

Litter quality mediated nitrogen effect on plant litter decomposition regardless of soil fauna presence

WEIDONG ZHANG,^{1,2} LIN CHAO,^{1,3} QINGPENG YANG,^{1,2} QINGKUI WANG,^{1,2} YUNTING FANG,¹ AND SILONG WANG^{1,2,4}

¹*Institute of Applied Ecology, Chinese Academy of Sciences, Key Laboratory of Forest Ecology and Management, Shenyang 110164 China*

²*Huitong Experimental Station of Forest Ecology, Chinese Academy of Sciences, Huitong 418307 China*

³*University of Chinese Academy of Sciences, Beijing 100049 China*

Abstract. Nitrogen addition has been shown to affect plant litter decomposition in terrestrial ecosystems. The way that nitrogen deposition impacts the relationship between plant litter decomposition and altered soil nitrogen availability is unclear, however. This study examined 18 co-occurring litter types in a subtropical forest in China in terms of their decomposition (1 yr of exposure in the field) with nitrogen addition treatment (0, 0.4, 1.6, and 4.0 mol·N·m⁻²·yr⁻¹) and soil fauna exclusion (litter bags with 0.1 and 2 cm mesh size). Results showed that the plant litter decomposition rate is significantly reduced because of nitrogen addition; the strength of the nitrogen addition effect is closely related to the nitrogen addition levels. Plant litters with diverse quality responded to nitrogen addition differently. When soil fauna was present, the nitrogen addition effect on medium-quality or high-quality plant litter decomposition rate was $-26\% \pm 5\%$ and $-29\% \pm 4\%$, respectively; these values are significantly higher than that of low-quality plant litter decomposition. The pattern is similar when soil fauna is absent. In general, the plant litter decomposition rate is decreased by soil fauna exclusion; an average inhibition of $-17\% \pm 1.5\%$ was exhibited across nitrogen addition treatment and litter quality groups. However, this effect is weakly related to nitrogen addition treatment and plant litter quality. We conclude that the variations in plant litter quality, nitrogen deposition, and soil fauna are important factors of decomposition and nutrient cycling in a subtropical forest ecosystem.

Key words: nitrogen addition; plant litter decomposition; plant litter quality; soil fauna; subtropical forest.

INTRODUCTION

In terrestrial ecosystems, plant litter plays an important role in maintaining soil fertility by regulating the nutrient cycle during decomposition (Fioretto et al. 2003, Pandey et al. 2007, Vivanco and Austin 2011). Therefore, understanding the decomposition dynamics of plant litters and the controlling factors of decomposition is important. Litter decomposition is generally driven by multiple factors, including climate, litter quality, and soil organisms (Chapin et al. 2002). Climate is the primary controlling factor of litter decomposition on a global scale because the biological process during decomposition is significantly regulated by temperature and moisture. However, plant litter decomposition is determined by litter quality, soil fertility, and soil organisms on a regional scale.

Anthropogenic reactive nitrogen inputs to the forest ecosystem through nitrogen deposition have increased threefold to fivefold over the past century (Galloway and Cowling 2002); these nitrogen inputs will still increase because nitrogen deposition is expected to continue to increase globally, particularly in China (Liu et al. 2013). Studies have shown that plant litter decomposition is

influenced by nitrogen deposition (Berg 1986, Fog 1988, Dijkstra et al. 2004, Bragazza et al. 2012). However, the direction of the nitrogen effect reported by different authors is inconsistent; various studies show that the addition of external nitrogen enhanced (Hunt et al. 1988, Hobbie 2000), suppressed (Carreiro et al. 2000), or had no significant effect on (Prescott 1995, Bryant et al. 1998) plant litter decomposition.

The different responses of plant litter decomposition to nitrogen addition might be partly explained by plant litter quality. On the basis of meta-analysis, Knorr et al. (2005) reported that nitrogen addition not only increased the decomposition of high-quality litters but also reduced the mass loss of low-quality litters. However, Jiang et al. (2014) presented the opposite result; nitrogen addition enhanced low-quality pine needle decomposition and decreased high-quality *Cryptocarya chinensis* leaf litter decomposition. This inconsistency might be attributed to the following reasons. First, most studies on this topic were conducted using few plant litter types; this limited method makes it difficult to build a reliable relationship between plant litter quality and nitrogen effect. Second, although meta-analysis study can partly overcome this limitation problem by involving a large number of litter species, defining litter quality is difficult because not all the chemistries of plant litters were reported in the original papers. For example, Knorr et al. (2005) placed each litter

Manuscript received 11 January 2016; revised 16 May 2016; accepted 13 June 2016. Corresponding Editor: J. B. Yavitt.

⁴E-mail: slwang@iae.ac.cn

type into three litter quality categories primarily based on lignin concentration. However, Coq et al. (2010) observed no correlation between initial litter lignin concentration and decomposition based on 16 co-occurring tropical rain forest tree species. Finally, soil fauna might alter the relationship between the nitrogen effect and litter quality; soil fauna can reportedly modify litter quality by enhancing the nitrogen concentration of litter in a forest ecosystem (Yang and Chen 2009). Therefore, further investigation should be performed considering many plant litter species and many litter properties indicated as litter quality to better understand the influence of nitrogen addition on litter decomposition.

Several mechanisms can explain the relationship between plant litter quality and nitrogen effect on plant litter decomposition, and two of these mechanisms are opposite of one another. The first mechanism is that decomposition can be determined by the stoichiometry of plant litter and microbial demands for resources; the decomposition rate often increases with the decrease in C/N ratio (Sterner and Elser 2002). Based on this theory, nitrogen addition can enhance plant litter decomposition by regulating its C/N ratio, particularly for low-quality litter species such as Chinese fir (Liao et al. 2000). The second mechanism is related to the “microbial nitrogen mining” hypothesis (Moorhead and Sinsabaugh 2006), which states that certain microbes use labile C to decompose recalcitrant organic matter to acquire N. If the external N meets the microbial demand for N, then the microbes can reduce the resources invested to decompose the recalcitrant organic component. In this case, nitrogen addition is expected to decrease plant litter decomposition, particularly for high-quality litters (Hobbie 2008).

Inconsistency in the effects of nitrogen addition on plant litter decomposition might also be related to soil fauna. On one hand, soil fauna can enhance mass loss by the fractionation and consumption of litter (Coleman and Crossley 1996). For example, several studies have reported a significant decrease in litter decomposition when soil fauna was excluded in forests (Heneghan et al. 1999, Yang and Chen 2009) and grasslands (Smith and Bradford 2003, Wall et al. 2008). On the other hand, soil fauna communities are sensitive to environmental changes, such as nitrogen deposition. For example, Gan et al. (2013) reported that long-term experimental N deposition reduced the abundance and composition of soil fauna in four sugar maple-dominated forests in North America. Ochoa-Hueso (2014) also reported that simulated N deposition had a significant influence on the total number of individuals in soil and on *Collembola* and *Paurodora* abundance in a semiarid Mediterranean shrubland. Therefore, we anticipate that the altered activity of soil fauna also interacts with nitrogen addition to influence the decomposition rates of plant litter.

