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Original article

Decomposition responses of pine (*Pinus massoniana*) needles with two different nutrient-status to N deposition in a tropical pine plantation in southern China

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Abstract –
• The effect of nitrogen (N) deposition on the decomposition of pine (*Pinus massoniana*) needles in a tropical pine plantation was studied. The pine needles with two different nutrient status (nutrient-rich and nutrient-poor) were used, followed by 3-levels of N treatments (Control: no N addition, Low-N: 5 g N m^{-2} y^{-1}, and Medium-N: 10 g N m^{-2} y^{-1} experimental inputs), which had been applied for 26 months continuously before this experiment and continued throughout the decomposition measurement.
• The main objective was to test the hypothesis that decomposition of nutrient-rich needles would be more sensitive to cumulative N deposition than the decomposition of nutrient-poor needles.
• Nitrogen addition had negative effect on mass loss, and the release of N and P from decomposing nutrient-rich needles but little or no effect on the decomposition of nutrient-poor needles. In addition, a negative effect in the initial decomposition phase and a positive effect in later decay stages were found on C release. The negative effect was stronger on nutrient-rich needles than on nutrient-poor needles, but the reverse was true for the positive effect.
• Our results suggest that response of litter decomposition to N deposition may vary depending on the nutrient status of the litter.

anthropogenic impacts / litter decomposition / carbon sequestration / nutrient status / tropics

Résumé – Décomposition des aiguilles de pin en relation avec différents apports de dépôts azotés dans une plantation de pins tropicaux (*Pinus massoniana*) du Sud de la Chine.
• Les effets des dépôts azotés (N) sur la décomposition des aiguilles de pin ont été étudiés dans une plantation de pins tropicaux (*Pinus massoniana*) du Sud de la Chine. Les aiguilles de pin différentes par leur statut nutritionnel (station riche en nutriments et station pauvre en nutriments) ont été utilisées dans une expérimentation comportant 3 niveaux de traitement azoté (témoin sans apport d'azote, apport faible d'azote : 5 g N m^{-2} an^{-1}, apport moyen d'azote : 10 g Nm^{-2} an^{-1}) qui ont été appliqués pendant une période de 26 mois avant et pendant cette expérimentation.
• L'objectif principal était de tester l'hypothèse que la décomposition des aiguilles riches en nutriments devrait être plus sensible à une déposition cumulée d'azote que la décomposition des aiguilles pauvres en nutriments.
• L'apport répété d'azote a eu un effet négatif sur la perte de masse et la libération d'azote et de phosphore des aiguilles de la station riche et un effet faible ou nul sur la décomposition des aiguilles de la station la plus pauvre. En outre, un effet négatif dans la phase initiale de la décomposition et un effet positif dans les phases plus tardives de la décomposition ont été mis en évidence pour la libération de carbone. L'effet négatif a été plus fort pour les aiguilles riches en nutriments que pour les aiguilles pauvres en nutriments, mais l'inverse était vrai pour ce qui concerne l'effet positif.
• Nos résultats, complétés par ceux obtenus au cours d'expérimentation antérieures sur le même site, suggèrent que l'accumulation de dépôts azotés peut changer les impacts sur la décomposition des litières et que cet effet peut varier en relation avec la fertilité de la station considérée.

perturbations anthropiques / décomposition de litière / séquestration du carbone / statut nutritif / tropiques

1. INTRODUCTION

Anthropogenic nitrogen (N) deposition is considered as an important factor regulating carbon (C) sequestration in terrestrial ecosystems (Hagedorn et al., 2003). Litter decomposition plays an important role in the formation of soil and humus and the availability of N and other nutrients to plants and microorganisms in forest ecosystems (Osono and Takeda, 2004; Vestgarden, 2001). The addition of exogenous N to the soil-litter subsystem has showed variable effects on the rate of litter decomposition in forest ecosystems, including increases, decreases, or no change (Ågren et al., 2001; Magill and Aber, 1998; Micks et al., 2004; Prescott, 1996; Prescott et al., 1999; Vestgarden, 2001; Vitousek, 1998). A positive response in the initial decomposition phase and a negative response in later decay stages have also been found (Berg and Matzner, 1997; Berg et al., 1998). The main reason for the positive effect of N deposition on litter decomposition lies that N stimulated

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microbial activity (Fog, 1998; Neff et al., 2002); and the prevailing hypothesis for the mechanism behind the variable response of decomposition to N addition tied to the interactions of N with the enzymes that catalyze degradation of cellulose and lignin, and hence to the composition of the litter (Carreiro et al., 2000). A "microbial nitrogen mining" hypothesis has also been proposed recently (Moorhead and Sinsabaugh, 2006).

