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Effects of nitrogen addition on litter decomposition and nutrient release in two tropical plantations with N_2-fixing vs. non-N_2-fixing tree species

Xiaomin Zhu · Hao Chen · Wei Zhang · Juan Huang · Shenglei Fu · Zhanfeng Liu · Jiangming Mo

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Abstract
Background and Aims Atmospheric nitrogen (N) deposition has elevated rapidly in tropical regions where N_2-fixing tree species are widespread. However, the effect of N deposition on litter decomposition in forests with N_2-fixing tree species remains unclear. We examined the effect of N addition on litter decomposition and nutrient release in two tropical plantations with *Acacia auriculiformis* (*AA*, N_2-fixing) and *Eucalyptus urophylla* (*EU*, non-N_2-fixing) in South China.
Methods Three levels of N additions were conducted: control, medium-N (50 kg N ha^{-1} $yr.^{-1}$) and high-N (100 kg N ha^{-1} $yr.^{-1}$) in each plantation.
Results Initial decomposition rate (k_a) for the control plots was faster in the *AA* plantation than in the *EU* plantation, but later in decomposition, larger fraction of slowly decomposing litter (*A*) remained in the former. N addition increased the slow fraction (*A*), decreasing soil microbial biomass and reducing acid-unhydrolyzable residue (AUR) degradation in the *AA* plantation. In the *EU* plantation, however, N additions significantly increased initial decomposition rate (k_a) and soil N availability. Furthermore, N addition decreased litter carbon and N release (in the *AA* plantation), while litter phosphorus release also decreased in both plantations.
Conclusions With ongoing N deposition in future, tropical plantations with N_2-fixing tree species would potentially increase carbon accumulation and nutrient retention in forest floor by slowing litter decomposition.

Keywords Litter decomposition · Release of carbon · Nitrogen and phosphorus · Microbial biomass · Nitrogen deposition · Nitrogen-fixing tree species

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Introduction

Litter decomposition is a fundamental ecosystem process that regulates nutrient cycling, humus formation and ecosystem carbon (C) storage (Wardle 2002; Osono and Takeda 2004; Berg and McClaugherty 2008). Several factors such as climate, soil properties, litter chemistry, and soil decomposer activity control litter decomposition rate and nutrient release (Trofymow and Moore 2002; Berg et al. 2010). Nitrogen (N), including endogenous (litter) and exogenous (soil) N availability, plays an important role in litter decomposition (Knorr et al. 2005). The initial C:N ratio is higher in the litter (71:1 on average) than that in the microbes (7:1 on average) (Yuan and Chen 2009; Xu

et al. 2013); therefore, microbes need to immobilize N from litter and soil to meet the requirements of growth and maintenance in the early stage of litter decomposition (Moore et al. 2006; Parton et al. 2007). In this stage, N may have a positive effect on litter decomposition (Hobbie 2005; Cornwell et al. 2008; Norris et al. 2013). However, during the later stages, when the rate of litter decomposition is dominated by the degradation of lignin and modified lignin-like humification products, N may have a negative influence on lignin degradation, leading to the production of substantial amounts of residue (Berg and McClaugherty 2008).

Global N deposition is expected to reach 200 Tg N $yr.^{-1}$ in 2050, with the greatest N deposition occurring in tropical regions (Galloway et al. 2004, 2008). In China, the rate of N deposition has reached 30–73 kg N ha^{-1} $yr.^{-1}$ in some tropical and subtropical forests (Fang et al. 2011). As a major source for exogenous N for forest ecosystems, N deposition was recently recognized as an important factor that controls litter decomposition. For example, N deposition can affect litter decomposition directly by raising soil N availability and changing the quantity and quality of litter inputs, or indirectly by altering plant or microbial community composition (Manning et al. 2008). The effects of N deposition on litter decomposition and nutrient release in forests depend on the forest nutrient status (N-rich vs. N-poor) and the stages of decomposition (early stage vs. later stages). Previous studies have indicated that N deposition accelerates the early stage of litter decomposition in the N-poor forests by increasing soil N availability or by decreasing litter C/N ratio (Hobbie 2000; Hobbie 2005; Norris et al. 2013). However, N deposition often suppresses litter decomposition in N-rich forests. In these forests, exogenous N may (1) inhibit the synthesis of oxidative enzymes involved in the degradation of acid-unhydrolyzable residue (AUR; formerly referred to as lignin) in the later stages of decomposition (Carreiro et al. 2000); (2) react with polyphenol compounds or products of microbial degradation to form more recalcitrant materials (Hobbie 2008); and (3) alter the composition and activity of decomposers to influence their degradability (e.g., shifting from a fungi-dominated to a bacteria-dominated community) (Frey et al. 2004; Cusack et al. 2011). In addition, some other studies have shown that N additions have no significant effect on litter decomposition rate in the phosphorus (P) limited forests, but significantly change nutrient dynamics by stimulating abiotic and/or microbial immobilization (Hobbie and Vitousek 2000; Kaspari et al. 2008; Mo et al. 2008).

Tropical regions have experienced a dramatic conversion from primary forests to secondary forests and plantations (FAOUN 2010). In China, the total area of forests is 208 million ha, approximately 59 % of which are natural forests and 33 % are plantations (available data from the eighth national forest resources inventory survey of China: http://english.forestry.gov.cn/index.php/information-services/forest-resources/2- forest-resources-in-China). The percentage of forest cover in the Pearl River Delta, Guangdong Province increased from 26 % in 1979 to 56 % in 2005, due to the ecological restoration of degraded land (Peng et al. 2009). Owing to symbiotic N_2-fixation, N_2-fixing tree species generally produce litter with high N concentration, and improve soil C storage and N availability (Forrester et al. 2005; Gei and Powers 2013). Therefore, N_2-fixing tree species have been widely used as pioneer plants to facilitate ecological restoration in eroded and degraded ecosystems (Ren et al. 2008). Based on previous studies (Mo et al. 2006; Hobbie 2008; Hobbie and Vitousek 2000), we concluded that N deposition might have a negative or no effect on litter decomposition in N-rich sites. However, these previous studies did not reveal any direct evidence in terms of the effects of N deposition on N_2-fixing species that play an important role in ecological restoration worldwide. Assessing the potential effect of elevated N deposition on litter decomposition and nutrient release is critical for predicating C accumulation and nutrient retention in tropical plantations, especially those with N_2-fixing tree species.

