



## Interspecific plant competition increases soil labile organic carbon and nitrogen contents

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### ABSTRACT

Plant competition can impose species-specific effects on the dynamics of soil carbon (C) and nitrogen (N) through rhizosphere processes and litter input. Therefore, it is crucial to quantify these effects in various terrestrial ecosystems for a better understanding of the mechanisms. Here, we collected subsoils containing low N from a subtropical forest and planted eight dominant tree species (two deciduous and six evergreens) in these soils in a greenhouse to explore the effects of interspecific plant competition on plant growth, soil C and N contents, and soil C and N mineralization rates after the plants had grown for 12 months. Soil labile organic C and N contents were represented by soil extractable organic C (EOC) and extractable organic N (EON) contents. We assessed the magnitude of the interspecific plant competition via the relative interaction intensity (RII) index, which was calculated from the biomass of seedlings in the mixed treatments compared with the single treatments. Our results showed that interspecific plant competition had species-specific effects on plant biomass and soil total C and N contents as well as soil C mineralization rates, whereas it tended to decrease soil N mineralization rates. However, interspecific plant competition significantly decreased plant C and N contents, and significantly increased soil EOC and EON contents with increasing RII. After classifying the communities into two functional types (i.e., deciduous–evergreen versus evergreen–evergreen), similar relationships were observed. These findings address the importance of quantifying interspecific plant competition on soil labile organic C and N contents, which is helpful for understanding soil C and N cycling in forest ecosystems.

### 1. Introduction

Relationships between plant diversity and ecosystem functions have attracted wide attention in terrestrial ecology. Generally, higher plant richness can increase ecosystem functions such as soil carbon (C) and nitrogen (N) storage (Isbell et al., 2011; MacDougall et al., 2013). The increased soil C and N storage under greater plant species richness might be attributed to higher plant litter and root exudation resulting from enhanced plant productivity (Fornara and Tilman, 2008; Cong et al., 2014; Laganière et al., 2015). Numerous studies have suggested that plant competition might play an important role in soil C and N dynamics (Cheng et al., 2013; Craine and Dybzinski, 2013). However, the findings are often inconsistent because plant competition has

positive as well as negative effects on soil C and N dynamics (Fan et al., 2011; Cheng et al., 2013; Yin et al., 2018).

Two different mechanisms underlying these phenomena have been proposed. First, the positive effects of plant competition could be attributed to increases in microbial activity derived from increased root exudation and litter input to enhance N availability by accelerating N mineralization of soil organic matter (i.e., the ‘microbial N mining’ hypothesis) (Craine et al., 2007; Fontaine et al., 2011). Second, the negative effects of plant competition might be explained by the ‘nutrient competition’ hypothesis in N-poor soils (Kuzakov, 2002). This hypothesis suggests that plant growth uses up soil mineral N, thus decreasing microbial growth and metabolism, and further reducing C and N mineralization of the soil organic matter. However, the magnitude

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and direction of the effects of plant competition on soil microbial activity and C and N mineralization could be species-specific, depending on the plant species' traits. As a result, the effects of plant species on soil C and N dynamics in monocultures cannot be extrapolated to predict the effects of plant species in mixtures (De Long et al., 2019). This indicates that plant interactions are very complex and may mask relationships between plant richness and soil C and N cycling. Therefore, it is important to quantify the effects of plant competition on soil C and N dynamics for a better understanding of how plant species' interactions affect soil organic C dynamics. Here, we introduced a plant competition intensity index, namely the relative interaction intensity (RI) to quantify the effects of interspecific plant competition on soil C and N dynamics.

It is very difficult to detect significant changes in soil organic C and N contents under different management practices over short-term periods (Bu et al., 2018). In contrast to soil C and N contents, changes in labile C and N contents are easy to measure; for example, many studies have shown that soil labile organic C and N contents can be changed by competition among herbaceous and shrub plants (Ashton et al., 2008; Dijkstra et al., 2010; De Deyn et al., 2012). In this respect, labile C and N contents, measured as hot water extracts, are often used as an index because they can reflect microbial biomass well (Zhou et al., 2013) and also act as a short-term reservoir of labile resources for plants and microbes (Zhou et al., 2017). However, it remains unclear how plant competition among tree species affects soil labile organic C and N contents (Yin et al., 2018).

On the basis of previous studies (Dijkstra et al., 2010; Yin et al., 2018), we hypothesized that strong plant competition could reduce soil C and N mineralization rates and thus increase labile organic C and N contents, proportional to plant competition intensity. To test this hypothesis, we collected subsoils with low N content from a subtropical forest and planted eight dominant tree species in this soil in a greenhouse. After harvest, we determined changes in plant and soil total C and N contents, soil labile organic C and N contents, and soil C and N mineralization rates with increasing RI.

## 2. Materials and methods

### 2.1. Experimental design

We conducted the experiment in a greenhouse near a subtropical evergreen broadleaf forest station at Ningbo City, Zhejiang Province, Eastern China (29°52'N, 121°39'E). According to the past 20 years' meteorological records, the mean annual temperature and mean annual precipitation were 16.2 °C and 1374.7 mm, respectively (Yan et al., 2018). The soil type in this region was an Acrisol according to the world soil classification, with a medium-heavy loam texture (Gao et al., 2014).

Eight native tree species were selected in this experiment (Fig. S1), including the deciduous species *Quercus chenii* Nakai (QC) and *Hovenia acerba* Lindl. (HA), and six evergreen species: *Cyclobalanopsis myrsinifolia* (Blume) Oerst. (CM), *Cyclobalanopsis glauca* (Thunb.) Oerst. (CG), *Lithocarpus harlandii* (Hance ex Walp.) Rehder (LH), *Castanopsis sclerophylla* (Lindl. & Paxton) Schottky (CS), *Phoebe sheareri* (Hemsl.) Gamble (PS) and *Schima superba* Gardner & Champ. (SS). Detailed information and the phylogeny of the tree species are presented in Table 1 and Fig. S2. Seeds of these tree species were collected in November 2016. We sterilized the seeds with insecticide (imidacloprid and carbendazim) and stored them at low temperature for 5 months to break their dormancy. In April 2017, large numbers of seeds from each plant species were planted in the nursery to allow tree seedlings to grow for 2 months. Tree seedlings with similar height (approximately 10 cm for evergreen species and approximately 15 cm for deciduous species) were selected and transplanted into pots (diameter, 15 cm; height, 15 cm). Infertile soils were collected from the subsurface layer (15–30 cm) of a natural forest nearby. Each pot was filled with ~ 2 kg of soil, which had

the following properties: a pH of 5.21, 0.61% total C and 0.04% total N; the concentrations of  $\text{NH}_4^+-\text{N}$  and  $\text{NO}_3^--\text{N}$  were 1.81 and 12.71 mg  $\text{kg}^{-1}$ , respectively.

The experiment included three planting treatments (Fig. S1): a single individual species treatment (i.e., one individual of each species per pot), eight monoculture treatments (i.e., six individuals of the same species per pot) and eight two-species mixed treatments (i.e., three individuals of two species per pot, resulting in six individuals of two species per pot). Based on differences in tree functional traits and phylogenies, we selected eight mixtures as shown below: (1) one deciduous–deciduous species mixture: QC–HA; (2) four deciduous–evergreen species mixtures: QC–CG, QC–PS, QC–CM and PS–HA; and (3) three evergreen–evergreen species mixtures: CG–CS, CG–CM and LH–SS. There were 120 pots in total and every treatment had five replicates.

During the experiment, all pots received the same management practices: we watered them once a week and rearranged them every 2 weeks to prevent artefact effects from variations in glasshouse conditions. The tree seedlings were grown for 12 months in the glasshouse until harvest in August 2018. At the end of the experiment, we harvested all plants and collected soil samples from every pot (see below).

### 2.2. Measurements of plant shoot and root C, and shoot and root N contents

All plants were separated according to shoots and roots. Roots were carefully washed in clean water. Shoot and root samples were dried at 65 °C for 24 h and then weighed. Plant materials were then finely ground in a ball mill, and plant C and N contents were determined on a Vario MICRO cube elemental analyzer (Elementar, Germany).

