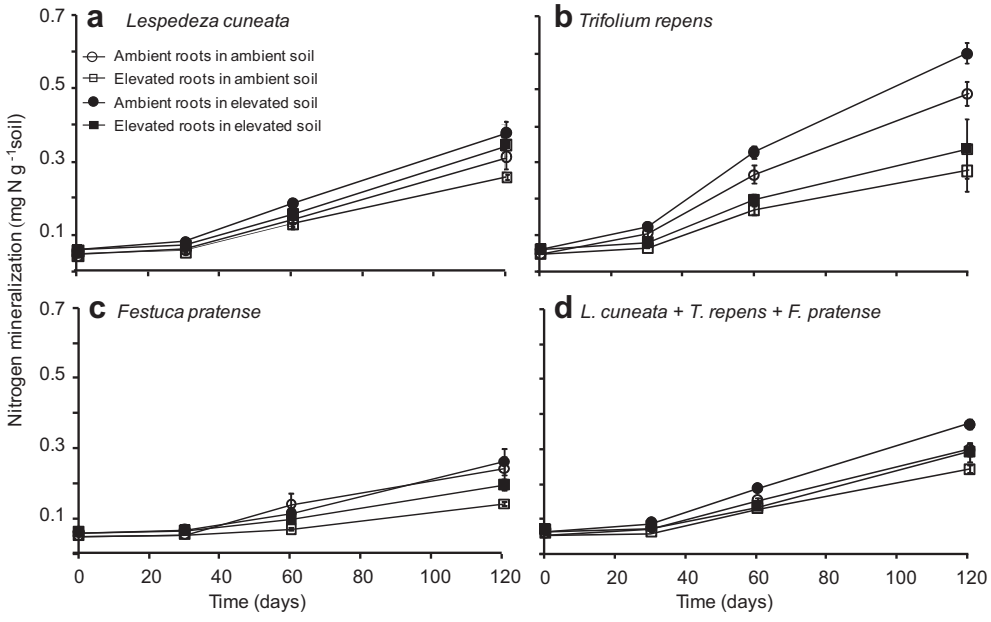

Fig. 3. Cumulative N mineralization over a 120-day incubation experiment with soils previously exposed to ambient or elevated CO_2 amended with roots previously exposed to ambient or elevated CO_2. The reciprocal transplant design enabled us to tease apart the impact of soil origin versus the impact of substrate quality on CO_2-C respiration. Roots grown under ambient or elevated atmospheric CO_2 conditions were incubated in soils developed under ambient or elevated atmospheric CO_2 conditions. Roots of three species were incubated individually and in mixture: (a) N mineralization in soil amended with *Lespedeza cuneata* roots; (b) N mineralization in soil amended with *Trifolium repens* roots; (c) N mineralization in soil amended with *Festuca pratense* roots; (d) N mineralization in soil amended with a mix of the three species. Values are means ± SE ($n = 3$). Shading conventions as in Fig. 2.