In this study, we conducted a simulated N deposition experiment to explore the effects of N additions on litter decomposition rate, nutrient release, and soil microbial biomass in two tropical plantations with *Acacia auriculiformis* (*AA*, N_2-fixing tree species) and *Eucalyptus urophylla* (*EU*, non-N_2-fixing tree species) in South China, where recent estimates of atmospheric N deposition range from 16.2 to 38.2 kg N ha^{-1} $yr.^{-1}$ (Fang et al. 2011). We hypothesized that: (1) in the initial stage of decomposition, *AA* litter would decompose faster but leave a larger slowly decomposing fraction in the later stages than *EU* litter due to high litter N concentration and soil N availability in the *AA* plantation; (2) external N addition would decrease initial decomposition rate for *AA* litter because of the negative N effect on soil microbial biomass. However, N addition

would increase initial decomposition rate for *EU* litter due to the improvement of N availability. In the later stages of decomposition, N addition would have negative effects on decomposition for both litter types, because of the slowdown in AUR degradation; (3) N addition would delay litter N and P release because of chemical immobilization and microbial assimilation in both plantations.

Materials and methods

Site description

The study was conducted at the Heshan National Field Research Station of Forest Ecosystems (112°50 E, 22°34 N), which is located in the middle of Guangdong Province in South China. The region has a tropical monsoon climate with a distinct wet and dry season. Mean annual precipitation is 1543 mm, with the wet season extending from April to September. Mean annual temperature is 22.5 °C, with an average temperature in the coldest (January) and hottest (July) months of 10.9 and 28.0 °C, respectively (Wu et al. 2011). Ambient atmospheric N deposition in precipitation was about 43.1 ± 3.9 kg N ha^{-1} $yr.^{-1}$ in 2011 with 1:1 ratio for NH_4^+ to NO_3^-, which is almost five-fold higher than that in 1995 (8.31 kg N ha^{-1} $yr.^{-1}$; Huang et al. 2014). The mean annual increase in the N deposition rate is nearly 2.17 kg N ha^{-1} $yr.^{-1}$. We established two research sites, a N_2-fixing plantation and a non-N_2-fixing plantations, which were located 500 m apart. These plantations were both established on a degraded grassland site in 1984, and each had an area of approximately 5–8 ha. A survey conducted in July 2010 (before the first N addition) showed that the dominant tree species in the N_2-fixing plantation was *Acacia auriculiformis* (*AA*), and *Eucalyptus urophylla* (*EU*) dominated the non-N_2-fixing plantation. The soils in both plantations are classified as acrisols (FAOUN 2006). The soil in this area has eroded seriously due to long-term disturbances. The mean annual litter production was 694 ± 25 g air-dried mass m^{-2} $yr.^{-1}$ and 590 ± 14 g air-dried mass m^{-2} $yr.^{-1}$ in the *AA* plantation and the *EU* plantation, respectively (Zhang et al. 2012). Leaf litter production accounted for 75–90 % of the total litter production in the two plantations (measured in 2011, Unpublished data).

Experimental treatments

Nitrogen addition treatments were initiated within these two plantations in July 2010. Three treatments (three replicate plots per each treatment) were established: control (no fertilizer), medium N (MN: 50 kg N ha^{-1} $yr.^{-1}$), high N (HN: 100 kg N ha^{-1} $yr.^{-1}$). The MN treatment and HN treatment received a total of 100 kg N $ha^{-1}yr^{-1}$ and 150 kg N ha^{-1} $yr.^{-1}$, respectively, via background N deposition plus N addition. The levels of N addition were reasonable to simulate the average projected increase in N deposition rate in the studied region for the years 2030 and 2060. The levels of N addition were comparable to other studies in the tropical zone (Mo et al. 2006; Hall et al. 2003). Eighteen plots with dimensions (10 m × 10 m) were established in these two plantations (nine plots per plantation). All plots and treatments were laid out randomly. Each plot was surrounded by a 10 m wide buffer strip. Ammonium nitrate (NH_4NO_3) solution has been sprayed every 30 days onto the forest floor as 12 equal doses over the whole year since August 2010. In each N addition event, fertilizers were weighed, dissolved in 10 L water for each plot, and sprayed evenly using a backhand sprayer. The control plots received 10 L water without N added.

Litter decomposition and soil sampling

We collected leaf litter in litter traps located within the control plots during August 2010. All the leaves were air-dried and stored at room temperature. The leaves of an individual species were mixed to obtain a uniform mixture before being placed in the mesh bags. Six subsamples of each species (about 10 g per subsample) were dried at 105 °C to obtain the initial oven-dry weight (conversion factor from air-dry to 105 °C; Mo et al. 2008). Other subsamples for each tree species were dried at 65 °C to a constant weight (Hobbie 2005), and then divided into two parts: one part was dried at 105 °C to obtain the conversion factor (conversion rate from 65 to 105 °C); the other part was analyzed for initial nutrient concentrations and AUR concentration (all results are reported on a 105 °C oven-dried weight basis). A total of 324 litterbags (162 bags for *AA* leaves, 162 bags for *EU* leaves) were prepared at the beginning of the study in November 2011. Approximately 10 g air-dried leaf litter was placed in a polyvinyl screen mesh bag (20 cm × 20 cm, mesh size was 0.5 mm at the bottom

and 2 mm at the top). Only one litter type was filled in each bag. At the end of November 2011, these litterbags were evenly distributed across each plot (18 bags for each plot) in the appropriate plantation. Three litterbags were retrieved from each plot (nine bags for each treatment) after 3, 6, 9, 12, 15 and 18 months. After collection, we returned the bags to the laboratory for separation and analysis.

Mineral soils (0–10 cm depth) were collected from all plots in December 2011 and July 2012. In each plot, three soil cores (3.5 cm inner diameter) were collected randomly and composited into one sample (a total of 18 samples). The soil samples were delivered to the laboratory as soon as possible for the determination of soil chemistry and microbial biomass carbon, nitrogen, and phosphorus.

Laboratory procedures

In the laboratory, litter was cleaned from any ingrown roots and other material, and then oven-dried in paper bags at 65 °C to a constant weight (Hobbie 2005). Each bag was individually weighed. Litters from the same plot in each plantation were mixed thoroughly and combined into one sample. Subsamples of the 65 °C-dried litter were grounded to pass through a 0.15-mm mesh sieve, and then analyzed for litter nutrient concentrations. The other subsamples for AUR concentration analysis were ground and passed through a 0.5-mm mesh sieve.