### 2.3. Measurements of soil labile organic C and N contents

Soil samples were collected with a soil auger (2.5 cm in diameter) to a depth of 10 cm within every pot. The soil cores were immediately mixed thoroughly and kept in a cooler at 4 °C. After passing the samples through a 2-mm sieve to remove roots and stones, the soil samples were stored at 4 °C prior to analysis. Soil total C and N contents were determined on a Vario MICRO cube elemental analyzer (Elementar, Germany).

We measured labile organic C and N contents in hot water extracts as described in Zhou et al. (2013). Briefly, soil samples (6 g) were extracted with 50 mL of hot water and incubated at 70 °C for 16 h in 50-mL Falcon tubes. After that, the Falcon tubes were rotated in an end-to-end shaker at 120 rpm for 1 h and then the supernatant was filtered through Whatman No. 42 paper. The inorganic N contents (the sum of  $\text{NH}_4^+-\text{N}$  and  $\text{NO}_3^--\text{N}$ ) were measured with a Smartchem Discrete Auto Analyzer (Smartchem 200, AMS, Italy). Soil extractable organic C (EOC) and total soluble N in hot water extracts were determined on a Multi N/C 3100 total organic C analyzer fitted with a total N unit (Analytik Jena, Germany). Soil extractable organic N (EON) was calculated by subtracting extractable inorganic N from total soluble N for every soil sample.

### 2.4. Measurements of soil C and N mineralization rates

Soil samples were incubated in the laboratory to estimate the soil C mineralization rates. About 12 g (dry weight equivalent) of field-moist soil was incubated in a 1-L sealed flask in the dark at 22 °C for 2 weeks. Gas samples of 30 mL from the headspace of the flasks were taken before and after the incubation. The  $\text{CO}_2$  concentrations in the gas samples were analyzed on a gas chromatograph (Agilent 7890A GC, USA). Soil C mineralization rates were calculated from the differences in the concentrations in the gas samples between two sampling times.

Soil net N mineralization rates were determined through a 7-day anaerobic incubation under laboratory conditions as described in Chen et al. (2002) and Zhou et al. (2012). Briefly, two 6-g portions of moist

**Table 1**  
Main properties of the eight tree species used in pot experiments in the greenhouse.

Tree species	Abbreviation	Family	Genus	Functional group
<i>Quercus chenii</i>	QC	Fagaceae	<i>Quercus</i>	Deciduous
<i>Hovenia acerba</i>	HA	Rhamnaceae	<i>Hovenia</i>	Deciduous
<i>Cyclobalanopsis myrsinifolia</i>	CM	Fagaceae	<i>Cyclobalanopsis</i>	Evergreen
<i>Cyclobalanopsis glauca</i>	CG	Fagaceae	<i>Cyclobalanopsis</i>	Evergreen
<i>Lithocarpus harlandii</i>	LH	Fagaceae	<i>Lithocarpus</i>	Evergreen
<i>Castanopsis sclerophylla</i>	CS	Fagaceae	<i>Castanopsis</i>	Evergreen
<i>Phoebe sheareri</i>	PS	Lauraceae	<i>Phoebe</i>	Evergreen
<i>Schima superba</i>	SS	Theaceae	<i>Schima</i>	Evergreen

**Table 2**  
Mean  $\pm$  standard errors for plant total biomass (sum of above- and below-ground biomass) at the end of the experiment.

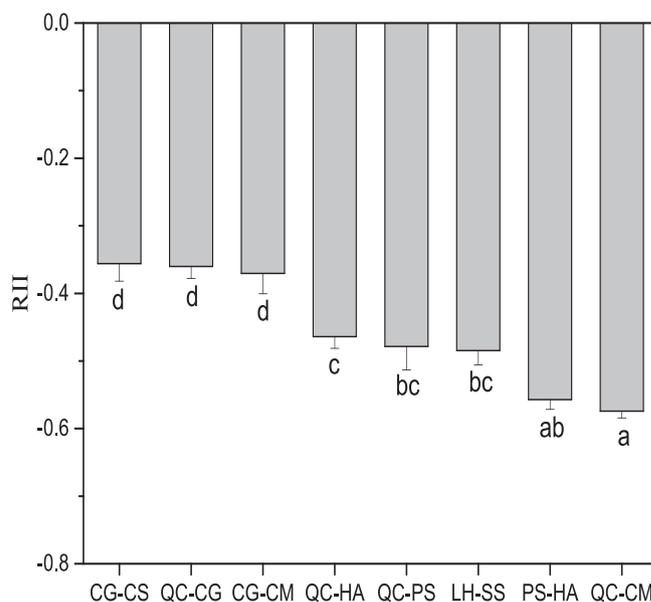
Treatments	Total biomass (g per plant per pot)
Single	
QC	8.35 $\pm$ 0.31
HA	7.16 $\pm$ 0.26
CM	5.21 $\pm$ 0.24
CG	3.79 $\pm$ 0.18
LH	5.76 $\pm$ 0.40
CS	2.68 $\pm$ 0.25
PS	2.50 $\pm$ 0.07
SS	4.87 $\pm$ 0.22
Monocultures (six seedlings)	
QC	2.29 $\pm$ 0.19
HA	1.29 $\pm$ 0.13
CM	1.35 $\pm$ 0.12
CG	2.23 $\pm$ 0.12
LH	2.94 $\pm$ 0.20
CS	1.32 $\pm$ 0.10
PS	0.96 $\pm$ 0.07
SS	1.65 $\pm$ 0.15
Mixtures (six seedlings)	
CG-CS	2.26 $\pm$ 0.27
CS	1.08 $\pm$ 0.25
QC-CG	3.82 $\pm$ 0.53
CG	1.90 $\pm$ 0.19
CG-CM	2.56 $\pm$ 0.13
CM	1.57 $\pm$ 0.24
QC-HA	3.27 $\pm$ 0.36
HA	2.54 $\pm$ 0.34
QC-PS	3.50 $\pm$ 0.27
PS	0.75 $\pm$ 0.09
LH-SS	2.53 $\pm$ 0.20
SS	1.36 $\pm$ 0.26
PS-HA	0.63 $\pm$ 0.05
HA	2.32 $\pm$ 0.25
QC-CM	2.99 $\pm$ 0.21
CM	1.03 $\pm$ 0.12

QC, *Quercus chenii*; HA, *Hovenia acerba*; CM, *Cyclobalanopsis myrsinifolia*; CG, *Cyclobalanopsis glauca*; LH, *Lithocarpus harlandii*; CS, *Castanopsis sclerophylla*; PS, *Phoebe sheareri*; SS, *Schima superba*.

soil were weighed, and one portion was amended with 20 mL of distilled water and incubated at 40 °C for 7 days, extracted with 20 mL of 4 M KCl in an end-to-end shaker for 1 h and then filtered through Whatman No. 42 filter paper. The other portion of soil was directly extracted as described above. The  $\text{NH}_4^+$ -N concentration was measured with a Smartchem Discrete Auto Analyzer (Smartchem 200, AMS, Italy).

## 2.5. Calculation of interspecific plant competition intensity and expected values

In order to quantify the effects of interspecific plant competition, we assessed the magnitude of potential competition among the seedlings in the eight mixed treatments as the RII (Armas et al., 2004):



**Fig. 1.** The relative interaction intensity (RII) of tree species in the mixed treatments. Different letters below the bars indicate significant differences in the mean values among the mixed treatments ( $P < 0.05$ ,  $n = 5$ ). QC, *Quercus chenii*; HA, *Hovenia acerba*; CM, *Cyclobalanopsis myrsinifolia*; CG, *Cyclobalanopsis glauca*; LH, *Lithocarpus harlandii*; CS, *Castanopsis sclerophylla*; PS, *Phoebe sheareri*; SS, *Schima superba*.