However, most studies of litter decomposition have been performed in temperate forest ecosystems, little information is available on decay responses to N deposition in tropical forests (Cleveland et al., 2006; McGroddy et al., 2004; Micks et al., 2004; Vitousek, 1998), especially in the forests of China (Mo et al., 2006; 2007). Nitrogen deposition in tropical and subtropical regions is projected to increase in the coming decades, due to the rapid expansion of industrial and agricultural activities (Galloway et al., 2004). Because tropical forests play an important role in the global ecosystems, even small changes in decomposition rates and subsequent CO_2 release could alter atmospheric composition and global climate. Thus identifying the effects of N deposition on litter decomposition will improve our understanding of the current and future global C balance (Cleveland et al., 2006).

In Asia, the use and emissions of reactive N increased from 14 Tg N y^{-1} in 1961 to 68 Tg N y^{-1} in 2000 and is expected to reach 105 Tg N y^{-1} in 2030 (Zheng et al., 2002). Currently, this leads to high atmospheric N deposition (30–73 kg ha^{-1} y^{-1}) in some forests of southern China (Huang et al., 1994; Ma, 1989; Ren et al., 2000; Xu et al., 2001; Zhou and Yan, 2001). In addition, most of the original primary forests has been cut and degraded by human activities during the past century (Wang et al., 1982). In extreme cases, the land became completely barren (He and Yu, 1984; Liu et al., 2000). Deforestation in China is estimated to be on the order of 0.61 million ha per year and the remnant mature native forest is less than 9 percent of the total territory in the 1990s (Liu et al., 2000). Attempts to reverse this process of land degradation have been initiated. Over the last decades, large areas have been reforested with a native pine species (*Pinus massoniana*). Logging of the trees is prohibited, but harvesting of understory and litter is generally allowed to satisfy local fuel needs. However, the interactive effects between N deposition and land-use changes on ecosystem processes are poorly known (Mo et al., 2007).

It is proposed that adding N to the soil-litter in N-limited ecosystems should increase microbial activity, but would not affect microbial activity in N-saturated ecosystems (Berg, 1986; Berg and Matzner, 1997; Berg et al., 1998). We have previously reported that, due to land-use history, tropical pine plantations in southern China are N limited for litter decomposition (Mo et al., 1995; 2006), and that N addition positively affected mass loss of decomposing litter (Mo et al., 2006), suggesting that N deposition may enhance C release to the atmosphere by increasing litter decomposition. In the same experiment, however, N addition significantly reduced litter decomposition in a N saturated old-growth forest (Mo et al., 2006). The results above imply when cumulative N deposition eliminates N limitation, further N input may reverse the N effect on litter decomposition, causing decomposition rates to decrease.

In addition, one of the consequences of N deposition is the increase of N concentration in leaf and litter, accompanying the change of other nutrient concentrations such as phosphorus and calcium (Berg and Matzner, 1997; Magill et al., 2000). The decomposition of litter with higher N concentration may respond differently to further N deposition, as substrate quality strongly influences the rates of decay (Berg and Eckbohm, 1991; Berg and Matzner, 1997; Cleveland et al., 2006) and net N release from decomposing litter is overwhelmingly driven by the initial N status (Parton et al., 2007). Results from laboratory experiments showed that low soil N availability can increase litter decomposition but high soil N supply or substrate N concentration decrease litter decomposition due to "microbial N mining" (Craine et al., 2007). However, to our knowledge, information regarding the decomposition response of litter to increased N deposition from the same species but with different nutrient-status is non-existent.

In this paper, we report the effect of continuous N addition on changes in mass remaining, concentrations of C, N and P of decomposing pine (*Pinus massoniana*) needles with two different nutrient-status in a tropical pine plantation in southern China. The objective was to test the following hypotheses: (1) cumulative N deposition changes needle decomposition in this tropical plantation; and (2) decomposition of nutrient-rich needles would be more sensitive to cumulative N deposition than the decomposition of nutrient-poor needles.

2. MATERIALS AND METHODS

2.1. Site description

This study was conducted in a 70-year pine forest in the Dinghushan Biosphere Reserve (DHSBR). The reserve lies in the middle part of Guangdong Province in southern China (112° 10' E, 23° 10' N) and occupies an area of approximately 1200 ha. The pine forest at about 50–200 m asl occupies 20% of the reserve (Mo et al., 2003; 2006; Zhang et al., 2000). This pine forest originated from the 1930's pine plantation after a clear-cut (Mo et al., 1995). The original site was badly eroded and degraded (Mo et al., 1995) and the forest was under constant human disturbances (generally the harvesting of understory and litter) from 1930 to 1998 so that the tree layer remained dominated by *P. massoniana* (Mo et al., 1995; 2007). Since 1998, there has been no or very little harvesting (Mo et al., 2007). The plantation is N-limited due to such site history including previous human disturbance (Mo et al., 1995; 2003; 2006; 2007).