Litter carbon concentration was measured by potassium dichromate oxidation titration with Fe^{2+} solution (Liu et al. 1996). Litter N and P concentration was determined using Kjeldahl digestion followed by the indophenol blue method and the Mo-Sb colorimetric method, respectively, on a UV-8000 spectrophotometer (Metash Instruments Corp., Shanghai, China) (Liu et al. 1996; Hobbie and Vitousek 2000). For the initial potassium (K), calcium (Ca), magnesium (Mg) and manganese (Mn) concentration, subsamples were determined by atomic absorption spectroscopy (AAS, contrAA 700, Analytik Jena, German), after digestion with a sulfuric acid-perchloric acid (H_2SO_4–$HClO_4$) solution (Liu et al. 1996). The AUR concentration in the litter samples was estimated by gravimetric analysis after using a hot sulfuric acid digestion (King and Heath 1967) and following the methods used by Hagiwara et al. (2012). AUR integrates the least soluble and most hydrolysis-resistant organic structures, including true lignin, cutin, waxes, and condensed tannins in varying proportions (Preston et al. 2009).

Soil from each treatment was sieved (2 mm) and mixed thoroughly by hand. Soil pH was determined in a 1:2.5 ratio of soil to water suspension using a pH meter (Meltler-Toledo Instruments Co., Ltd., Shanghai, China). One 20 g subsample from each composite sample was shaken for 1 h in 100 ml 2 M KCl solution, filtered through Whatman no.1 filters, and then frozen immediately for later analysis. Soil NH_4^+ concentration was determined by the indophenol blue colorimetric analysis, and NO_3^- concentration was determined after cadmium reduction to NO_2^-, followed by sulfanilamide-NAD reaction (Liu et al. 1996). Soil available N was the sum of NH_4^+ and NO_3^-. Soil organic carbon (SOC), soil total nitrogen (TN) and soil total phosphorus (TP) was determined by the same methods used for the litter C, N and P concentration. Soil available P was determined by colorimetric analysis after extraction with 50 mL Bray-1 (0.03 M NH_4F-0.025 M HCl) extractants (Anderson and Ingram 1989). Microbial biomass C, N and P were estimated after chloroform fumigation. Briefly, fresh samples of fumigated and no-fumigated soil were shaken with 0.5 M K_2SO_4 for 1 h (soil to solution ratio was 1:5; Brookes et al. 1985; Vance et al. 1987). Organic C and TN were determined simultaneously on a TOC-V_{CHN} analyzer (Shimadzu Corp., Japan) after a ten-fold dilution of the extracts. Microbial biomass P was extracted in a 1:4 soil to solution ratio with Bray-1 extractants based on a method described by Oberson et al. (1997) as modified in Wu et al. (2000). TP was determined spectrophotometrically after digestion with 0.2 mL H_2O_2 (30 %, *v*/*v*) and 0.5 mL $HClO_4$ (70 %, *v*/*v*). Microbial biomass C, N and P were calculated from the difference between the fumigated and non-fumigated samples using the conversion factors $k_C = k_N = 0.45$ and $k_P = 0.40$ (Jenkinson et al. 2004). The C:N, N:P and C:P ratios in soil microbial biomass were expressed on a molar basis. Subsamples of litter and soils were dried at 105 °C to a constant weight (at least 24 h), and all results are reported on a 105 °C dry weight basis (Mo et al. 2006).

Data calculations and analyses

We fitted the fraction of initial litter mass remaining (and litter carbon remaining) against time to two exponential decay models (Olson 1963; Weider and Lang 1982): a single-exponential decay model, $X = e^{-k_s t}$, and an asymptote function, $X = A + (1 - A)\, e^{-k_a t}$, where X is the fraction of mass remaining at time t (year), "e"

is the base of natural logarithm. In the single-exponential model, k_s is the decomposition constant (year $^{-1}$) over the whole decomposition period. In the asymptotic model, A is the fraction of the initial mass with a decomposition rate of zero (i.e., the asymptote), and k_a is the initial decomposition rate (year^{-1}). Berg and McClaugherty (2008) and Hobbie et al. (2012) suggested that the decomposition rate would never actually equal zero, so A can be regarded to the "slow fraction", (1 - A) is equal to the "limit value" and represents the maximum accumulated mass loss before decomposition gradually slows to zero. These two decomposition models were fitted to the data using least squares regression of the natural logarithm of fractional mass remaining or carbon remaining (Jensen and Nybroe 1999). Litter C, N and P contents were determined by multiplying the nutrient concentration by the mass and were expressed as the fraction of the original nutrient content remaining (Hobbie and Vitousek 2000).

One-way analysis of variance (ANOVA) with Tukey's HSD test was used to test the difference in soil chemical characteristics, microbial biomass and its nutrient ratios, decomposition parameters (k_s, k_a, and A), litter mass and nutrient (C, N and P) remaining, and AUR remaining among N treatments. Repeated measure ANOVA with the MIXED model was performed to examine the effect of N additions on litter mass and nutrient remaining over time in both plantations. The t-test was performed to test the difference in soil chemistries, initial litter quality and decomposition parameters between the two plantations. Linear regressions were performed using the GLM model in SAS. All data were tested to fulfill the assumptions of normality and homogeneity of variance, and transformations were carried out when necessary. All analyses were conducted using SAS 8.0 for Windows (SAS Institute Inc., Cary NC, USA). Graphic illustrations were generated using Origin 8.0 software (Origin Lab Corporation, Northampton, MA, USA). Statistically significant differences were identified when P-values <0.05, unless otherwise stated. Mean values ±1 standard error are reported in the text.

Results

Soil characteristics and initial litter quality

The control plots in the *AA* plantation had higher soil available N, SOC and TN, but a lower pH value than in the *EU* plantation in mineral soil layer (0–10 cm) (all $P < 0.05$; Table 1). In the *AA* plantation, HN addition significantly increased soil TN concentration ($P = 0.001$) and soil available N concentration ($P = 0.003$), but decreased soil C:N ratio ($P = 0.0001$). However, there was no significant difference between the control and MN addition plots. There was also no significant difference in the concentrations of SOC, soil available P, and soil pH among treatments in the *AA* plantation (all $P > 0.05$; Table 1). In the *EU* plantation, soil available N concentration was significantly increased by N additions ($P = 0.0016$; Table 1). However, N addition had no significant effect on SOC, TN, the C:N ratio and pH value in the 0–10 cm mineral soil layer in the *EU* plantation (all $P > 0.05$; Table 1).