The present study examined the potential effects of plant litter quality, nitrogen availability, soil fauna, and their interaction on plant litter decomposition. A field incubation experiment was conducted with nitrogen

addition and soil fauna exclusion treatments in a subtropical forest. Nitrogen addition (0, 0.4, 1.6, and 4.0 mol·N·m⁻²·yr⁻¹) and soil fauna exclusion were applied independently and in combination on 18 dominant tree species litters with different qualities. This study hypothesizes that (1) the plant litter quality would control the response of litter decomposition to nitrogen addition; (2) the nitrogen effect on litter decomposition can be modified by soil fauna; and (3) the soil faunas have a significant effect on the decomposition of high-quality plant litters because of feeding preferences.

MATERIALS AND METHODS

Study site

The study site is located at the Huitong Natural Research Station of Forest Ecosystem (26°40' to 27°09' N and 109°26' to 110°08' E) in Hunan Province, China. The study area has a humid mid-subtropical monsoon climate. Mean annual temperature is 16.5°C; mean annual precipitation is 1,200 mm. The secondary broad-leaved forest is dominated by native evergreen broad-leaved tree species, namely, *Castanopsis hystrix*, *Cyclobalanopsis glauca*, *Machilus pauhoi*, *Liquidambar formosana*, and *Juglans cathayensis*. The tree density in 2005 was 937 stems/ha with 14.0 m for tree height and 22.2 cm for tree diameter at breast height. The soil was approximately 80 cm deep and was classified as an oxisol based on US soil taxonomy (Soil Survey Staff, 1999). The soil was medium-clay loam (sand, 32%; silt, 22%; and clay, 46%) with a pH (water extract) of 4.27. The soil C/N ratio was 10.9 (calculated from concentrations) with a total N of 2.35 g/kg soil and total P of 0.15 g/kg soil (all values correspond to the top 0 cm to 10 cm of soil).

Plant material

Freshly fallen plant leaf litters were used from 18 common and relatively abundant tree species in the area of the study site (Appendix S1: Table S1). Freshly fallen litter was collected at the forests near Huitong Natural Research Station of Forest Ecosystem. Only freshly fallen leaf litter was selected, whereas leaves with obvious signs of herbivory, galls, or fungal attacks or with atypical coloration were excluded. All litters were dried at 35°C immediately after collection and then stored dry.

Litter chemistry

The oven-dried leaf litter was ground, sieved through a 0.5 mm mesh, and analyzed for chemical properties. Total N concentration was determined by the micro-Kjeldahl method; a 0.5 g sample was digested in a 10 mL concentrated H₂SO₄ by using a catalyst mixture (CuSO₄, K₂SO₄, and selenium powder) and then distilled. A 0.2 g litter sample was digested in a 10 mL triacid mixture (nitric, perchloric, and sulphuric acid, 5:1:1) and then

cooled to measure P. Total P in the digested samples was colorimetrically determined by the ammonium molybdate stannous chloride method (Olsen and Sommers 1982). For condensed tannins, the poranthocyanidin method described by Howell et al. (1976) was used. A 0.01 g sample was briefly mixed with 95% alcohol and centrifuged for 10 min at $1,776 \times g$. The residue was washed three times with acetone; then, the precipitate was air-dried after centrifugation. A 3 mL mixture (*n*-butanol and HCl, 95:5) was then added and mixed. The solution was brought to room temperature; its absorbance was read at 550 nm. Lignin content and hemicelluloses were determined following the procedure described by Fukushima and Hatfield (2001). A 100 mg plant litter was digested with a 4.0 mL AcBrHAc reagent at 50°C for 2 h with occasional mixing. After cooling the sample, the volume was made to reach 16.0 mL with HAc, and this solution was centrifuged ($3,000 \times g$, 15 min); 0.5 mL of this solution was added to a tube containing 2.5 mL of HAc and 1.5 mL of 0.3 mol/L NaOH. After shaking the sample, 0.5 mL of 0.5 mol/L hydroxylamine hydrochloride solution was added; the volume was 10 mL with HAc. The solution was then read in a spectrophotometer at 280 nm. All chemical analyses of the litter samples were conducted in triplicate for each sample; the mean of each triplicate was taken. The details of leaf litter chemistry are shown in Appendix S1: Table S2.

Plant litter decomposition

The decomposition of the same litter material used for the chemical analyses was assessed for the 18 tree species by using litterbags exposed in the secondary forest. Two different types of litterbags were constructed to determine the contribution of soil fauna to decomposition. Mesh bags of 0.1 mm were used to exclude mesofauna and macrofauna access, whereas mesh bags of 2 mm were used to allow mesofauna access but not macrofauna access. This mesh was only employed for the upper part of the litter bags to avoid the loss of litter fragments in mesh with large widths during field exposure (Coq et al. 2010, Garcia-Palacios et al. 2016). A 0.1 mm mesh is selected for the lower part facing the soil surface. Both types of litter bags were 15×15 cm and were filled with 2 g of air-dried litter. A total of 24 plots (1×1 m) were established in an evergreen broad-leaved forest in May 2013; the understory was

removed to allow the litter bags to touch the soil. The 36 litterbags with different litter species and mesh sizes were then placed in each plot on May 21, 2013. The total number of litterbags was 864 (18 species \times 2 mesh sizes \times 4 nitrogen addition levels \times 6 replicates). Thereafter, sodium nitrate was applied in pellet form 12 times per year at 1 month intervals in each plot. The total dose is equivalent to 0, 0.4, 1.6, and 4.0 mol·N·m⁻²·yr⁻¹. Each nitrogen treatment is replicated six times. All litter bags were retrieved after a total of 365 d of field exposure. Upon harvesting, the remaining litter was gently rinsed with tap water to remove soil particles, dried at 65°C to constant mass, and weighed. The annual decomposition rate constant (k , 1/yr) was calculated by the following equation: $k = \ln(M_t/M_0)/t$, where M_t is the final mass, M_0 is the initial mass converted to the equivalent mass at 65°C, and t is the incubation time (in years). By using k , a first-order exponential decay is assumed for all litter species. The soil fauna effect and nitrogen addition effect were calculated according to the following equation: $ES = 100 \times (k_t - k_c)/k_c$, where k_t is the plant litter decomposition rate in soil fauna absence or with nitrogen addition treatment and k_c is the decomposition rate in the associated control.