The survey conducted in June 2003 (before the start of N addition experiment) showed that the plantation was dominated by *P. massoniana*. Stem density, tree height, and diameter at the breast height in the forest were described by Fang et al. (2006). The mean annual litter production was 3.3 Mg ha^{-1} y^{-1}; about 91% of total leaf litter was from *P. massoniana* (Zhang et al., 2000). Standing floor litter (excluding humus) measured in June 2003 was 23.7 ± 4.8 Mg ha^{-1} (mean ± standard error, $n = 3$) (Fang et al., 2006). The soils in the study site are oxisols, with depth generally less than 30 cm to bedrock (Mo et al., 1995, 2003). Ratio of (humic acid)/(fulvic acid) in the humus layer was 0.9 (He et al., 1982).

The reserve has a monsoon climate and is located in a subtropical/tropical moist forest life zone (Holdridge, 1967). The mean annual rainfall of 1927 mm has a distinct seasonal pattern, with 75% of it falling from March to August and only 6% from December to February (Huang and Fan, 1982). Nitrogen deposition in precipitation was 36–38 kg N ha^{-1} y^{-1} in 1990s (Huang et al., 1994; Zhou and Yan, 2001), and was measured at 34 and 32 kg N ha^{-1} y^{-1} in 2004 and 2005, respectively (Fang et al., 2007). Annual mean relative humidity is 80%. Annual mean temperature is 21.0 °C, with average temperature of the coldest (January) and warmest (July) month at 12.6 °C and 28.0 °C, respectively (Huang and Fan, 1982).

2.2. Experimental treatments

Nitrogen addition experiments were initiated in 2003 (Mo et al., 2006). Three N addition treatments (each with three replicates) were established: Control (no added N, atmospheric deposition = 36–38 kg N ha^{-1} y^{-1} in the 1990s), Low-N (additional input of 5 g N m^{-2} y^{-1}), and Medium-N (additional input of 10 g N m^{-2} y^{-1}). Low-N treatment (86–88 kg ha^{-1} y^{-1}, atmospheric deposition plus experimental N input) corresponded to a value similar to the projected N deposition (45–113 kg ha^{-1} y^{-1}) in 2030 in southern China (Zheng et al., 2002). Nine plots (three N treatments, each with three replicates) of dimensions 20 m by 10 m were randomly established, each surrounded by a 10-m-wide buffer strip. NH_4NO_3 solution was sprayed monthly by hand onto the forest floor of these plots as 12 equal applications over the whole year beginning in July 2003 and continued ever since. Fertilizer was weighed, mixed with 20 L of water, and applied to the plots below the canopy using a backpack sprayer. Two passes were made across each plot to ensure an even distribution of fertilizer. The Control plots received 20 L water without N (Mo et al., 2006).

Two types of litter with contrasting nutrient status (nutrient-rich needle and nutrient-poor needle) were collected from the reserve. The pine needles were collected using litter traps and nylon mesh placed on the forest floor under the pine trees, and collected in August and September 2005, time of peak litterfall (Zhang et al., 2000). The nutrient-rich needle was collected from plots receiving continuous experimental N inputs in the past 26 months while nutrient-poor litter was collected from Control plots and areas without experimental N inputs in the plantation.

All needles were air-dried to a constant weight. The needles of each litter type were mixed to obtain a uniform mixture before filling the mesh bags. Six sub-samples (about 12.00 g per sub-sample) from each type of needles were analyzed for the initial oven-dry weight (conversion rate from air-dried to 105 °C), and initial C, N and P concentrations. We measured the soil properties of plots where the needles were collected using soil samples collected in September 2005 (Lu et al., 2008). The results showed that available N (NH_4^+-N and NO_3^--N) concentrations in the soil generally increased with increasing levels of N addition (Tab. I, from Lu et al., 2008). These increases were more pronounced in NO_3^--N than in NH_4^+-N. There was no significant effect of N addition on total N, total P, total C and C/N ratios in the 0-10cm mineral soils. Nor was the difference found on the concentrations of exchangeable base cations except for $Ca^{2+}$and Mg^{2+}. Soil pH value decreased with increasing levels of N addition, but the difference among N treatments was not statistically significant (Tab. I). The pine needles collected from plots receiving experimental N inputs were higher in nutrients (nutrient-rich needles) than the pine needles collected from Control plots and areas without experimental N inputs (nutrient-poor needles) (Tab. II).