The *AA* leaf litter had significantly higher litter C, N, and AUR concentrations, as well as higher C:P, N:P (all $P < 0.001$), and AUR:N ($P = 0.014$) ratios, but lower P, K, Ca, Mg, and Mn concentration (all $P < 0.05$), and C:N ratios ($P = 0.0030$) than the *EU* leaf litter (Table 2).

Decomposition rates and patterns

All data of litter mass remaining gave reasonably good fits to both two functions (the adjusted-R^2 values ranged from 0.81 to 0.95 for the single-exponential model, and from 0.80 to 0.97 for the asymptotic model). The patterns of litter mass loss among treatments were characterized by a fast initial decomposition rate (k_a; ranged from 1.50 to 2.21 y^{-1}), followed by a subsequent slow rate because a large "slow fraction" (A; ranged from 0.3 to 0.59) remained in the later stages of decomposition for both litter types (Fig. 1).

Patterns of leaf litter decomposition in the control plots reflected the natural decomposition process without N addition. Compared to the *EU* litter, the *AA* litter had higher initial decomposition rate (k_a: 1.94 ± 0.04 y^{-1} and 1.50 ± 0.22 y^{-1} for the *AA* and *EU* plantation, respectively; $P = 0.02$), and a larger "slow fraction" (A: 0.50 ± 0.02 and 0.30 ± 0.06 for the *AA* and *EU* plantations, respectively; $P = 0.039$; Fig. 1a). The pattern of k_s (which is a constant rate over the whole decomposition period) did not reflect the pattern of k_a, because a large slow fraction remained in both litter types.

In the *AA* plantation, N addition had no significant effect on the initial decomposition rate (k_a, y^{-1}), while HN addition significantly increased the value of A (the asymptote, the fraction of litter whose decomposition

Table 1 Mineral soil properties (0-10 cm depth) in the *AA* and *EU* plantations in Heshan, South China

Plantation	Treatment	Av. N (mg kg^{-1})	SOC (g kg^{-1})	TN (g kg^{-1})	C:N	Av. P (mg kg^{-1})	pH (H_2O)
AA	CK	14.3 (0. 3) Aa	23.8 (1.7) A	2.0(0.1) A a	12.5 (0.5) a	2.5 (0.2)	3.69 (0.01) A
	MN	16.3 (0.7) ab	22.6 (1.4)	2.3(0.2) a	12.0 (2.9) a	2.9 (0.3)	3.63 (0.01)
	HN	18.2 (0.5) b	23.3 (0.3)	2.7(0.1) b	7.9 (0.3) b	2.6 (0.1)	3.66 (0.00)
EU	CK	13.8 (0.4) B a	18.5 (0.4) B	1.5 (0.2) B	12.9 (2.5)	2.1 (0.1) ab	3.75 (0.01) B
	MN	19.7 (0.3) b	19.6 (1.6)	1.7 (0.2)	12.2 (2.5)	1.9 (0.1) a	3.66 (0.03)
	HN	19.8 (0.2) b	21.7 (1.8)	1.8 (0.1)	11.9 (1.3)	3.1 (0.1) b	3.67 (0.04)

Soil samples were collected in December 2011. Values are means ±1SE in parentheses, $n = 3$. Different capital letters indicated significant difference in control plots between two plantations (T-test, $P < 0.05$) Different lowercase indicated significant difference between treatments at each plantation (ANOVA with Tukey's HSD, $P \leq 0.05$). *AA*: *Acacia auriculiformis*; *EU*: *Eucalyptus urophylla*; Av. N: soil available nitrogen (the sum of NH_4^+ and NO_3^-); SOC: soil organic carbon; TN: total nitrogen; Av. P: soil available phosphorus. CK: control (without N addition), MN: medium-N addition (50 kg N ha $yr.^{-1}$), HN: high-N addition (100 kg N ha $yr.^{-1}$)

rate approached zero) from 0.50 ± 0.02 in the control plots to 0.59 ± 0.02 in the HN-addition plots ($P = 0.019$; Fig. 1b). Therefore, it is reasonable that the result showed that the values of k_s decreased following increasing levels of N addition, and the difference between the HN-addition plots and the control plots was significant ($P = 0.004$; Fig. 1b). Repeated measures ANOVA with Tukey's HSD test also indicated that litter mass loss was significantly slower in the HN plots than in the control plots on the third, fourth, and fifth sampling dates ($P = 0.0001$; Fig. 2a).

In the *EU* plantation, N addition had a positive effect on the initial decomposition rate (k_a, y^{-1}; $P = 0.0034$), which increased from 2.24 ± 0.11 (the control plots) to 3.7 ± 0.17 (the MN-addition plots) and 2.9 ± 0.09 (the HN-addition plots), while the slow fraction (A) showed a decreasing trend with increasing levels of N addition ($P = 0.035$; Fig. 1c). The pattern of k_s was inconsistent with those for k_a, showing a reduction following the increasing of A (Fig. 1c). Furthermore, repeated measures ANOVA with Tukey's HSD test showed that the effect of N addition on litter mass loss was not significant over the entire experimental period ($P = 0.083$; Fig. 2b).

Litter C, N and P content in decomposing litter

All data of litter C remaining gave reasonably good fits to both two functions (the adjusted-R^2 values ranged from 0.78 to 0.99 for the single-exponential model, and from 0.88 to 0.99 for the asymptotic model). Patterns of k_s, k_a, and A (here A means the fraction of initial carbon that decomposed very slowly) for carbon decomposition for the control plots in these two plantations were similar to those for litter mass decomposition, but the differences were not significant (Fig. 1d). The effect of N addition on litter C decomposition was consistent with that for litter mass decomposition in both plantations. In the *AA* plantation, HN-addition significantly increased A ($P = 0.005$), and decreased k_s ($P = 0.021$; Fig. 1e). Repeated measures ANOVA showed that the effect of N addition on litter C remaining was significant ($P = 0.023$). After 18-months of decomposition, the

Table 2 Initial litter chemistry for the *AA* and *EU* litters used in the litter decomposition experiment

Litter type	Total C (mg g^{-1})	Total N (mg g^{-1})	Total P (mg g^{-1})	K (mg g^{-1})	Ca (mg g^{-1})	Mg (mg g^{-1})	Mn (mg g^{-1})	AUR (mg g^{-1})	C:N	C:P	N:P	AUR:N
AA	549 (10.1)	16.3 (0.4)	0.354 (0.006)	1.812 (0.074)	8.036 (0.108)	1.322 (0.011)	0.120 (0.001)	429 (14)	34.4 (1.2)	1552 (31)	45 (1.6)	28.1 (0.7)
EU	487 (13.5)	11.6 (0.3)	0.378 (0.006)	2.295 (0.083)	9.357 (0.143)	1.407 (0.013)	0.437 (0.003)	304 (9)	42.6 (1.6)	1290 (21)	31 (1.2)	25.1 (0.6)
P value	***	***	*	**	***	***	***	***	**	***	***	*