Data analysis

Three-way ANOVA was used to analyze the effects of plant litter quality, nitrogen addition, and soil fauna exclusion on plant litter decomposition and nitrogen addition. Two-way ANOVA was employed to examine the effect of plant litter quality and nitrogen addition on soil fauna effects. Type III sums of squares were used to assess the significance of main effects and interactions. Significant differences among treatment means were investigated by using the least significant difference multiple-comparison post hoc test. All statistical analyses were performed by using the SPSS software (version 16.0; SPSS Inc., Chicago, Illinois, USA).

RESULTS

Nitrogen addition effect on soil chemical properties

Soil nitrogen availability was significantly affected by nitrogen addition ($P < 0.05$; Table 1). The NH_4^+ -N concentration in soil was substantially higher under a 4.0 mol

TABLE 1. Nitrogen addition effect on the concentration of soil organic carbon, total nitrogen, NH_4^+ -N, NO_3^- -N, and pH.

Treatment	SOC (g/kg)	TN (g/kg)	NH_4^+ -N (mg/kg)	NO_3^- -N (mg/kg)	pH (water)
N0	16.4 (2.0) ^a	2.0 (0.1) ^a	9.5 (1.2) ^b	3.8 (0.5) ^d	4.26 (0.21) ^a
N1	15.8 (3.2) ^a	1.9 (0.2) ^a	9.2 (0.6) ^b	7.6 (2.3) ^c	4.11 (0.26) ^a
N2	14.3 (1.5) ^a	2.0 (0.2) ^a	9.5 (1.1) ^b	12.6 (1.2) ^b	4.35 (0.22) ^a
N3	14.0 (1.6) ^a	1.8 (0.2) ^a	14.9 (4.6) ^a	25.2 (1.9) ^a	4.23 (0.25) ^a

Notes: Values are the means and standard errors of six replicates per treatment. Significant differences between the mean values of the treatments in each column are indicated by different lowercase letters ($P < 0.05$).

N addition level than under other N addition levels ($P < 0.05$). The NO_3^- -N concentration increased from 3.8 ± 0.5 mg/kg in the control to 7.6 ± 2.3 , 12.6 ± 1.2 , and 25.2 ± 1.9 mg/kg under 0.4, 1.6, and 4.0 mol N addition levels, respectively. However, nitrogen addition had an insignificant influence on soil organic carbon, total nitrogen concentration, and pH ($P > 0.05$).

Plant litter quality and chemical properties

When pooled across soil fauna presence and absence, plant litters from the studied species varied significantly in plant litter decomposition rate when no nitrogen was amended (Fig. 1). Decomposition rates ranged from 0.35 per year for *Phoebe bournei* to 3.07 per year for *Sapium sebiferum*. High-quality plant litters were assumed to have fast decomposition rates. Thus, the plant litter species were divided into three groups according to the decomposition rate, namely, low-quality litter, medium-quality litter, and high-quality litter. Each group contained six plant litter species. Figure 2 shows that plant litter quality had a significant effect on litter chemistries. Compared with low-quality or medium-quality litters, high-quality litters were generally characterized by a significantly high phosphorus concentration, specific leaf area (SLA), and low lignin content and lignin/N ratio ($P < 0.05$). Furthermore, the nitrogen, cellulose, and hemicelluloses concentrations showed similar patterns among different litter quality groups; the differences were statistically insignificant though ($P > 0.05$).

Plant litter decomposition

After 1 yr of exposure in the field, the plant litter decomposition rate was significantly controlled by litter quality ($F_{2,120} = 101.5$, $P < 0.001$; Table 2), nitrogen

addition ($F_{3,120} = 15.5$, $P < 0.001$), and soil fauna exclusion ($F_{1,120} = 14.1$, $P < 0.001$). A significant interaction between litter quality and nitrogen addition ($F_{6,120} = 3.0$, $P = 0.01$) was also observed. Furthermore, the interaction between litter quality and soil fauna ($F_{2,120} = 2.1$, $P = 0.13$), as well as between nitrogen and soil fauna ($F_{3,120} = 0.4$, $P = 0.74$), were insignificant.

When pooled across different nitrogen and soil fauna treatments, the plant litter decomposition rate consistently increased with the increase in litter quality (Fig. 3). Overall, plant litter decomposition was inhibited by nitrogen addition. When soil fauna was present, the plant litter decomposition rate across all species decreased from 1.4 ± 0.2 per year under 0 mol N addition level to 1.2 ± 0.1 , 1.1 ± 0.1 , and 0.8 ± 0.1 per year under 0.4, 1.6, and 4.0 mol N addition levels, respectively. The pattern was similar when soil fauna was absent; the litter decomposition rates were 1.1 ± 0.1 , 1.0 ± 0.1 , 0.9 ± 0.1 , and 0.7 ± 0.1 per year under 0, 0.4, 1.6, and 4.0 mol N addition levels, respectively. Furthermore, nitrogen addition had a neutral or negative effect on litter decomposition in each litter quality group; these results showed similar patterns. Soil fauna plays an important role in plant litter decomposition. When pooled across different nitrogen treatment and plant litter quality groups, the plant litter decomposition rate was significantly higher when soil fauna (1.1 ± 0.1 per year) was present than when soil fauna was absent (0.9 ± 0.1 per year).

Nitrogen addition effect on plant litter decomposition

The effects of nitrogen addition on litter decomposition rates (across plant litter species and nitrogen addition treatments) varied significantly; the effects ranged from positive to neutral and even negative. The strongest positive effect occurred in *P. bournei* (50.1%)

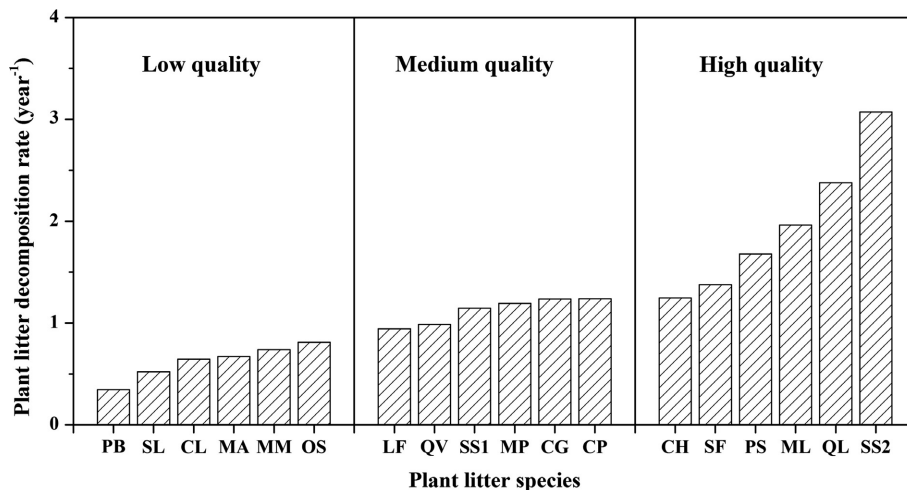


FIG. 1. Decomposition rate (1/yr) of 18 studied plant litters after 365 d of field exposure pooled across soil fauna presence and absence in ambient N treatment. According to the decomposition rate, the 18 studied plant litters were divided into three groups, namely, low quality, medium quality, and high quality. See Appendix S1 for full species names.