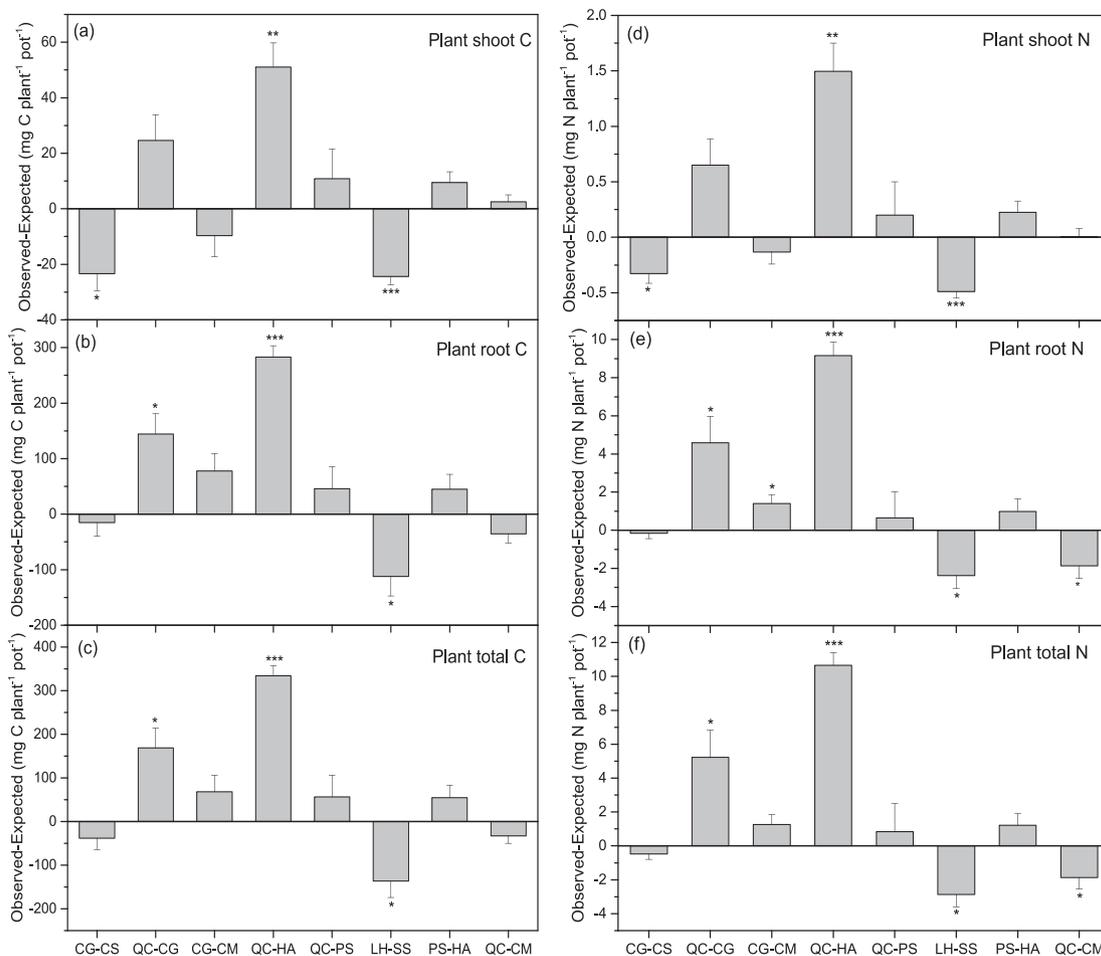
$$RII_{ij,i} = \frac{B_{mix,i} - B_{sin,i}}{B_{mix,i} + B_{sin,i}} \quad (1)$$

where  $B_{mix,i}$  is the average total biomass (shoot + root) of the three seedlings of species  $i$  in the mixed treatments and  $B_{sin,i}$  is the total biomass (shoot + root) of the seedling of species  $i$  in the single treatment. The RII values ranged from  $-1$  to  $1$ . When the RII value is negative, this indicates interspecific competition. Regarding each mixed treatment, two relative competitive intensity values were derived:  $RII_{ij,i}$  and  $RII_{ij,j}$ . We calculated the mean of these two values and used them to calculate the relationships between RII and soil properties.

We determined the effect of interspecific plant competition on shoot and root C and N contents, soil EOC and EON contents, and soil C and N mineralization rates by comparing the differences in the observed values and expected values ( $Expected_{mix}$ ) in the mixed treatments. We calculated the  $Expected_{mix}$  values of plant and soil properties based on measurements in the monocultures weighted by the plant biomass of each species in the mixtures:

$$Expected_{mix} = (TotBio_{mix,i} \times Observed_{mono,i} + TotBio_{mix,j} \times Observed_{mono,j}) / TotBio_{mix} \quad (2)$$

where  $TotBio_{mix,i}$  is the total biomass of species  $i$  in the mixture,  $Observed_{mono,i}$  is the measured plant and soil properties for species  $i$  in the monoculture and  $TotBio_{mix}$  is the total biomass in each mixed treatment. If the observed values for the mixed treatment are lower



**Fig. 2.** Observed minus expected values of plant shoot C (a), root C (b), plant total C (c), shoot N (d), root N (e) and plant total N (f) in the mixed treatments. Expected values in the mixtures were calculated from the average values of the corresponding properties in the monocultures, adjusted for species-specific plant biomass in the mixtures. The two-tailed *t*-test was used to determine significant deviations from zero. \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ . Data represent the mean and standard errors ( $n = 5$ ). QC, *Quercus chenii*; HA, *Hovenia acerba*; CM, *Cylobalanopsis myrsinifolia*; CG, *Cylobalanopsis glauca*; LH, *Lithocarpus harlandii*; CS, *Castanopsis sclerophylla*; PS, *Phoebe sheareri*; SS, *Schima superba*.

than the expected values, the interspecific competition effect is negative; if it is larger, the effect is positive.

## 2.6. Statistical analyses

We used one-way analysis of variance (ANOVA) to compare differences in tree shoot and root C and N contents, soil EOC and EON contents, and soil C and N mineralization rates among the monoculture and mixed treatments (Tables S2–S3). We also used ANOVA to compare differences in RII among the eight mixed treatments. We used the two-tailed *t*-test to test if the difference between the observed and expected values of plant C and N contents, soil EOC and EON contents, and soil C and N mineralization rates deviated significantly from zero. Simple linear regressions were used to calculate the relationships between RII (every pot) and plant and soil properties. All statistical analyses were performed with SAS and the significance level was set at  $P < 0.05$ .

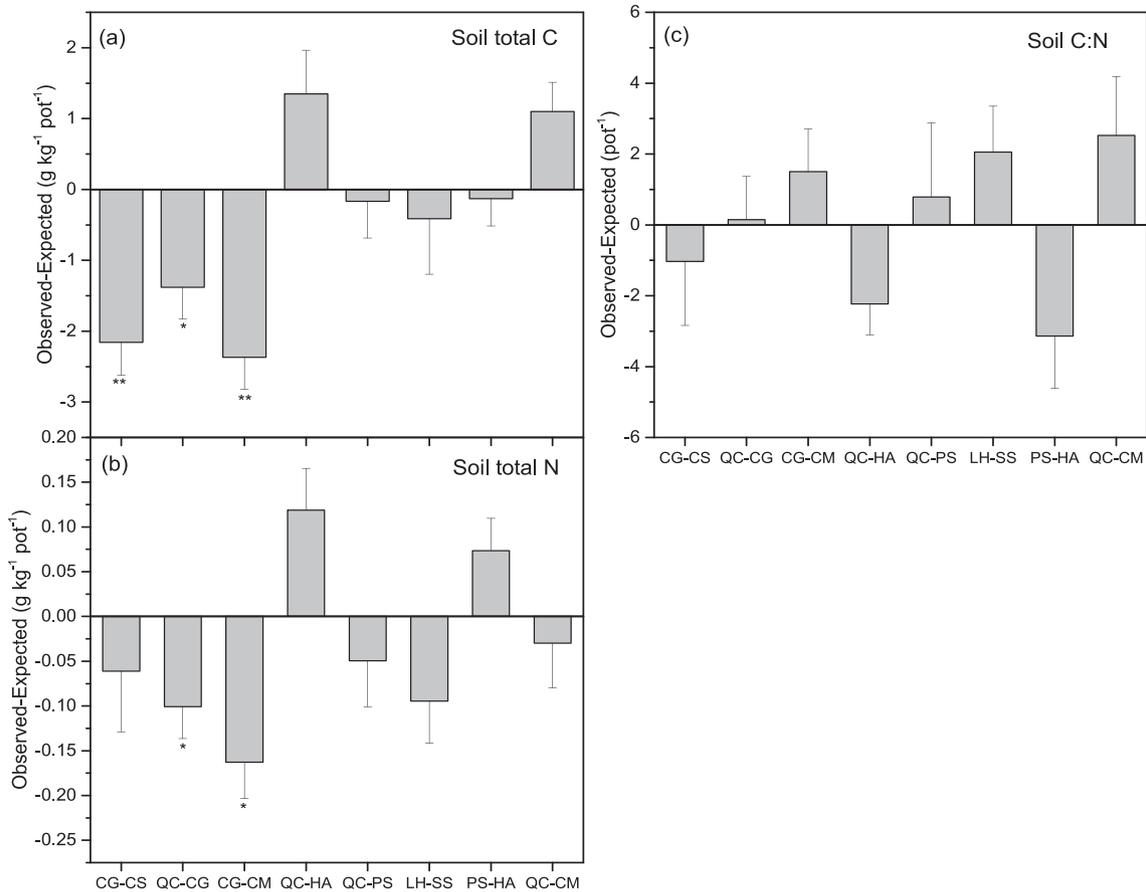
## 3. Results

### 3.1. Effects of interspecific plant competition on plant biomass, and C and N contents

When grown as a single seedlings, QC and HA had the highest total plant biomass, significantly higher than those of the other single seedlings (Table 2). However, CS and PS had the lowest total plant