Values are presented as means ±1SE in parentheses, $n = 6$. Significance: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. AUR: acid-unhydrolyzable residue

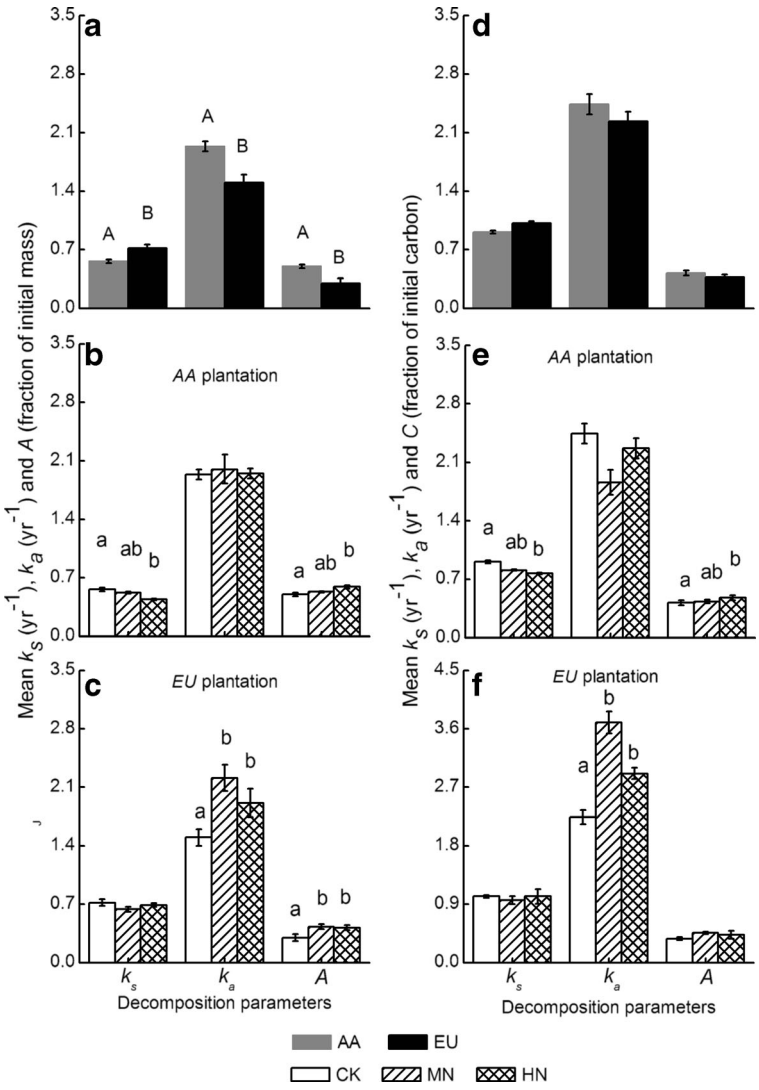

Fig. 1 Mean decomposition parameters obtained from fitting the single-exponential and asymptotic models to data of litter mass remaining (**a**, **b**, and **c**) and carbon remaining (**d**, **e**, and **f**) to show differences between two plantations, and among treatments in the *AA* plantation and *EU* plantation. Values are means ±1SE in parentheses. Decomposition parameters are indicated as: k_s, single-exponential model decomposition rate; k_a, asymptotic model decomposition rate; A, asymptotes (the fraction of initial litter with the decomposition rate of zero). Different capital letter indicated significant difference in control plots between two plantations ($P < 0.05$). Different low letter indicated significant difference among treatments in each plantation ($P < 0.05$). CK: control (without N addition), MN: medium-N addition (50 kg N ha yr.$^{-1}$), HN: high-N addition (100 kg N ha yr.$^{-1}$)

fraction of initial C remaining in MN and HN addition plots was 17 and 22 % higher than that in the control plots, respectively (Fig. 2c). In the *EU* plantation, N addition (both MN and HN addition) significantly increased k_a ($P = 0.004$), but had no significant effect on A (Fig. 1f).

N additions had a positive effect on litter N content in the *AA* plantation over the whole study period ($P < 0.001$; Fig. 2e), but there was no significant effect in the *EU* plantation (Fig. 2f). For the *AA* plantation, net N accumulation occurred in the MN-addition and HN-addition plots on the first sampling date, following a net N release over the next nine months. After 12-months of decomposition, the fraction of initial N remaining in the MN and HN plots were 25 and 23 % higher than that in the control plots, respectively.

The suppressing effect of N additions on litter P release was more pronounced in the *AA* plantation than in the *EU* plantation ($P = 0.0001$ for the *AA* plantation and $P = 0.003$ for the *EU* plantation; Fig. 2g, h). In the *AA* plantation, the fractions of initial P remaining in the MN and HN plots were 32 and 39 % higher than in the control plots, respectively, after 12-months of decomposition. However, in the *EU* plantation, the fractions of initial P content in the MN and HN plots were 19 and 22 % higher than in the control plots, respectively, on the fourth sampling date.