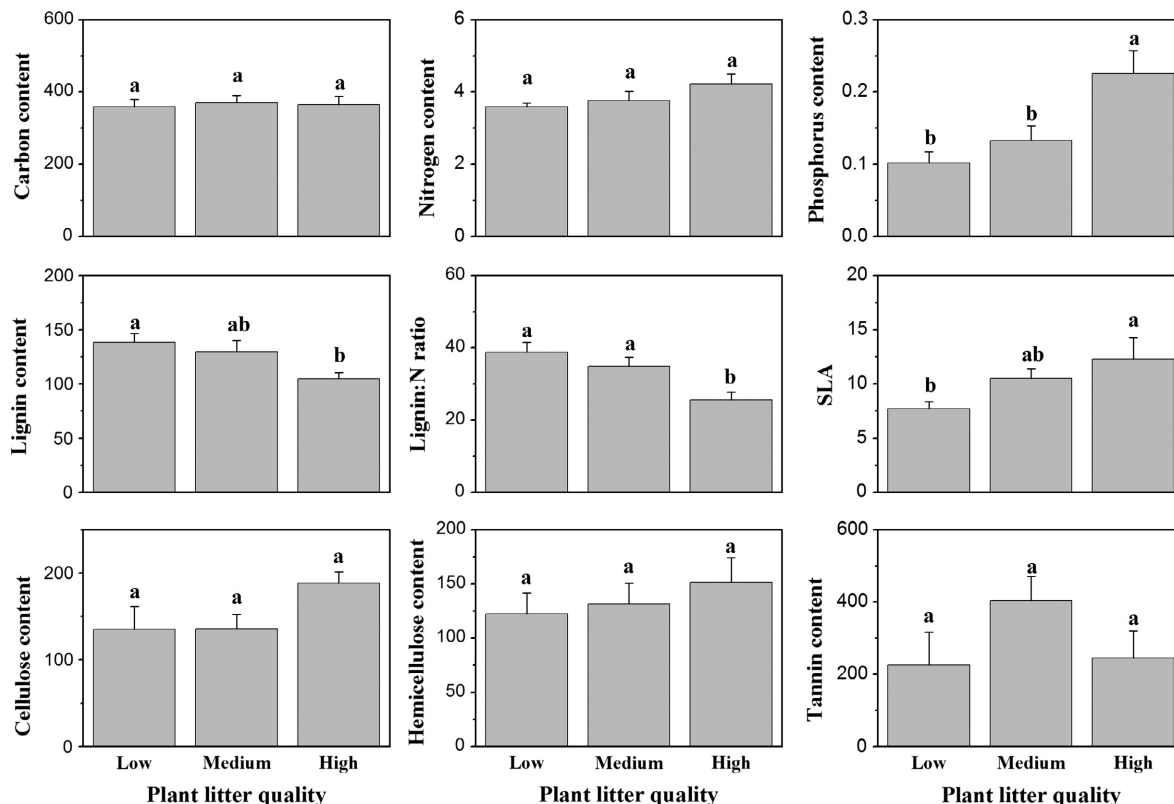


FIG. 2. Variation in litter chemistry among different litter quality groups. Data are expressed as the mean \pm 1 SE ($N = 6$). Significant differences in mean values between different plant litter quality groups are indicated by different lowercase letters ($P < 0.05$).

under a 0.4 mol N addition level when soil fauna was present and in *Cunninghamia lanceolata* (97.0%) under a 4.0 mol N addition level when soil fauna was absent. Both species were in the low-quality litter group. The strongest negative effect occurred in *Quercus laevis* under a 4.0 mol N addition level when soil fauna was present (-55.5%) or absent (-58.6%); *Q. laevis* belongs to the high-quality litter group. The strength of the nitrogen addition effect was closely related to the nitrogen addition levels ($F_{2,90} = 22.6$, $P < 0.001$; Table 3). Across all species, the nitrogen addition effect under the 1.6 and 4.0 mol N addition levels was significantly higher than that under the 0.4 mol N addition level ($P < 0.05$) when soil fauna was present. The nitrogen addition effect showed a similar pattern when soil fauna was absent with $-2.6\% \pm 4\%$, $-12.8\% \pm 4\%$, and $-33.9\% \pm 3\%$ inhibition under 0.4, 1.6, and 4.0 mol N addition levels, respectively. Plant litters with diverse qualities also responded to nitrogen addition differently ($F_{2,90} = 24.1$, $P < 0.001$). The nitrogen addition effect on plant litter decomposition with low-quality was insignificant from zero across all nitrogen addition levels ($P > 0.05$) in the presence of soil fauna. However, this effect was substantially different from zero in medium-quality and high-quality litters ($P < 0.05$) with an

average of $-26\% \pm 5\%$ and $-29\% \pm 4\%$, respectively (Fig. 4). Furthermore, the difference in the nitrogen addition effect among different litter quality groups followed the same trend when soil fauna was absent. The nitrogen effect was not influenced by the presence of soil fauna ($F_{1,90} = 0.4$, $P = 0.53$).

TABLE 2. Results of a full factorial ANOVA analysis on decomposition rate constant k (1/yr).

Effects	df	SS	MS	F	P
Fauna	1	1.5	1.5	14.1	<0.001
Quality	2	22.1	11.1	101.5	<0.001
Nitrogen	3	5.1	1.7	15.5	<0.001
Fauna \times quality	2	0.5	0.2	2.1	0.13
Fauna \times nitrogen	3	0.1	0.05	0.4	0.74
Quality \times nitrogen	6	2.0	0.3	3.0	0.01
Fauna \times quality \times nitrogen	6	0.1	0.01	0.1	0.99
Error	120	13.1			

Note: Degree of freedom, type III sum of squares, mean square, F statistic, and statistical significance are given for all the main effects, namely, soil fauna, plant litter quality, and nitrogen addition treatment.