biomass among the single seedlings (Table 2). In the monoculture treatments, LH had the highest total plant biomass, which was significantly higher than that of the other monocultures, whereas PS had the lowest biomass (Table 2). We found that QC–HA ( $2.91 \pm 0.35$  g) and QC–CG ( $2.86 \pm 0.36$  g) had the highest total plant biomass, significantly higher than those of the other mixed treatments, whereas PS–HA had the lowest total plant biomass ( $1.48 \pm 0.15$  g) among the mixed treatments (Table 2).

QC–CM and PS–HA had the highest RII values, which were significantly higher than those in the other mixed treatments, which showed a decreasing trend of LH–SS > QC–PS > QC–HA > CG–CM > QC–CG > CG–CS (Fig. 1, Table S1). Interspecific competition had positive or negative effects on plant properties in the eight mixed treatments (Fig. 2). Competition between QC and HA increased plant shoot C and N contents by 64.63% and 64.94% respectively; plant shoot C and N contents decreased under CG–CS and LH–SS (Fig. 2a, d). Plant root C and N contents significantly increased through interspecific competition under QC–CG and QC–HA, but significantly decreased under LH–SS (Fig. 2b, e). Significantly higher plant root N content was seen under CG–CM but significantly lower plant root N contents under QC–CM compared with the expected values (Fig. 2e). Interspecific competition significantly increased plant total C and N contents under QC–CG and QC–HA, whereas total C and N significantly decreased under LH–SS (Fig. 2c, f). Plant total N contents were significantly lower under QC–CM compared with the expected values in the mixed treatments (Fig. 2f).



**Fig. 3.** Observed minus expected values of soil total C (a), soil total N (b), and the ratio of C to N (c) in the mixed treatments. Expected values in the mixtures were calculated from the average values for soil total C, soil total N and the ratio of C to N in the monocultures, adjusted for species-specific plant biomass in the mixtures. The two-tailed *t*-test was used to determine significant deviations from zero \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ . Data represent the mean and standard errors ( $n = 5$ ). QC, *Quercus chenii*; HA, *Hovenia acerba*; CM, *Cylobalanopsis myrsinifolia*; CG, *Cylobalanopsis glauca*; LH, *Lithocarpus harlandii*; CS, *Castanopsis sclerophylla*; PS, *Phoebe sheareri*; SS, *Schima superba*.

### 3.2. Effects of interspecific plant competition on soil EOC and EON contents, and C and N mineralization rates

Interspecific competition significantly decreased soil total C and N contents under QC–CG and CG–CM (Fig. 3a, b), whereas the soil total C contents were significantly lower under CG–CS compared with the expected values (Fig. 3a). No differences in soil C:N ratios were observed among the treatments (Fig. 3c).

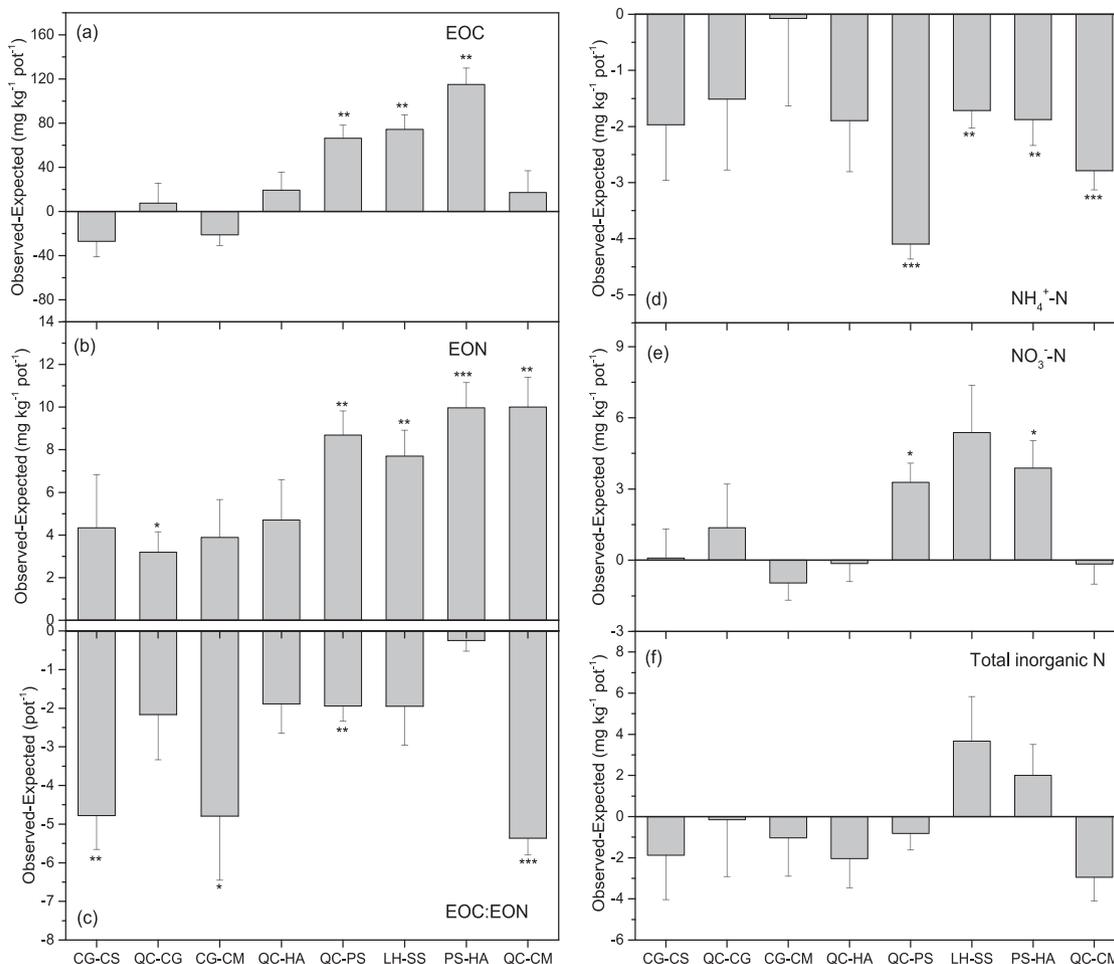
Interspecific competition significantly increased soil EOC contents by 32.41–57.31% under QC–PS, LH–SS and PS–HA (Fig. 4a, b), and markedly increased soil EON contents by 20.80–67.10% under QC–CG, QC–PS, LH–SS, PS–HA and QC–CM (Fig. 4b). The soil EOC:EON ratios were significantly lower under CG–CS, CG–CM, QC–PS and QC–CM (Fig. 4c). In general, interspecific competition decreased soil  $\text{NH}_4^+$ –N contents by 1.85–85.40% (Fig. 4d), though it tended to increase soil  $\text{NO}_3^-$ –N contents (Fig. 4e). Moreover, interspecific competition significantly decreased soil  $\text{NH}_4^+$ –N contents under QC–PS, LH–SS, PS–HA and QC–CM (Fig. 4d), whereas it significantly increased soil  $\text{NO}_3^-$ –N contents under QC–PS and PS–HA (Fig. 4e).