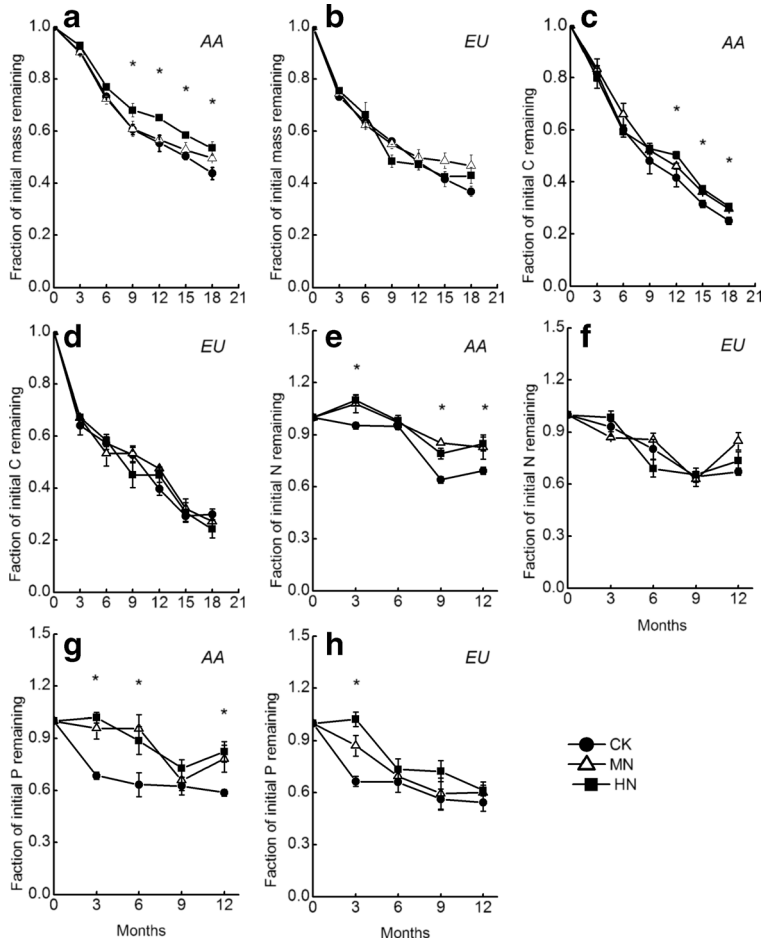

Fig. 2 Changes of litter mass loss and nutrient release (represented as fraction of initial remaining) in decomposing litter for two dominant tree species in various N treatments in the *AA* plantation (**a**, **c**, **e**, and **g**) and the *EU* plantation (**b**, **d**, **e**, and **f**). Values are means; Bars are ±1SE. Fraction of original nutrient remaining >1 means net nutrient accumulation; fraction of original nutrient remaining <1 means net nutrient release. Asterisk (*) indicates significant difference at least between two treatments at $P < 0.05$ ($n = 3$)

Soil microbial biomass and nutrient ratios

Microbial biomass C in the control plots was no significantly different between the two plantations. N addition significantly decreased microbial biomass C in the *AA* plantation ($P = 0.031$; Fig. 3a). The mean microbial biomass C concentration was 334 ± 16 mg C kg^{-1} in the control plots, 301 ± 18 mg C kg^{-1} in the MN plots, and 253 ± 13 mg C kg^{-1} in the HN plots, which represented a decrease of 10 and 24 %, respectively (Fig. 3a). In the *EU* plantation, microbial biomass C also decreased with N addition, but the differences were not statistically significant (Fig. 3a).

Microbial biomass N was not affected by N addition in either plantation (Fig. 3b), while N addition significantly increased microbial biomass P in the *AA* plantation ($P = 0.0001$; Fig. 3c). The mean microbial biomass P concentration was 14.5 ± 0.6 mg P kg^{-1} in the control plots, 20.0 ± 0.3 mg P kg^{-1} in the MN plots and 21.6 ± 0.6 mg P kg^{-1} in the HN plots, which represented an increase of 38 and 49 %, respectively. However, no significant differences were found in the *EU* plantation.

Nitrogen addition significantly decreased the microbial biomass C:N ratio in the *AA* plantation ($P = 0.0097$; Fig. 3d). The mean microbial biomass C:N ratio was 6.1 ± 0.2 in the control plots, 5.0 ± 0.2 in the MN-addition plots, and 4.8 ± 0.1 in the HN-addition plots. The microbial biomass C:P ratio significantly decreased with N addition in the *AA* plantation from 63 ± 4 in the control plots to 31 ± 1 in the HN-addition plots ($P = 0.0003$; Fig. 3f). The microbial biomass N:P ratio in the *AA* plantation also significantly decreased with N addition from 10.4 ± 0.5 to 6.4 ± 0.6 ($P = 0.004$; Fig. 3f). However, the microbial biomass C:N, C:P, and N:P ratios were not affected by N addition in the *EU* plantation (Fig. 3d, e, f).

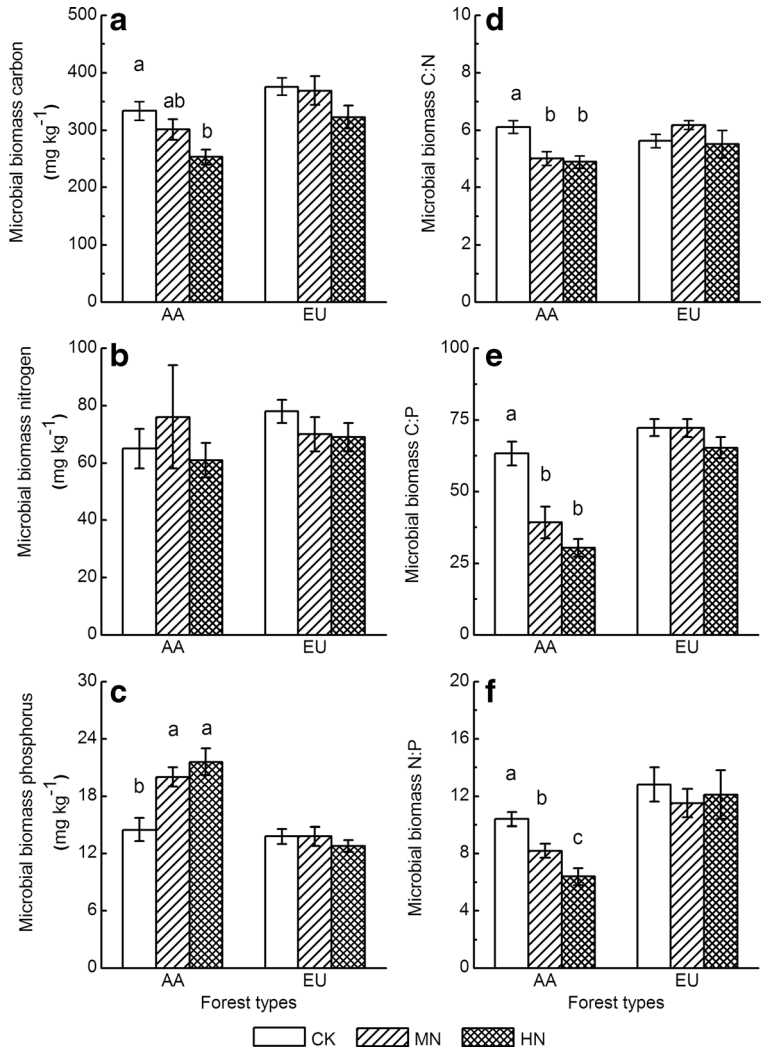

Fig. 3 Soil microbial biomass carbon, nitrogen, phosphorus, and their stoichiometric ratios in CK and N fertilized plots in the *AA* and *EU* plantations. Soil samples (0-10 cm) were collected in July 2012. Different letters indicated a significant difference ($P < 0.05$) between treatments in each plantation ($n = 3$)