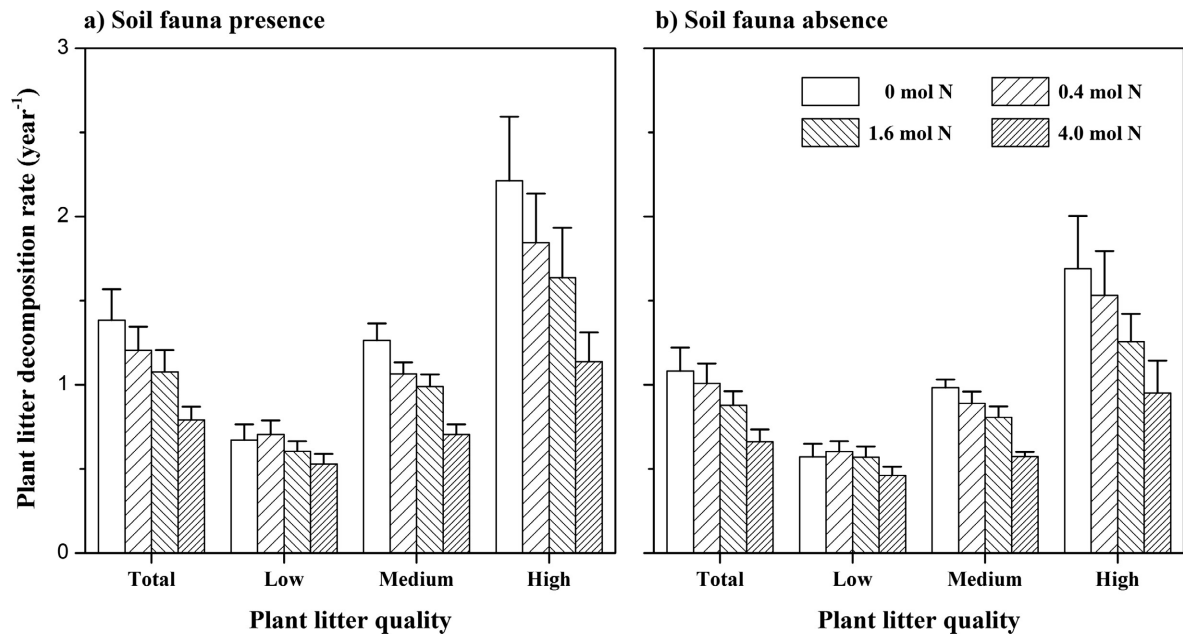


FIG. 3. Effects of nitrogen addition, plant litter quality, and soil fauna on plant litter decomposition after 365 d of field exposure.

Soil fauna effect on plant litter decomposition

Soil fauna exclusion decreased the plant litter decomposition rate with an average inhibition of $-17\% \pm 1.5\%$ across nitrogen addition treatment and litter quality groups. However, this effect was largely unpredictable; the nitrogen addition level ($F_{3,60} = 0.7, P = 0.56$), plant litter quality ($F_{2,60} = 1.2, P = 0.31$), and their interaction ($F_{6,60} = 0.7, P = 0.67$; Table 4) were not observed on soil fauna effect. When pooled across nitrogen addition levels, the soil fauna effect increased with plant litter quality with an average of $-13.9\% \pm 2.8\%$, $-17.8\% \pm 2.6\%$, and $-19.4\% \pm 2.3\%$ in plant litters with low, medium, and high quality, respectively (Fig. 5). However, these trends were statistically insignificant ($P > 0.05$).

DISCUSSION

The NO_3^- -N content of the soil responds stronger than NH_4^+ -N in the current study. This phenomenon may be attributed to the following reasons: First, the strong nitrification process in a subtropical forest (Chen and Mulder 2007, Zhang and Wang 2012) can weaken the nitrogen addition effect on the NH_4^+ -N content in soil. Second, microbes preferentially take up inorganic nitrogen as NH_4^+ -N than NO_3^- -N because of different energy costs during the assimilation process (Lavelle and Spain 2003). However, no statistically significant nitrogen addition effect is observed on the total carbon and nitrogen content of the soil; this insignificance is possibly due to the short duration of the present study. Although Waldrop et al. (2004) reported a significant gain (10%) in soil C in an oak-dominated ecosystem because of nitrogen

addition, more than 3 yr were needed to detect this positive effect. In addition, the present study determined that soil pH does not vary among N treatments, thus indicating that nitrogen availability and unchanged pH accounts for the nitrogen effect on plant litter decomposition in this study.

Plant litter chemistries related to decomposition

Previous studies have shown that plant litter decomposition is often correlated with the initial chemistry of litters, such as N content, C/N ratio, and lignin/N ratio (Aber et al. 1990, Aerts 1997). The results of the current study partly support this statement: High-quality plant litter has a high decomposition rate and has a significantly low initial lignin content and lignin/N ratio. However, the plant phosphorus content mostly varies and has the

TABLE 3. Results of the three-way analysis of variance for nitrogen addition effect using soil fauna, nitrogen addition level, plant litter quality, and their interaction as fixed factors.

Effects	df	SS	MS	F	P
Fauna	1	116.9	116.9	0.4	0.53
Nitrogen	2	13,545.0	6,772.5	22.6	<0.001
Quality	2	14,427.0	7,213.5	24.1	<0.001
Fauna × nitrogen	2	442.5	221.2	0.7	0.48
Fauna × quality	2	175.3	87.6	0.3	0.75
Nitrogen × quality	4	1,378.1	344.5	1.2	0.34
Fauna × nitrogen × quality	4	771.0	192.7	0.6	0.63
Error	90	26,947.9	299.4		

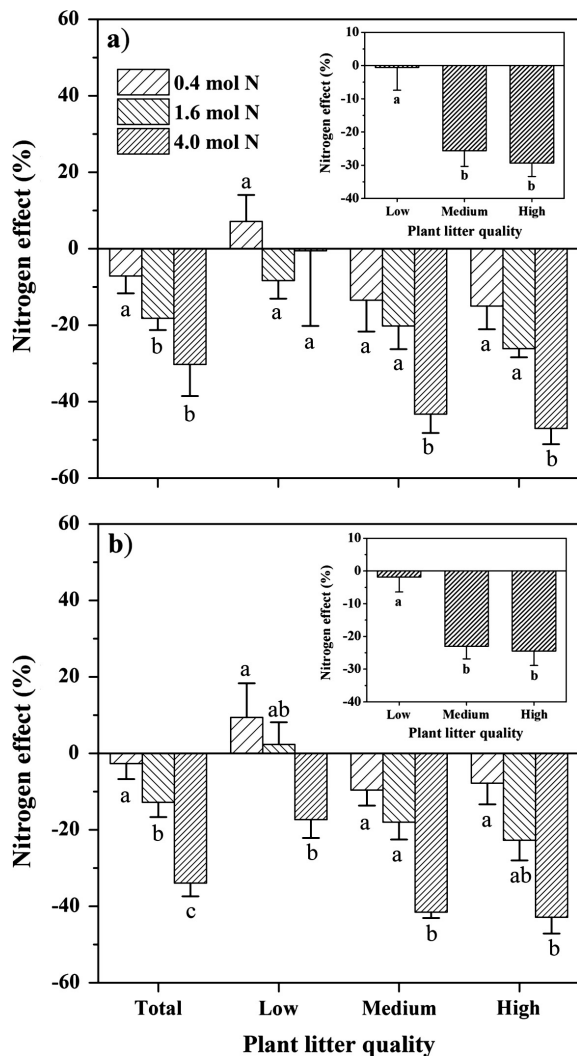


FIG. 4. Effects of nitrogen addition level, plant litter quality, and soil fauna on nitrogen addition effect.

strongest effect on litter decomposition across all chemistries. This result indicates that the subtropical forest ecosystem is limited by phosphorus because of accumulated P losses during long-term soil development in this area (Vitousek et al. 2010). Furthermore, SLA is another important determinant of plant litter decomposition. This finding is in line with the results of Huang et al. (2007); their study revealed a strong positive correlation between litter decomposition and SLA in an evergreen broad-leaved forest in eastern China.