Interspecific competition significantly decreased the soil net N mineralization rates by 31.32–31.61% under CG–CS, QC–HA and PS–HA, and significantly increased them under QC–CM (Fig. 5b). No marked differences were found in soil C mineralization rates among the treatments (Fig. 5a).

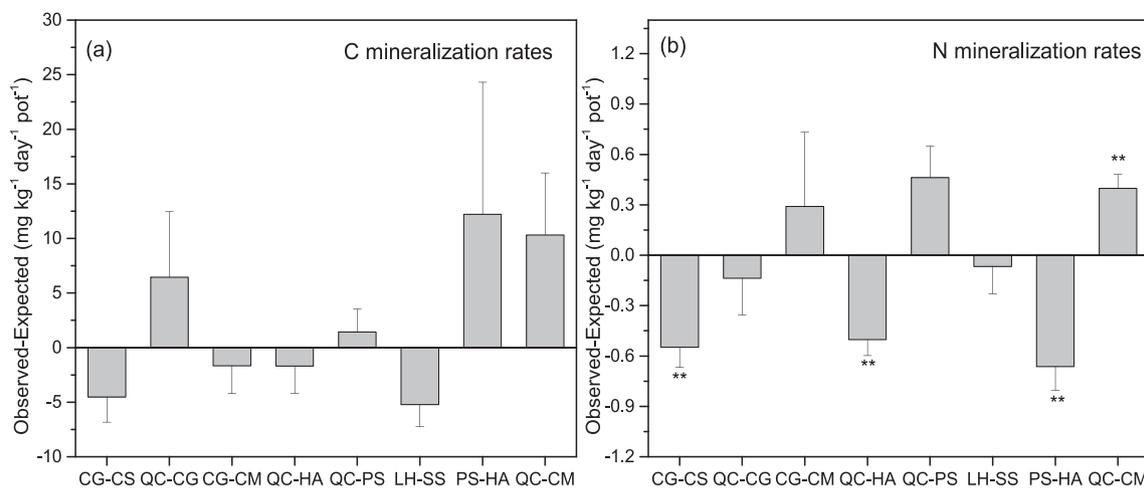
### 3.3. Relationships between competition intensity, and plant and soil properties

Significantly negative relationships were found between RII and plant root C and N contents as well as between RII and plant total C and N contents (root C:  $R^2 = 0.13$ , root N:  $R^2 = 0.12$ , plant total C:  $R^2 = 0.10$ , plant total N:  $R^2 = 0.10$ ;  $P < 0.05$ ) (Fig. 6b, c, e, f; Table S4). For deciduous–evergreen species mixtures, RII was significantly and negatively related to plant shoot C and N contents, root C and N contents, and total C and N contents (shoot C:  $R^2 = 0.54$ , shoot N:  $R^2 = 0.54$ , root C:  $R^2 = 0.71$ , root N:  $R^2 = 0.67$ , plant total C:  $R^2 = 0.71$ , plant total N:  $R^2 = 0.68$ ;  $P < 0.001$ ) (Fig. 6; Table S5). Similarly, RII was significantly and negatively related to plant shoot C and N contents, root C and N contents, and total C and N contents in the evergreen–evergreen species mixtures (shoot C:  $R^2 = 0.31$ ,  $P < 0.05$ ; shoot N:  $R^2 = 0.56$ , root C:  $R^2 = 0.70$ , root N:  $R^2 = 0.71$ , plant total C:  $R^2 = 0.71$ , plant total N:  $R^2 = 0.73$ ;  $P < 0.001$ ) (Fig. 6; Table S6).

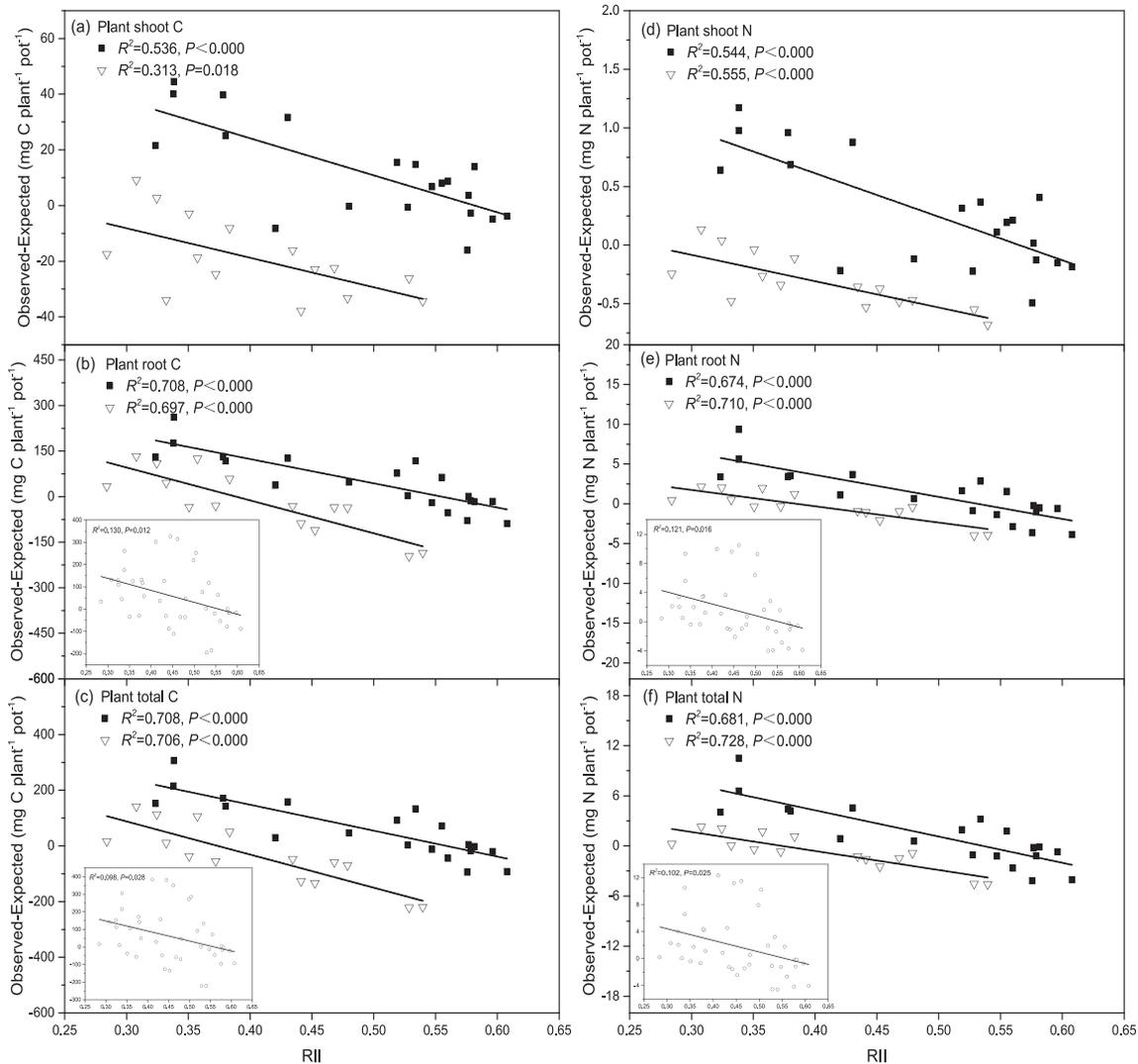
Significantly positive relationships were found between RII and soil EOC and EON contents within the treatments (EOC:  $R^2 = 0.25$ , EON:  $R^2 = 0.18$ ;  $P < 0.01$ ) (Fig. 7a, b; Table S4). The RII values were significantly and positively related to soil EON contents in deciduous–evergreen species mixtures ( $R^2 = 0.33$ ,  $P < 0.01$ ) (Fig. 7b, Table S5) but were significantly and positively related to soil EOC contents and the EOC:EON ratio in evergreen–evergreen species mixtures (EOC:  $R^2 = 0.35$ , EOC:EON ratio:  $R^2 = 0.26$ ;  $P < 0.05$ ) (Fig. 7a, c; Table S6).