Discussion

By fitting the asymptotic model to our data (litter mass and C remaining), we found significant residue (*C*, the asymptotic residue ranging from 0.3 to 0.59 for litter mass, and from 0.39 to 0.48 for litter carbon) in all plots. Furthermore, the asymptotic model enables us to estimate the effects of N addition on initial rates of decomposition and the value of the asymptotes (the fraction of initial mass decomposing very slowly), as mentioned by Berg and Matzner (1997) and Hobbie et al. (2012). Therefore, we mainly discuss the decomposition patterns between the control plots of two plantations, and the patterns among treatments in each plantation using the decomposition parameters calculated from the asymptotic model unless otherwise stated.

The litter decomposition patterns in the control plots

The litter decomposition rates (k_s, yr.$^{-1}$) observed in the control plots of the *AA* and *EU* plantations (0.514–0.768 yr.$^{-1}$) were similar to previous results for temperate sites (from 0.3 to 0.7 yr.$^{-1}$; Hobbie 2008), whereas they were higher than those in some coniferous forests/plantations (from 0.17 to 0.31 yr.$^{-1}$; Mo et al. 2006; Perakis et al. 2012; Hobbie 2008). Higher rates of litter decomposition than our result were also reported from other tropical forests (ranging from 1.12 to 4.41 yr.$^{-1}$), probably due to the higher microbial biomass and

activity in those forests (Mo et al. 2006; Cleveland et al. 2006).

The initial decomposition rate (k_a) for *AA* litter was significantly higher than that for *EU* litter, which was consistent with our hypothesis and supported previous reports which showed that the litter mass loss of N_2-fixing species (e.g. grey alder (*Alnus incana*), red alder (*Alnus rubra*)) was faster than that of non-N_2-fixing species (e.g. white birch (*Betula pubescens*), Scots pone (*Pinus sylvestris*), lodgepole pine (*Pinus contorta*) and Douglas-fir (*Pseudotsuga menziesii*)) in early stage of decomposition (Berg and Ekbohm 1991; Perakis et al. 2012). As mentioned in the results, *AA* litter had significantly higher initial N concentration than the *EU* litter. Moreover, the *AA* plantation had higher soil N availability than the *EU* plantation due to symbiotic N_2-fixation. Under these conditions, decomposers (who often have higher C:N ratio than litter) can acquire sufficient N resource from litter and soil, and secrete more enzymes to degrade carbon (Allison and Vitousek 2005; Hobbie et al. 2012).

The *AA* litter had a larger slow fraction (the asymptote, *A*) than the *EU* litter in the later stages of decomposition, as expected, and consistent with some previous studies which showed that the higher N concentration in litter, the larger the slow fraction remained (or the lower level of "limit value") (Berg and Ekbohm 1991, Berg and McClaugherty 2008 and Hobbie et al. 2012). Previous studies have shown that the slowly decomposing fraction (*A*) was positively correlated with initial litter N concentration, but negatively correlated with litter Mn and Ca concentrations (Berg 2000; Berg et al. 2010; Davey et al. 2007). In the present study, initial AUR concentration was significantly higher in the *AA* litter (429 ± 14 mg g^{-1}) than in the *EU* litter (303 ± 9 mg g^{-1}). High litter N and soil inorganic N concentrations can suppress the colonization by ligninolytic fungi and the secretion of ligninolytic enzymes by white-rot fungi, and thus slow the degradation of AUR (Keyser et al. 1978; Berg and Matzner 1997; Berg and McClaugherty 2008; Hagiwara et al. 2012). Soil inorganic N might bond with AUR to form more recalcitrant substances, leading to a hampering effect on decomposition in the later stages of decomposition (Fog 1988; Davidson et al. 2003). In the present study, Mn concentration, which plays an important role in the synthesis of Mn-peroxidase by white rot fungi (Berg and McClaugherty 2008; Hatakka and Hammel 2010), was significantly higher in the *EU* litter than that in the *AA* litter ($P < 0.0001$; Table 2). Litter Ca and P concentrations, which likely are positively related to limit values (Berg and Ekbohm 1991; Berg and Matzner 1997; Hobbie et al. 2012), were higher in the *EU* litter than in the *AA* litter. We suggest that the difference in the decomposition rates for the whole decomposition period between the two plantations depends on the combined effect of factors controlling litter decomposition in the initial period (N and P) and in the later period (N, AUR, Ca, Mn).

Effects of N additions on litter decomposition rates

Consistent with our hypothesis and some previous studies (Berg and Ekbohm 1991; Berg and Matzner 1997; Hobbie et al. 2012), our results showed that N addition had no effect on the initial decomposition rate (k_a) in the *AA* plantation, but significantly decreased the level of "limit value" (the accumulated mass loss before the decomposition rate approached zero; which decreased from 50 % in the control plots to 41 % in the HN-addition plots), and increased the fraction of slowly decomposing litter in the later stages of litter decomposition. Therefore, the value of k_s for the HN-addition plots was lower than that for the control plots during the overall decomposition period.

The inhibitory effect of N addition on lignin-degrading enzymes can partly explain the negative N effect on litter decomposition. In our study, we found that (1) the negative N effect on litter decomposition was more significant in the *AA* litter (high AUR:N ratio) than in the *EU* litter (low AUR:N ratio; Table 2) and (2) a determination of AUR after 12-months of decomposition showed that 78 % of the initial AUR was remained in the HN-addition plots, while 66 % of the initial AUR was remained in the control plots in the *AA* plantation. The reduction in AUR decay may be due to the suppressive effect of N addition on the formation of ligninases (Edwards et al. 2011, Hobbie et al. 2012). Sinsabaugh et al. (2002) indicated that N addition (20 kg N ha^{-1} $yr.^{-1}$ and 80 kg N ha^{-1} $yr.^{-1}$) significantly depressed the activity of polyphenol oxidase, leading to a decrease in litter mass loss of red oak in a mixed deciduous woodland in Armonk, NY, USA. Another reason for a slowdown in AUR decay is that inorganic N reacts with AUR chemically, which is the first step of the humification process (Gerber et al. 2010).