Nitrogen effect on plant litter decomposition

The results of the present study showed that nitrogen addition generally decreases plant litter decomposition after 1 yr of exposure in the field. The nitrogen effect increases with the increase in nitrogen addition rate; this

result is in line with the finding in the grassland ecosystem reported by Liu et al. (2010). Furthermore, Knorr et al. (2005) also demonstrated that the negative nitrogen effect increases with the increase in added nitrogen when the addition rate is less than 20 times of the ambient N depositions in a meta-analysis. Clearly, the pattern of nitrogen effect in the current study cannot be explained by basic stoichiometric decomposition theory.

We determined that the nitrogen effect on high-quality plant litter decomposition is significantly larger than that on lower-quality plant litter decomposition; this result is consistent with hypothesis (1) of the present study. Several possible mechanisms can explain the relationship between nitrogen effect and plant litter quality. The first possible mechanism is that added N can react with the breakdown products of lignin degradation to form other decay-resistant complexes (Fog 1988, Berg and McClaugherty 2003). Thus, nitrogen is expected to have a more significant inhibition effect on the decomposition of plant litters with higher lignin concentrations than those with lower lignin concentrations. However, the results of the present study showed that nitrogen addition has a minor effect on the decomposition of plant litter with low quality as partly characterized by the high lignin content. Clinton et al. (1995) reported that only a small amount of the added N is incorporated into recalcitrant organic matter by the ^{15}N isotope tracer technique. Knicker (2004) also discovered that the formation of heteroaromatic N has no major effect on organic nitrogen stabilization in their studied soils and sediments. Therefore, the evidence for this hypothesis is weak; this first mechanism is unlikely to explain the rapid decrease in plant litter decomposition observed in the present study. The second possible mechanism is that other nutrients, such as P, become limiting for decomposer microbes after the addition of inorganic nitrogen (Ostertag and Hobbie 1999, Hobbie and Vitousek 2000). If this hypothesis works, then a high inhibition effect should be expected for litters with low P concentration. However, the opposite trend was observed. Therefore, this second mechanism is also unlikely to explain the result of the present study. The third mechanism is related to "nitrogen mining" theory: when no nitrogen is added, lignin decomposition is a mechanism that releases N from the cell wall. Thus, microbes need to produce enzymes, such as phenol oxidase and peroxidase, to break down lignin to assess protected N. When nitrogen is added, N is readily available in mineral form; lignin

TABLE 4. Results of the two-way analysis of variance for soil fauna effect using nitrogen addition treatment, plant litter quality, and their interaction as fixed factors.

Effects	df	SS	MS	F	P
Nitrogen	3	338.3	112.8	0.7	0.56
Quality	2	388.6	194.3	1.2	0.31
Nitrogen \times quality	6	659.4	109.9	0.7	0.67
Error	60	9,853.5	164.2		

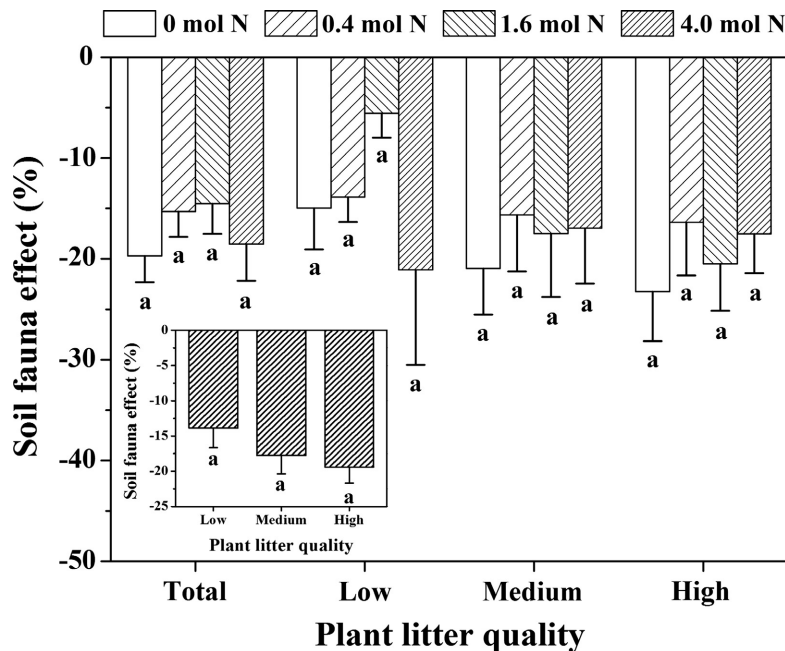


FIG. 5. Effect of plant litter quality and nitrogen addition on soil fauna effect.

degraders have little incentive to invest resources to produce lignolytic enzymes. The microbes are assumed to benefit from nitrogen mining in high-quality litter decomposition under ambient conditions. Therefore, a high nitrogen inhibition is expected on high-quality plant litters. Therefore, the results of this study are consistent with the predictions of “nitrogen mining” theory; this mechanism seems to be the most believable explanation at present.

In contrast to hypothesis (2) of the present study, the nitrogen addition effect on litter decomposition has a limited relationship with soil fauna. This result is in line with the results from the meta-analysis of Knorr et al. (2005); their results showed that the between-group heterogeneity of the nitrogen effect is insignificant for mesh size categories. These results imply that nitrogen availability regulates plant litter decomposition equally regardless of soil fauna presence.