**Fig. 4.** Observed minus expected values of soil extractable organic C (EOC) (a) and extractable organic N (EON) contents (b), the ratio of EOC to EON (c),  $\text{NH}_4^+\text{-N}$  (d) and  $\text{NO}_3^-\text{-N}$  contents (e), and the total inorganic N content (f) in the mixed treatments. Expected values in the mixtures were calculated from the average values of the corresponding properties in the monocultures, adjusted for species-specific plant biomass in the mixtures. The two-tailed *t*-test was used to determine significant deviations from zero. \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ . Data represent the mean and standard errors ( $n = 5$ ). QC, *Quercus chenii*; HA, *Hovenia acerba*; CM, *Cylobalanopsis myrsinifolia*; CG, *Cylobalanopsis glauca*; LH, *Lithocarpus harlandii*; CS, *Castanopsis sclerophylla*; PS, *Phoebe sheareri*; SS, *Schima superba*.



**Fig. 5.** Observed minus expected values of soil C mineralization rates (a) and net N mineralization rates (b) in the mixed treatments. Expected values in the mixtures were calculated from the average soil C and N mineralization rates in the monocultures, adjusted for species-specific plant biomass in the mixtures. The two-tailed *t*-test was used to determine significant deviations from zero. \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , \*\*\*:  $P < 0.001$ . Data represent the mean and standard errors ( $n = 5$ ). QC, *Quercus chenii*; HA, *Hovenia acerba*; CM, *Cylobalanopsis myrsinifolia*; CG, *Cylobalanopsis glauca*; LH, *Lithocarpus harlandii*; CS, *Castanopsis sclerophylla*; PS, *Phoebe sheareri*; SS, *Schima superba*.



**Fig. 6.** Linear relationship of interspecific plant competition versus the observed minus expected values for shoot C (a), root C (b), plant total C (c), shoot N (d), root N (e) and plant root N (f) in the mixed treatments. We also analyzed the linear relationship between interspecific competition and the observed minus expected values of the corresponding plant C and N contents in different functional types. (○) represents all mixed treatments, (■) represents deciduous–evergreen species mixtures and (▽) represents evergreen–evergreen species mixtures. RII, relative interaction intensity (absolute values).

#### 4. Discussion

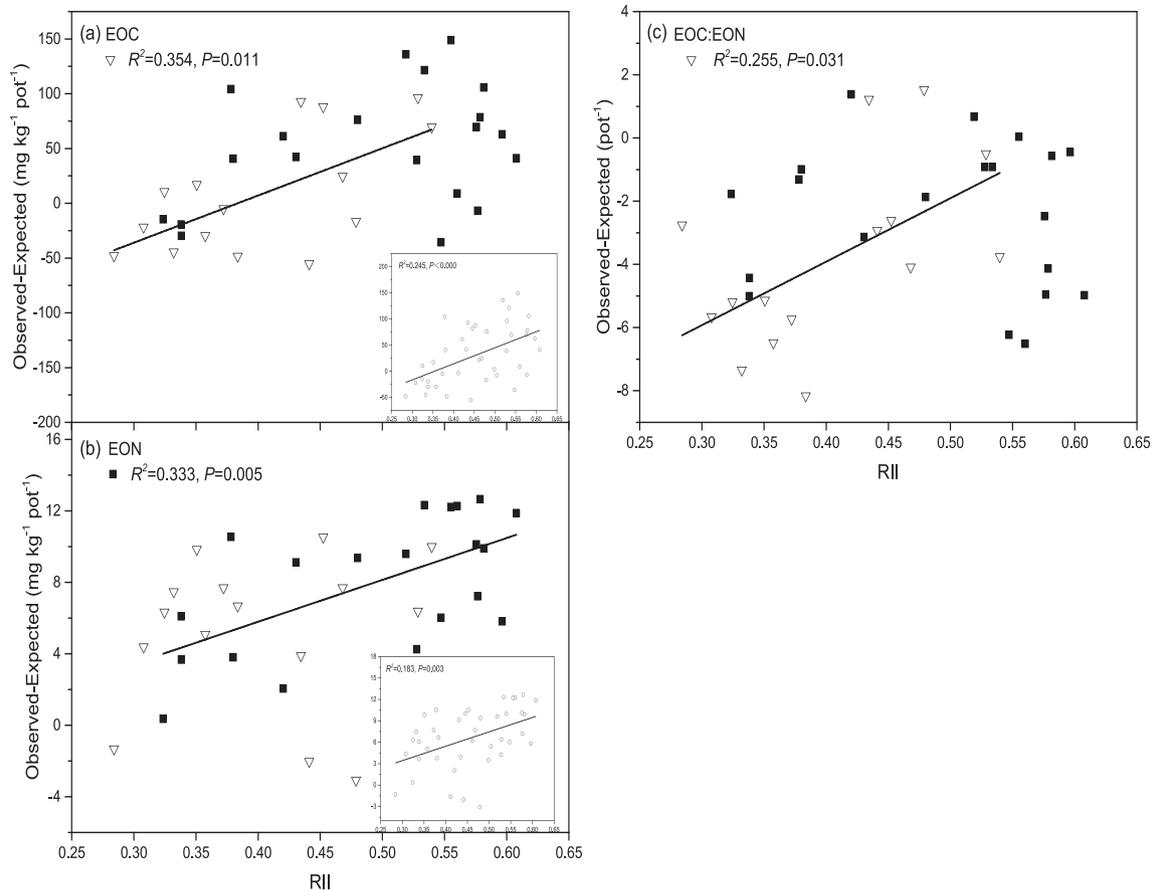
Numerous studies have explored the effects of plant competition on plant and soil C and N contents, showing species-specific effects (Dijkstra et al., 2010; Cheng et al., 2013; Yin et al., 2018). However, it remains unclear how plant competition intensity influences plant and soil C and N contents. In this study, we found that plant competition significantly decreased plant C and N contents, but significantly increased soil labile organic C and N contents with increasing plant competition intensity (Figs. 8, S7 and S8). To our knowledge, this is the first time the effects of interspecific plant competition on soil labile organic C and N contents have been quantified, which is helpful for improving our understanding of soil C and N cycling in forest ecosystems.

##### 4.1. Effects of interspecific plant competition on plant biomass, and C and N contents

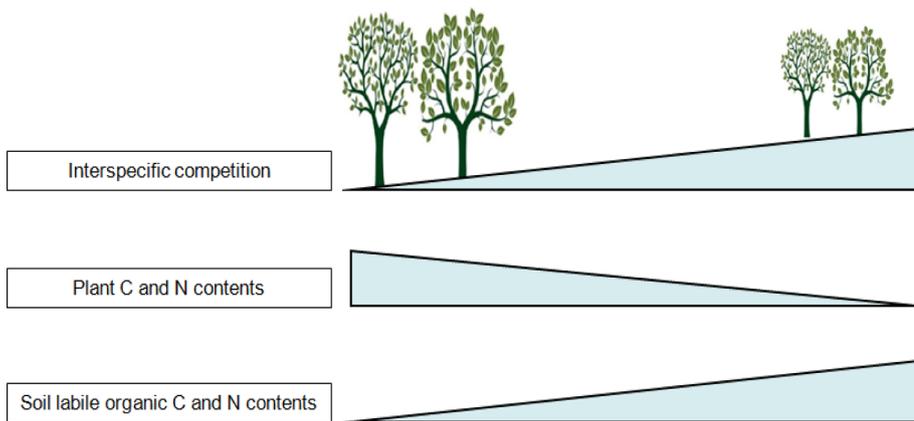
Similar to previous studies (Dijkstra et al., 2010; Medina-Roldan and Bardgett, 2012), plant competition had species-specific effects on plant biomass, and C and N contents (Fig. 2). These results were confirmed by other calculation methods (Fig. S3), as we determined the

$Expected_{mix}$  of plant C and N contents in the mixed treatments based on their averages in the monocultures (Dijkstra et al., 2010). Previous studies have shown that deciduous trees tend to have faster growth and N uptake than evergreen tree species in the early stages of growth (Seyoum et al., 2012; Chi et al., 2015). In the mixed treatments, deciduous–evergreen tree species could produce higher plant total biomass than the monocultures, as plant complementarity may override the effects of plant competition (Xu et al., 2011). This is supported by the positive effect of plant competition on root and plant total C and N contents under the deciduous–evergreen species mixture *Q. chenii*–*C. glauca* (Fig. 2), as well as by the negative effects on plant shoot C and N contents under the evergreen–evergreen mixture of *C. glauca*–*S. sclerophylla*, and the negative effects on plant shoot, root and total C and N contents under the evergreen–evergreen mixture of *L. harlandii*–*S. superba*. On the other hand, we found that the deciduous–deciduous mixture of *Q. chenii*–*H. acerba* had significantly positive effects on plant shoot, root and total C and N contents, which was in contrast with what we expected. This could be attributed to different niches of N uptake forms (Ashton et al., 2010; Zeng et al., 2014), but further studies are needed to explore the underlying mechanisms.