Another explanation is that microbial decomposers synthesize phenolic compounds and/or break down litter lignin and other polyphenolic compounds during

decomposition. The by-products of these processes then react with inorganic N to form more resistant compounds (Davidson et al. 2003). There was a close relationship among inorganic N addition, formation of resistant compounds, and litter decomposition rate (Berg and Matzner 1997). In our study, we also found that litter decomposed in the HN-addition plots had less accumulated mass loss than that in the control plots in the *AA* plantation (41 and 50 % in the control and HN-addition plots, respectively; Fig. 1b).

Nitrogen can alter the microbial biomass and community composition, which are closely related to decomposition ability. Excess N input alters the balance between the N and P concentrations and/or decreases the pH value of the soil environment, which affects microbial growth and maintenance (Smolander et al. 1994; Ramirez et al. 2012). A study conducted in four sugar maple (*Acer saccharum* Marsh)-dominated northern hardwood sites distributed across lower and upper Michigan, USA, showed that atmospheric NO_3^- deposition exerted a direct and negative effect on microbial activity, which slowed the decomposition of aboveground litter and led to the accumulation of soil organic matter and N in the forest floor (Zak et al. 2008). Our results show that high N addition significantly decreased soil microbial biomass C and microbial biomass C:N ratios in soil (Fig. 3a, d). This may suggest that there has been a shift in the microbial decomposers community from a highly efficient, fungus-dominated community with high overall C:N ratio to a less efficient, bacteria-dominated community with low C:N ratio (Frey et al. 2004; Högberg et al. 2007). A meta-analysis also show that microbial biomass declined 15 % on average under N addition, and the abundance of microbes and fungi declined significantly when chronic, high amounts of N had been added (Treseder 2008).

The negative effect of N addition on the later stages of decomposition in the *AA* plantation suggests that ongoing N deposition would increase the fraction of slowly decomposing litter (or reduce the "limit value"), leading to an increase in the accumulation of carbon in the forest floor. This inference is supported by the results of C loss from decomposing litter in the HN-addition plot (a higher fraction of recalcitrant carbon remained in the HN-addition plots), and by some previous reports (Keeler et al. 2009; Gerber et al. 2010; Whittinghill et al. 2012).

In contrast to the *AA* plantation, N addition had a positive effect on initial litter decomposition (k_a) in the *EU* plantation. Due to land-use history and high nutrient depletion by the growth of *EU* species (fast-growing species), the *EU* plantation had lower soil N availability than the *AA* plantation (Table 1), which suggests that there may be a strong competition for N uptake between plants and microbes in the *EU* plantation. N addition significantly increased soil available N concentration (from 18.5 to 21.7 mg kg^{-1}) in this plantation, which supplied sufficient N resource for decomposers in the early stage of decomposition. In this case, decomposers can invest more energy and N to produce polysaccharide hydrolases, causing an acceleration in litter decomposition (Sinsabaugh et al. 2002; Allison and Vitousek 2005). This result is similar to those from a tropical forest in southern China, which showed that N addition had a positive effect on decomposition in N-poor pine forest (Mo et al. 2006) and some temperate forests with low-N status (Berg and Matzner 1997; Hobbie et al. 2012).

Effects of N addition on litter net release of N and P

Our results indicated that N addition significantly inhibited litter N release in the *AA* plantation, but not in the *EU* plantation. One possible explanation is that N addition altered the balance of C, N, and P requirement in microbial decomposers by improving soil N availability. Because N addition decreased the microbial biomass C:N ratio (Fig. 3d), the gap between the microbial biomass C:N ratio and the litter C:N ratio was wider in the N-addition plots than in the control plots at the beginning of litter decomposition. This gap gradually narrowed in the decomposing process. When N-limitation of litter decomposition was relieved by N addition, the negative effect of N on litter N release was stronger in the N-rich litter types than the N-poor litter types (Mo et al. 2008). Moore et al. (2006) suggested that litter nutrient loss was slower in nutrient-rich sites than in nutrient-poor sites. N can be retained in litter by microbial uptake (fungal hyphae transfer N from decomposing litter and soil) or chemical immobilization (ammonia-N can react with by-products of microbial breakdown and humus) (Vitousek and Hobbie 2000; Hobbie 2008).

The suppressing effect of N addition on litter P release can also be explained by the nutrient-status theory. The initial litter C:P and N:P ratios were significantly higher for the *AA* litter than for the *EU* litter (Table 2). However, N addition significantly reduced the soil microbial biomass C:P ratio in the *AA* plantation

(Fig. 3e). For this reason, N addition may further enhance P-limitation during litter decomposition and stimulate microbial P immobilization in the decomposing litter in the *AA* plantation. Our results indicate that N addition had a stronger suppressing effect on litter P release in the *AA* plantation than in the *EU* plantation. Our results were consistent with other studies in tropical forests, which reported that N fertilization can induce litter nutrient retention due to the fairly plastic C:N and C:P ratios of decomposers, even when additional N has no (or a negative) effect on litter decomposition (Hobbie and Vitousek 2000; Mo et al. 2008).

Conclusion

In the present study, litter decomposition and nutrient release following N addition were measured in two tropical plantations with N_2-fixing and non-N_2-fixing tree species. Since litter N concentration and soil inorganic N was higher in the *AA* plantation, litter decomposition in this plantation was faster in the early stage of decomposition, but a larger, very slowly decomposing fraction was found in later stages, compared to the *EU* plantation. N addition did not increase the initial decomposition rate (k_a), but significantly slowed down decomposition in later stages in the *AA* plantation. In the *EU* plantation, N addition significantly increased the initial decomposition rate, but had a negative effect in the later stages. For the *AA* plantation, the negative effect of N addition on litter decomposition was due to a reduction in the decay of AUR and microbial biomass. In addition, N addition also affected the loss of litter C, N (only in the *AA* plantation) and P (in both plantations) during the decomposition process by stimulating chemical and microbial immobilization. To the best of our knowledge, our study is the first to investigate the effect of N deposition on litter decomposition and nutrient dynamics between plantations with N_2-fixing tree species and non-N_2-fixing species. Our results suggest that future N deposition would potentially decrease litter decomposition, which would lead to increasing carbon accumulation and nutrients' retention in the forest floor in plantations with N_2-fixing tree species. Considering that N deposition and expansion of reforestation worldwide will continue to increase in the future, similar studies should be conducted in other regions to explore the selection of tree species for reforestation and relevant forest management.

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