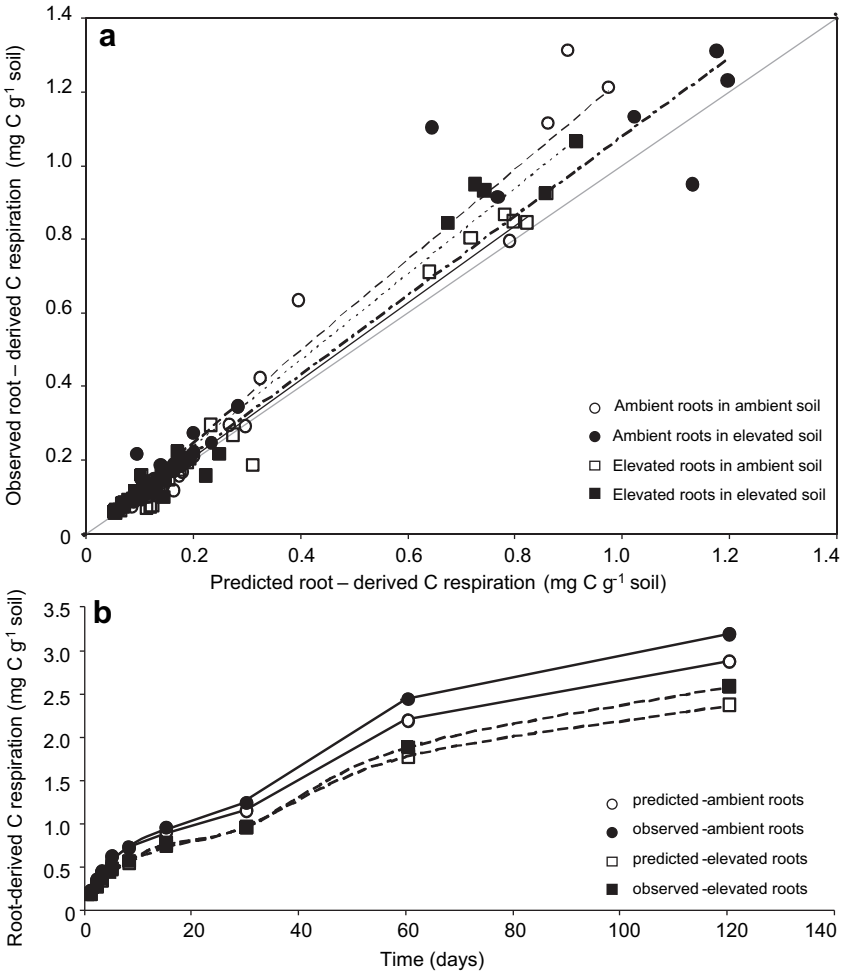

Fig. 4. Difference between the 'predicted' decomposition rates (mean of individual species' decomposition rate) and the observed decomposition rates of species decomposed in a mixture. Roots grown under ambient or elevated atmospheric CO_2 conditions were incubated in soils developed under ambient or elevated atmospheric CO_2 conditions. (a) The relationship between 'predicted' and 'observed' root-derived CO_2-C respiration data across time compared to a 1:1 line, and (b) cumulative 'predicted' and 'observed' decomposition of roots exposed to ambient *versus* elevated CO_2.

findings: (1) elevated atmospheric CO_2 did not alter C:N ratios of any of the species. However, roots grown under elevated atmospheric CO_2 had slower decomposition and N-mineralization rates in soils amended with N-fixer roots (*T. repens* and *L. cuneata*), but not grass roots (*F. pratense*); (2) decomposing roots in a mixture of the three species grown under ambient CO_2 together significantly increased decomposition rates relative to the average decomposition rates of the same species decomposed alone; (3) when roots from three plant species produced under elevated CO_2 were decomposed together, decomposition was not enhanced in a non-additive manner. Our data suggest the impact of climatic change on plant communities may be particularly important belowground where the roots of different species intermingle and are in direct contact with the soil.

4.1. Elevated CO_2 impacts on root tissue C and N mineralization

When roots of the two N-fixers, *T. repens* and *L. cuneata*, were grown under elevated atmospheric CO_2, they decomposed more slowly than roots grown under ambient CO_2 conditions. In contrast, roots of the grass species, *F. pratense*, decomposed at similar rates independent of the CO_2 treatment. Our results agree with others who found that exposure to elevated atmospheric CO_2 can slow (Ball and Drake, 1998; Gorissen et al., 1995; van Groenigen et al., 2006) or have no impact on root tissue decomposition (Dilustro et al., 2001; King et al., 2001; Knops et al., 2007). The reduction in decomposition of *T. repens* and *L. cuneata* roots grown under elevated atmospheric CO_2 was surprising given that the impact of elevated CO_2 on tissue quality and decomposition is generally small (de Graaff et al., 2006a,b; Luo et al., 2006; Norby et al., 2001). In addition, the plant tissue response to elevated CO_2 is often even smaller in legumes, due to the symbiotic relationship between legumes and the N_2-fixing *Rhizobium* bacteria (de Graaff et al., 2004; Hartwig et al., 2000). Indeed, we found no change in root tissue C:N ratios between treatments suggesting that another chemical constituent, such as lignin or suberin content may be driving the patterns observed in this system (Gorissen and Cotrufo, 2000; van Ginkel et al., 1996; van Ginkel and Gorissen, 1998).

Our speculation that changes in root tissue quality other than C:N ratios of the roots may have caused the reduction in decomposition under elevated CO_2 is supported by the N mineralization and lignin data. While not statistically tested in this study, it appears that elevated CO_2 enhanced the lignin concentration of *F. pratense* (from 16% to 18%), and *L. cuneata* (from 14% to 16%) roots in our experiment, and these changes may have contributed to the reduction in decomposition rates of *L. cuneata* root tissue grown under elevated CO_2. The reductions in root decomposition rates under elevated CO_2 were accompanied by reductions in N-mineralization rates: N mineralization was not affected by elevated CO_2 in soils amended with *F. pratense* roots, but was significantly reduced

under elevated CO_2 in soils amended with *T. repens* and in soils with *L. cuneata* roots. These results suggest that exposure to elevated CO_2 increased the abundance of recalcitrant C-rich compounds in root tissue of both legumes, which reduced N-mineralization rates.