Additionally, we noticed that RII not only significantly decreased plant C and N contents across the mixed treatments but also had similar



**Fig. 7.** Linear relationship of interspecific plant competition versus the observed minus expected values for soil extractable organic C (EOC) (a), extractable organic N (EON) (b) and the ratio of EOC to EON (c) in the mixed treatments. We also analyzed the linear relationship between interspecific competition and the observed minus expected values of EOC, EON and the ratio of EOC to EON in different functional types. (○) represents all mixed treatments, (■) represents deciduous–evergreen species mixtures and (▽) represents evergreen–evergreen species mixtures. RII, relative interaction intensity (absolute values).



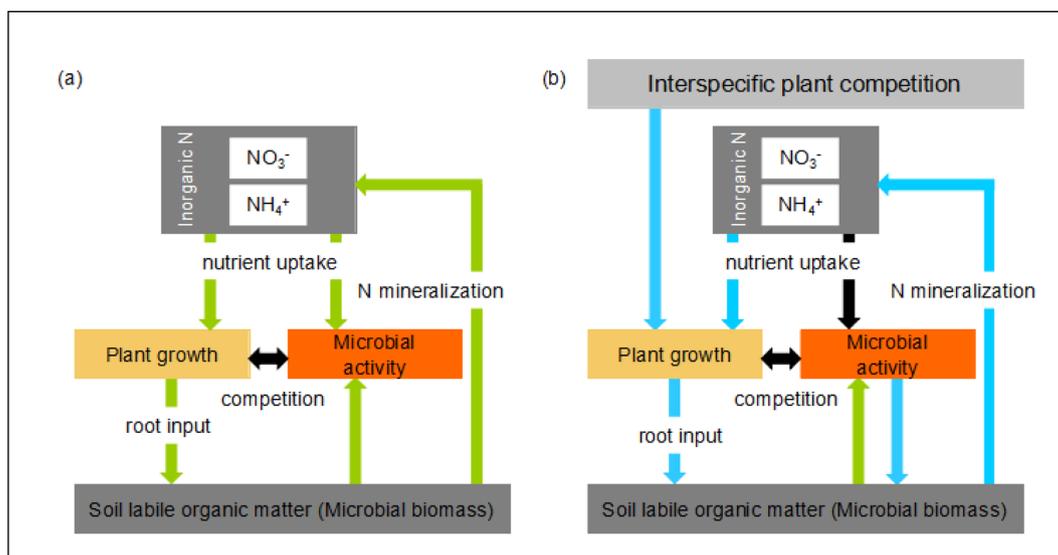
**Fig. 8.** Conceptual framework depicting the effects of interspecific competition among tree species on soil labile organic C and N pools. The blue wedges show increases and decreases (left to right). Soil labile organic C and N contents are indicated by the extractable organic C (EOC) and extractable organic N (EON) contents. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

effects on plant C and N contents in mixtures with different functional types (deciduous–evergreen species and evergreen–evergreen species) (Fig. 6). This indicates that deciduous–evergreen and evergreen–evergreen tree species mixtures have similar strategies to adapt to competition.

**4.2. Effects of interspecific plant competition on soil EOC and EON contents, and the underlying mechanisms**

Generally, soil EOC and EON contents include microbial biomass and mobile soil soluble organic matter derived from root exudation and litterfall (Zhou et al., 2017; Bu et al., 2018; Gu et al., 2019). Soil EON

can be converted to  $\text{NH}_4^+ \text{-N}$  via soil N mineralization (Shen et al., 2014). In this study, we hypothesized that under N-poor conditions, plant competition might decrease soil  $\text{NH}_4^+ \text{-N}$  through increased uptake (Fig. 9). As we expected, we found that plant competition had negative effects on soil  $\text{NH}_4^+ \text{-N}$  contents. However, in contrast to our expectation, we found that there were no differences in soil microbial activities (i.e., C mineralization rates) (Fig. 5). Our results address the importance of nutrient competition between plant and soil microorganisms, which may override the impact of microbial N mining, and play an important role in driving soil C and N dynamics under N-poor conditions in this subtropical forest. These results were supported by the significantly positive relationships among interspecific plant



**Fig. 9.** Impacts of interspecific plant competition on soil C and N dynamics and the underlying mechanisms relative to (a) common conditions and (b) interspecific competition. Green lines show positive impacts, blue lines indicate negative impacts and black lines indicate neutral impacts. Soil labile organic matter was represented by soil extractable organic C (EOC) and extractable organic N (EON) contents in hot water extracts in this study. Soil EOC and EON contents mainly included microbial biomass and can be used to represent microbial biomass (Gu et al., 2019). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

competition, soil EON contents and lower soil N mineralization rates (Figs. 5 and 9).

Unlike the effects of plant competition on plant C and N contents, plant competition mostly increased soil EOC and EON contents (Fig. 4). Consistent with previous studies (De Deyn et al., 2012), we found species-specific effects of plant competition on soil inorganic N contents. These results were also confirmed by another calculation method (Figs. S4–S6), as we determined the  $Expected_{mix}$  values of soil C and N contents in the mixed treatment based on their averages in the monocultures (Dijkstra et al., 2010). However, significant relationships between RII and soil EON contents were only observed in the deciduous–evergreen species mixtures, not in the evergreen–evergreen species mixtures (Fig. 7b). Such results could be related to the growth strategies of the two functional groups of plants (Mueller et al., 2012; Zeng et al., 2014). Further studies should focus on the effects of plant competition at different developmental stages or growth stages on soil C and N contents for a better understanding of the mechanisms behind this phenomenon.

## 5. Conclusions

Overall, plant competition had species-specific effects on plant C and N contents, soil inorganic N contents and C mineralization rates, but it tended to decrease soil N mineralization rates. However, there were significantly negative relationships between RII and plant C and N contents, but significantly positive relationships between RII and soil EOC and EON contents. Similar results were observed in deciduous–evergreen and evergreen–evergreen tree species mixtures. These results can be explained adequately by the ‘nutrient competition’ hypothesis, which may override the impact of the ‘microbial N mining’ hypothesis in this N-poor subtropical forest. Our findings address the importance of quantifying the effects of interspecific plant competition on soil labile organic C and N contents, which can improve our understanding of soil C and N cycling in forest ecosystems. Future studies should focus on rhizosphere priming effects in this respect.

## CRedit authorship contribution statement

**Miaoying Wang:** Conceptualization, Visualization, Writing-

Original draft preparation. **Jing Yang:** Investigation, Methodology, Formal analysis. **Hailun Gao:** Investigation. **Wenshi Xu:** Investigation. **Mingqiu Dong:** Investigation. **Guochun Shen:** Conceptualization, Methodology. **Juan Xu:** Methodology. **Xingliang Xu:** Writing-Reviewing and Editing. **Jianming Xue:** Writing-Reviewing and Editing. **Cheng-Yuan Xu:** Writing-Reviewing and Editing. **Xiaoqi Zhou:** Conceptualization, Supervision, Funding acquisition, Writing-Reviewing and Editing.

## Data availability

All research data from this study have been available online in the Mendeley Dataset. Website: <https://data.mendeley.com/datasets/sy6552x4d3/draft?a=c968cd9a-69d4-403f-9672-626e79c0860a>.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foreco.2020.117991>.

## References

Armas, C., Ordiales, R., Pugnaire, F.I., 2004. Measuring plant interactions: a new comparative index. *Ecology* 85, 2682–2686.