Soil fauna effect on plant litter decomposition

Plant litter decomposition rates were reduced by 17% because of soil fauna exclusion across all litter species and nitrogen addition treatments in this study; the soil fauna effect in the present study was significantly lower than the global soil fauna effect in the previous meta-analysis study (Zhang et al. 2015). Based on the results from 75 papers that cover 197 plant species with 543 cases of plant litter decomposition experiments, plant litter decomposition is observed to decrease by 35% when soil fauna is excluded by physical or chemical exclusion techniques. This phenomenon might be attributed to the following two reasons: First, the results of the current study are based on excluding soil mesofauna (0.01 mm to 2 mm in size). However, soil

macrofauna (larger than 2 mm in size) can also contribute to litter decomposition. Therefore, the soil fauna effect on plant litter decomposition might be underestimated to some extent in the current study. Second, the larger mesh size (2 mm) was only employed for the upper part of the litter bags. This approach overcame the risk that some narrow-leaved species litter might be lost through the litter bags with larger mesh sizes. However, this approach also presents another problem: The smaller mesh size for the lower part might restrict soil fauna access to the litter, although soil fauna can get access easily to the litter through the upper layer. In fact, Kampichler and Bruckner (2009) argued that the difference in the decomposition rates in different mesh size treatments represent not only the pure soil fauna effect, but also the additive mesh size effect. Thus, the soil fauna effects observed in previous studies might be overestimated. Therefore, evaluating the contribution of the “true” soil fauna effect on litter decomposition still needs further study in the future, despite the fact that this topic has been investigated for more than 50 yr.

The soil fauna effect was observed to increase with the increase in plant litter quality across nitrogen addition treatments; this pattern was not statistically significant though. This phenomenon is related to the feeding preference of soil fauna among litter species (Spain and Le Feuvre 1987, Coq et al. 2010). High-quality litters with more nutrients and energy can be palatable to soil fauna. Thus, the soil fauna effect on litter decomposition is enhanced. By contrast, low-quality litters with more defensive materials, such as tannin, can avoid the feeding of soil fauna and subsequently lower the soil fauna effect (Coq et al. 2010). The results of a global meta-analysis has also shown that high-quality plant litters have a strong soil

fauna effect because the log effect size is negatively related to nitrogen and phosphorus content and positively related to lignin content (Zhang et al. 2015). In addition, nitrogen addition was observed to have a minor influence on the soil fauna effect. This result implies that soil fauna might contribute equally to nutrient and energy cycling in forest ecosystems in the future nitrogen deposition scenario.

In this study, the effects of nitrogen addition, soil fauna, and initial litter quality on plant litter decomposition were investigated in a subtropical forest. In general, plant litter decomposition rate was significantly reduced because of nitrogen addition; the inhabitation effect was related to litter quality. This finding implies that high-quality plant litter is more sensitive to nitrogen deposition for future climate change in this studied area than low-quality litter. The increased decomposition rate of plant litter because of soil fauna presence indicates that soil fauna plays an important role in mediating litter decomposition and consequently in nutrient return and plant productivity. However, the soil fauna effect is still largely unpredictable, because it is weakly related to nitrogen addition and plant litter quality. Overall, the present study provides evidence that the direction and extent of the nitrogen effect on plant litter decomposition can be largely explained by litter quality regardless of soil fauna presence.

ACKNOWLEDGMENTS

This research was funded by the National Key Research and Development Program of China (Grant no. 2016YFA0600800) and National Basic Research Program of China (Grant no. 2012CB416905) We are grateful to the anonymous reviewers for their comments and suggestions that helped improve this manuscript. We thank Ke Huang and Munan Zhu for their laboratory support and litter bag assemblage.

LITERATURE CITED

- Aber, J. D., J. M. Melillo, and C. A. McLaugherty. 1990. Predicting long-term patterns of mass-loss, nitrogen dynamics, and soil organic-matter formation from initial fine litter chemistry in temperate forest ecosystems. *Canadian Journal of Botany-Revue Canadienne De Botanique* 68:2201–2208.
- Aerts, R. 1997. Climate, leaf litter chemistry and leaf litter decomposition in terrestrial ecosystems: a triangular relationship. *Oikos* 79:439–449.
- Berg, B. 1986. The influence of experimental acidification on nutrient release and decomposition rates of needle and root litter in the forest floor. *Forest Ecology and Management* 15:195–213.
- Berg, B., and C. McLaugherty. 2003. *Plant litter: decomposition, humus formation, carbon sequestration*. Springer, Berlin, Germany.
- Bragazza, L., A. Buttler, J. Habermacher, L. Brancaloni, R. Gerdol, H. Fritze, P. Hanajik, R. Laiho, and D. Johnson. 2012. High nitrogen deposition alters the decomposition of bog plant litter and reduces carbon accumulation. *Global Change Biology* 18:1163–1172.
- Bryant, D. M., E. A. Holland, T. R. Seastedt, and M. D. Walker. 1998. Analysis of litter decomposition in an alpine tundra. *Canadian Journal of Botany* 76:1295–1304.
- Carreiro, M. M., R. L. Sinsabaugh, D. A. Repert, and D. F. Parkhurst. 2000. Microbial enzyme shifts explain litter decay responses to simulated N deposition. *Ecology* 81:2359–2365.
- Chapin III, S. F., P. Matson, and H. A. Mooney. 2002. *Principles of terrestrial ecosystem ecology*. Springer-Verlag, New York, New York, USA.
- Chen, X. Y., and J. Mulder. 2007. Indicators for nitrogen status and leaching in subtropical forest ecosystems, South China. *Biogeochemistry* 82:165–180.
- Clinton, P. W., R. H. Newman, and R. B. Allen. 1995. Immobilization of ¹⁵N in forest litter studied by N-15 CPMAS NMR spectroscopy. *European Journal of Soil Science* 46: 551–556.
- Coleman, D. C., and D. A. Crossley. 1996. *Fundamentals of soil ecology*. Academic, New York, New York, USA.
- Coq, S., J.-M. Souquet, E. Meudec, V. Cheynier, and S. Hättenschwiler. 2010. Interspecific variation in leaf litter tannins drives decomposition in a tropical rain forest of French Guiana. *Ecology* 91:2080–2091.
- Dijkstra, F. A., S. E. Hobbie, J. M. H. Knops, and P. B. Reich. 2004. Nitrogen deposition and plant species interact to influence soil carbon stabilization. *Ecology Letters* 7: 1192–1198.
- Fioretto, A., S. Papa, and A. Fuggi. 2003. Litterfall and litter decomposition in a low Mediterranean shrubland. *Biology and Fertility of Soils* 39:37–44.
- Fog, K. 1988. The effect of added nitrogen on the rate of decomposition of organic matter. *Biological Reviews* 63: 433–462.
- Fukushima, R. S., and R. D. Hatfield. 2001. Extraction and isolation of lignin for utilization as a standard to determine lignin concentration using the acetyl bromide spectrophotometric method. *Journal of Agricultural and Food Chemistry* 49:3133–3139.
- Galloway, J. N., and E. B. Cowling. 2002. Reactive nitrogen and the world: 200 years of change. *Ambio* 31:64–71.
- Gan, H. J., D. R. Zak, and M. D. Hunter. 2013. Chronic nitrogen deposition alters the structure and function of detrital food webs in a northern hardwood ecosystem. *Ecological Applications* 23:1311–1321.
- García-Palacios, P., E. A. Shaw, D. H. Wall, and S. Hättenschwiler. 2016. Temporal dynamics of biotic and abiotic drivers of litter decomposition. *Ecology Letters* 19:554–563.
- Heneghan, L., D. C. Coleman, X. Zou, D. A. Crossley Jr., and B. L. Haines. 1999. Soil microarthropod contributions to decomposition dynamics: tropical-temperate comparisons of a single substrate. *Ecology* 80:1873–1882.
- Hobbie, S. E. 2000. Interactions between litter lignin and soil N availability during leaf litter decomposition in a *Hawaiian montane* forest. *Ecosystems* 3:484–494.
- Hobbie, S. E. 2008. Nitrogen effects on decomposition: a five-year experiment in eight temperate sites. *Ecology* 89:2633–2644.
- Hobbie, S. E., and P. M. Vitousek. 2000. Nutrient limitation of decomposition in Hawaiian forests. *Ecology* 81:1867–1877.
- Howell, C. R., A. A. Bell, and R. D. Stipanovic. 1976. Effect of aging on flavonoid content and resistance of cotton leaves to verticillium wilt. *Physiological Plant Pathology* 8: 181–188.
- Huang, J., X. Wang, and E. Yan. 2007. Leaf nutrient concentration, nutrient resorption and litter decomposition in an evergreen broad-leaved forest in eastern China. *Forest Ecology and Management* 239:150–158.
- Hunt, H. W., E. R. Ingham, D. C. Coleman, E. T. Elliott, and C. P. P. Reid. 1988. N limitation of production and decomposition in prairie, mountain meadow, and pine forest. *Ecology* 69:1009–1016.
- Jiang, X., L. Cao, R. Zhang, L. Yan, Y. Mao, and Y. Yang. 2014. Effects of nitrogen addition and litter properties on litter decomposition and enzyme activities of individual fungi. *Applied Soil Ecology* 80:108–115.