Our reciprocal experimental design allowed us to test whether differences in the rate of decomposition or N mineralization were controlled by root tissue quality or by changes in the innate soil microbial activity. If the microbial community had caused any differences in decomposition of roots within the same treatment we should find a significant interaction between soil and root CO_2 treatments. We did not find such an interaction for soil N mineralization and the only significant interaction between soil CO_2 and root CO_2 treatments for root C decomposition was found in the soils incubated with *T. repens* roots. Our results suggest that the microbial community and its activity was not sufficiently altered in the elevated CO_2 treatments to affect root decomposition rates or soil N-mineralization rates in most treatments, although we did not directly measure the microbial community directly. Prior work in this study also found no impact of elevated CO_2 on the abundance and composition of the microbial community (Castro et al., 2010), so we feel comfortable with the assertion that CO_2-induced shifts in root quality are more important in driving changes in decomposition rates under elevated CO_2, than shifts in the microbial community.

4.2. Interactions among elevated CO_2, root tissue diversity, and decomposition

Changes in species diversity or the quality of a species in a mix might have a disproportionate impact on root decomposition and subsequent soil organic matter accrual because roots are in direct contact with the soil matrix and thus the decomposer community. In general, plant species diversity has been a poor predictor of leaf litter decomposition rates (Bardgett and Shine, 1999; Hattenschwiler et al., 2005; Meier and Bowman, 2008; Spehn et al., 2005; Wardle et al., 1997), but the chemical diversity among tissue types is a good predictor of C-mineralization rates (Epps et al., 2007; Hoorens et al., 2002; Meier and Bowman, 2008; Orwin et al., 2006). In our study, mixing the roots of three species produced under ambient CO_2 significantly enhanced C-mineralization rates relative to the predicted decomposition rate for each species. The species used in this study are chemically diverse, ranging from relatively labile roots of N-fixers with a low C:N ratio (i.e. *T. repens*) to grass roots with a high C:N ratio (i.e. *F. pratense*).

The mechanism that controls synergistic effects of tissue decomposition with enhanced tissue chemical diversity is still unknown. A likely explanatory process for this effect is priming, which is the process whereby the addition of labile compounds to soil enhances decomposition rates of more recalcitrant SOM (Kuzyakov et al., 2000; Kuzyakov, 2002; Parnas, 1976). Thus, labile compounds in high quality tissue could speed up decomposition of more recalcitrant compounds in lower quality tissue when the tissue types are mixed. Under elevated CO_2, there was no synergistic effect of tissue mixing on decomposition rates. We hypothesize that elevated CO_2 reduced the chemical diversity of the root tissue by increasing the recalcitrance of the most labile tissue in the mix. Thus, we suspect, the priming effect resulting from mixing tissue was too small to cause a significant non-additive increase in decomposition. A similar hypothesis was tested by Hoorens et al., (2002) with plant species that vary widely in tissue C:N ratios grown in the laboratory under ambient and elevated CO_2 levels. Similar to our results, i.e. elevated CO_2 reduced individual decomposition rates of the most labile tissue species in our mix (i.e. *L. cuneata* and *T. repens*), they found that elevated CO_2 removed the synergistic effects of mixing tissue only when elevated CO_2 reduced the tissue quality and individual decomposition rate of the most labile tissue species.

Since we found no significant effect of elevated CO_2 on the root quality of any of plant species in our study, we cannot unequivocally conclude that a CO_2-induced reduction of priming in the species mixture is the mechanism responsible for the observed reduction of decomposition rates. If elevated CO_2 interacts with plant species diversity to alter decomposition rates, elevated CO_2 may have a greater impact on overall belowground decomposition rates than previously predicted. Further, elevated CO_2 may indirectly affect tissue quality by causing a shift in plant species composition (Dukes and Hungate, 2002; Teyssonneyre et al., 2002). In our project we see large shifts in plant species composition over time (Engel et al., 2009; Kardol et al., 2010a,b) and these shifts result in changes in soil communities (Kardol et al., 2010b; Kardol et al., 2011) and litter decomposition rates (Tyner and Classen, 2007). Such indirect effects on tissue quality can significantly alter soil C and N cycling (Chung et al., 2007), and may outweigh the direct effect of elevated CO_2 on overall root decomposition rates within a plant community.

5. Conclusion

Our results indicate that elevated atmospheric CO_2 can impact root decomposition rates and these changes can alter soil C and N cycling. If elevated CO_2 reduces root decomposition rates of select plant species in a community, it may lead to reductions in overall decomposition rates. A large percentage of NPP is allocated belowground and elevated CO_2 is predicted to increase belowground C allocation in many ecosystems. Given the importance of roots in regulating soil C and the potential of many combinations of root mixtures in the soils, an important next step is to understand how elevated CO_2 effects on a single species' root decomposition and chemistry translates to the decomposition of a root mixture.

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