- Ashton, I.W., Miller, A.E., Bowman, W.D., Suding, K.N., 2008. Nitrogen preferences and plant-soil feedbacks as influenced by neighbors in the alpine tundra. *Oecologia* 156, 625–636.
- Ashton, I.W., Miller, A.E., Bowman, W.D., Suding, K.N., 2010. Niche complementarity due to plasticity in resource use: plant partitioning of chemical N forms. *Ecology* 91, 3252–3260.
- Bu, X.L., Gu, X.Y., Zhou, X.Q., Zhang, M.Y., Guo, Z.Y., Zhang, J., Zhou, X.H., Chen, X.Y., Wang, X.H., 2018. Extreme drought slightly decreased soil labile organic C and N contents and altered microbial community structure in a subtropical evergreen forest. *For. Ecol. Manag.* 429, 18–27.
- Chen, C.R., Xu, Z.H., Hughes, J.M., 2002. Effects of nitrogen fertilization on soil nitrogen pools and microbial properties in a hoop pine (*Araucaria cunninghamii*) plantation in southeast Queensland, Australia. *Biol. Fertil. Soils* 36, 276–283.
- Cheng, W.X., Parton, W.J., Gonzalez-Meler, M.A., Phillips, R., Asao, S., McNickle, G.G., Brzostek, E., Jastrow, J.D., 2013. Synthesis and modeling perspectives of rhizosphere priming. *New Phytol.* 201, 31–44.
- Chi, X.L., Tang, Z.Y., Xie, Z.Q., Guo, Q., Zhang, M., Ge, J.L., Xiong, G.M., Fang, J.Y., 2015. Effects of size, neighbors, and site condition on tree growth in a subtropical evergreen and deciduous broad-leaved mixed forest. *China. Ecol. Evol.* 5, 5149–5161.
- Cong, W.-F., van Ruijven, J., Mommer, L., De Deyn, G.B., Berendse, F., Hoffland, E., 2014. Plant species richness promotes soil carbon and nitrogen stocks in grasslands without legumes. *J. Ecol.* 102, 1163–1170.
- Craine, J.M., Dybzinski, R., 2013. Mechanisms of plant competition for nutrients, water and light. *Funct. Ecol.* 27, 833–840.
- Craine, J.M., Morrow, C., Fierer, N., 2007. Microbial nitrogen limitation increases decomposition. *Ecology* 88, 2105–2113.
- De Deyn, G.B., Quirk, H., Oakley, S., Ostle, N.J., Bardgett, R.D., 2012. Increased plant carbon translocation linked to overyielding in grassland species mixtures. *PLoS One* 7, e45926.
- De Long, J.R., Jackson, B.G., Wilkinson, A., Pritchard, W.J., Oakley, S., Mason, K.E., Stephan, J.G., Ostle, N.J., Johnson, D., Baggs, E.M., Bardgett, R.D., 2019. Relationships between plant traits, soil properties and carbon fluxes differ between monocultures and mixed communities in temperate grassland. *J. Ecol.* 107, 1704–1719.
- Dijkstra, F.A., Morgan, J.A., Blumenthal, D., Follett, R.F., 2010. Water limitation and plant inter-specific competition reduce rhizosphere-induced C decomposition and plant N uptake. *Soil Biol. Biochem.* 42, 1073–1082.
- Fan, F.L., Zhang, F.S., Lu, Y.H., 2011. Linking plant identity and interspecific competition to soil nitrogen cycling through ammonia oxidizer communities. *Soil Biol. Biochem.* 43, 46–54.
- Fontaine, S., Henault, C., Aamor, A., Bdioui, N., Bloor, J.M.G., Maire, V., Mary, B., Revallot, S., Maron, P.A., 2011. Fungi mediate long term sequestration of carbon and nitrogen in soil through their priming effect. *Soil Biol. Biochem.* 43, 86–96.
- Fornara, D.A., Tilman, D., 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. *J. Ecol.* 96, 314–322.
- Gao, Q., Hasselquist, N.J., Palmroth, S., Zheng, Z.M., You, W.H., 2014. Short-term response of soil respiration to nitrogen fertilization in a subtropical evergreen forest. *Soil Biol. Biochem.* 76, 297–300.
- Gu, X.Y., Zhou, X.Q., Bu, X.L., Xue, M.D., Jiang, L.L., Wang, S.P., Hao, Y.B., Wang, Y.F., Xu, X.L., Wang, G.J., Krause, S.M.B., Smail, S.J., Clinton, P.W., 2019. Soil extractable organic C and N contents, methanotrophic activity under warming and degradation in a Tibetan alpine meadow. *Agr. Ecosyst. Environ.* 278, 6–14.
- Isbell, F., Calcagno, V., Hector, A., Connolly, J., Harpole, W.S., Reich, P.B., Scherer-Lorenzen, M., Schmid, B., Tilman, D., van Ruijven, J., Weigelt, A., Wilsey, B.J., Zavaleta, E.S., Loreau, M., 2011. High plant diversity is needed to maintain ecosystem services. *Nature* 477, 199–202.
- Kuzyakov, Y., 2002. Review: Factors affecting rhizosphere priming effects. *J. Plant Nutr. Soil Sci.* 165, 382–396.
- Laganière, J., Cavard, X., Brassard, B.W., Paré, D., Bergeron, Y., Chen, H.Y.H., 2015. The influence of boreal tree species mixtures on ecosystem carbon storage and fluxes. *For. Ecol. Manag.* 354, 119–129.
- MacDougall, A.S., McCann, K.S., Gellner, G., Turkington, R., 2013. Diversity loss with persistent human disturbance increases vulnerability to ecosystem collapse. *Nature* 494, 86–89.
- Medina-Roldan, E., Bardgett, R.D., 2012. Inter-specific competition, but not different soil microbial communities, affects N chemical forms uptake by competing graminoids of upland grasslands. *PLoS One* 7, e51193.
- Mueller, K.E., Hobbie, S.E., Oleksyn, J., Reich, P.B., Eissenstat, D.M., 2012. Do evergreen and deciduous trees have different effects on net N mineralization in soil? *Ecology* 93, 1463–1472.
- Seyoum, Y., Fetene, M., Strobl, S., Beck, E., 2012. Foliage dynamics, leaf traits, and growth of coexisting evergreen and deciduous trees in a tropical montane forest in Ethiopia. *Trees* 26, 1495–1512.
- Shen, J.-P., Xu, Z.H., He, J.-Z., 2014. Frontiers in the microbial processes of ammonia oxidation in soils and sediments. *J. Soils Sediments* 14, 1023–1029.
- Xu, B.-C., Xu, W.-Z., Huang, J., Shan, L., Li, F.-M., 2011. Biomass allocation, relative competitive ability and water use efficiency of two dominant species in semiarid Loess Plateau under water stress. *Plant Sci.* 181, 644–651.
- Yan, E.-R., Zhou, L.-L., Chen, H.Y.H., Wang, X.-H., Liu, X.-Y., 2018. Linking intraspecific trait variability and spatial patterns of subtropical trees. *Oecologia* 186, 793–803.
- Yin, L.M., Dijkstra, F.A., Wang, P., Zhu, B., Cheng, W.X., 2018. Rhizosphere priming effects on soil carbon and nitrogen dynamics among tree species with and without intraspecific competition. *New Phytol.* 218, 1036–1048.
- Zeng, Y.L., Xiang, W.H., Deng, X.W., Fang, X., Liu, C., Peng, C.H., 2014. Soil N forms and gross transformation rates in Chinese subtropical forests dominated by different tree species. *Plant Soil* 384, 231–242.
- Zhou, X.Q., Chen, C.R., Wang, Y.F., Xu, Z.H., Duan, J.C., Hao, Y.B., Smail, S., 2013. Soil extractable carbon and nitrogen, microbial biomass and microbial metabolic activity in response to warming and increased precipitation in a semiarid Inner Mongolian grassland. *Geoderma* 206, 24–31.
- Zhou, X.Q., Chen, C.R., Wu, H.W., Xu, Z.H., 2012. Dynamics of soil extractable carbon and nitrogen under different cover crop residues. *J. Soils Sediments* 12, 844–853.
- Zhou, X.Q., Dong, H.B., Lan, Z.M., Bacon, G., Hao, Y.B., Chen, C.R., 2017. Vertical distribution of soil extractable organic C and N contents and total C and N stocks in 78-year-old tree plantations in subtropical Australia. *Environ. Sci. Pollut. Res.* 24, 22312–22320.