- Kampichler, C., and A. Bruckner. 2009. The role of microarthropods in terrestrial decomposition: a meta-analysis of 40 years of litterbag studies. *Biological Reviews* 84:375–389.
- Knicker, H. 2004. Stabilization of N-compounds in soil and organic-matter-rich sediments—What is the difference? *Marine Chemistry* 92:167–195.
- Knorr, M., S. D. Frey, and P. S. Curtis. 2005. Nitrogen additions and litter decomposition: a meta-analysis. *Ecology* 86:3252–3257.
- Lavelle, P., and A. V. Spain. 2003. *Soil ecology*. Kluwer Scientific, Amsterdam, the Netherlands, 95p.
- Liao, L. P., H. Gao, S. L. Wang, Y. Q. Ma, Z. Q. Huang, and X. J. Yu. 2000. The effect of nitrogen addition on soil nutrient leaching and the decomposition of Chinese fir leaf litter. *Acta Phytocologica Sinica* 24:34–39.
- Liu, P., J. Huang, O. J. Sun, and X. Han. 2013. Enhanced nitrogen deposition over China. *Nature* 494:459–462.
- Liu, P., 2010. Litter decomposition and nutrient release as affected by soil nitrogen availability and litter quality in a semi-arid grassland ecosystem. *Oecologia* 162:771–780.
- Moorhead, D. L., and R. L. Sinsabaugh. 2006. A theoretical model of litter decay and microbial interaction. *Ecological Monographs* 76:151–174.
- Ochoa-Hueso, R., M. Arróniz-Crespo, M. A. Bowker, F. T. Maestre, M. E. Pérez-Corona, M. R. Theobald, M. G. Vivanco, and E. Manrique. 2014. Biogeochemical indicators of elevated nitrogen deposition in semi-arid Mediterranean ecosystems. *Environmental Monitoring and Assessment* 186:5831–5842.
- Olsen, S. R., and L. E. Sommers. 1982. Phosphorus. Pages 403–430 in A. L. Page, R. H. Miller, and D. R. Keeney, editors. *Methods of soil analysis, part 2*. Agronomy Society of America and Soil Science, Madison, Wisconsin, USA.
- Ostertag, R., and S. E. Hobbie. 1999. Early stages of root and leaf decomposition in Hawaiian forests: effects of nutrient availability. *Oecologia* 121:564–573.
- Pandey, R. R., G. Sharma, S. K. Tripathi, and A. K. Singh. 2007. Litterfall, litter decomposition and nutrient dynamics in a subtropical natural oak forest and managed plantation in northeastern India. *Forest Ecology and Management* 240:96–104.
- Prescott, C. E. 1995. Does N availability control rates of litter decomposition in forests? *Plant and Soil* 168–169:83–88.
- Smith, V. C., and M. A. Bradford. 2003. Litter quality impacts on grassland litter decomposition are differently dependent on soil fauna across time. *Applied Soil Ecology* 24:197–203.
- Soil Survey Staff. 1999. *Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys*. United States Department of Agriculture, Soil Conservation Service, U.S. Government Printing Office, Washington, D.C., USA.
- Spain, A. V., and R. P. Le Feuvre. 1987. Breakdown of four litters of contrasting quality in a tropical Australian rainforest. *Journal of Applied Ecology* 24:279–288.
- Sterner, R. W., and J. Elser. 2002. *Ecological stoichiometry*. Princeton University Press, Princeton, New Jersey, USA.
- Vitousek, P. M., S. Porder, B. Z. Houlton, and O. A. Chadwick. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. *Ecological Applications* 20:5–15.
- Vivanco, L., and A. T. Austin. 2011. Nitrogen addition stimulates forest litter decomposition and disrupts species interactions in Patagonia, Argentina. *Global Change Biology* 17:1963–1974.
- Waldrop, M. P., 2004. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecological Applications* 14:1172–1177.
- Wall, D. H., et al. 2008. Global decomposition experiment shows soil animal impacts on decomposition are climate-dependent. *Global Change Biology* 14:2661–2677.
- Yang, X. D., and J. Chen. 2009. Plant litter quality influences the contribution of soil fauna to litter decomposition in humid tropical forests, southwestern China. *Soil Biology & Biochemistry* 41:910–918.
- Zhang, W. D., and S. L. Wang. 2012. Effects of NH_4^+ and NO_3^- on litter and soil organic carbon decomposition in a Chinese fir plantation in South China. *Soil Biology & Biochemistry* 47:116–122.
- Zhang, W. D., S. F. Yuan, N. Hu, Y. L. Lou, and S. L. Wang. 2015. Predicting soil fauna effect on plant litter decomposition by using boosted regression trees. *Soil Biology & Biochemistry* 82:81–86.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at <http://onlinelibrary.wiley.com/doi/10.1002/ecy.1515/supinfo>