Table I. Soil properties (0–10 cm soil depth) in the Control, Low-N and Medium-N plots in a tropical pine plantation in southern China.

	Control	Low-N	Medium-N
NH_4^+ -N (mg kg^{-1})	2.8(0.4)a	2.6(0.4)a	3.7(0.8)b
NO_3^- -N (mg kg^{-1})	2.5(0.2)a	4.5(1.0)b	4.5(0.3)b
Total N (mg kg^{-1})	1.3(0.1)	1.2(0.1)	1.2(0.1)
Total P (mg kg^{-1})	0.18(0.01)	0.18(0.00)	0.17(0.02)
Total C (mg kg^{-1})	27(1)	24(2)	25(2)
C/N	20(1)	21(1)	21(1)
pH (H_2 O)	4.07(0.09)	3.96(0.02)	3.98(0.02)
K (mg kg^{-1})	61(3)	62(4)	58(2)
Ca (mg kg^{-1})	385(16)b	449(7)a	439(7)a
Na (mg kg^{-1})	43(4)	54(6)	44(3)
Al (mg kg^{-1})	321(11)	336(15)	303(19)
Mg (mg kg^{-1})	19(1)b	22(1)a	23(1)a
Mn (mg kg^{-1})	2.0(0.2)	1.8(0.3)	2.2(0.2)

Data are cited from Lu et al., 2008. Values are means with 1 SE in parentheses, $n = 6$; measured in September 2005. The Low-N and Medium-N plots have been receiving continuous experimental N additions for 26 months before the decomposition study. Different letter within a single row indicate significant difference ($p < 0.05$).

2.3. Field sampling

A total of 144 litter bags were prepared in October 2005, using polyvinyl screen mesh (0.5 mm in the bottom and 2 mm in the top) of approximately 25 cm × 25 cm in dimension. The number and contents of the bags were as follows: 72 bags for each type of needles, with each bag filled with about 12.00 g air-dried litter. The litter bags were placed on the forest floor along with the existing litter at the end of October 2005, in total 9 experimental plots.

Two litter bags of each litter type were collected from each plot at about 3, 6, 9 and 12 months after the start of the decomposition study and the data from two litter bags were pooled for each plot, yielding a sample size of three for each type of needles in each N treatment on each collection date. The bags were returned to the laboratory for further processing and later analysis.

2.4. Laboratory procedures

In the laboratory, litter was cleaned from any ingrown roots and other contaminants and oven-dried in paper bags first to a constant weight at 40 °C (Mo et al., 2006). After drying, the contents of each bag were weighed individually (Sundarapandian and Swamy, 1999). Subsamples of dried litter was grounded to pass a 0.15 mm mesh sieve and analyzed later for C, N, and P concentrations.

Total C was measured by dichromate oxidation before titration with Fe^{2+} solution (Liu et al., 1996). Total N concentration was determined with semimicro-Kjeldahl digestion followed by the detection of ammonium, and total P concentration was analyzed colorimetrically after digestion (Liu et al., 1996). Subsamples were dried at 105 °C to a constant weight (at least 24 h), and all results are reported on 105 °C basis (Liu et al., 1996).

Table II. Initial chemical properties of pine (*Pinus massoniana*) needles in a tropical pine plantation in southern China.

Needle type	N mg g^{-1}	C mg g^{-1}	P mg g^{-1}	C/N	C/P	N/P
Nutrient-poor	9.8(0.2)a	434(7)a	0.54(0.01)a	44(1)a	808(21)a	18.2(0.4)a
Nutrient-rich	11.5(0.1)b	445(8)a	0.63(0.01)b	39(1)b	708(17)b	18.3(0.2)a

* Values are means with 1 SE in parentheses, $n = 6$; Different letter within a single column indicate significant difference at $p < 0.001$; Nutrient-poor pine needles were grown in the control plots and areas without experimental N inputs; Nutrient-rich pine needles were grown in the plots receiving experimental N inputs continuously in the previous 26 months (including Low-N and Medium-N plots).

2.5. Data analyses

The model for decomposition that we used is represented by the following equations (Olson, 1963; Xu, 2006): (1) $X/Xo = e^{-kt}$ (exponential model); and (2) $X/X_o = a + kt$ (linear model).

Where X/Xo is the fractional mass remaining at time t, X the mass remaining at time t, Xo the original mass, "e" the base of natural logarithm, k the decomposition constant (y^{-1}), and "a" the intercept. We test the fitness of both linear and exponential models to our results. The exponential model was fit to the data using least squares regression of the natural logarithm of fractional mass remaining (litter mass, and content of C and P, respectively) (Jensen and Nybroe, 1999).

A repeated measure ANOVA with Tukey's HSD test was performed to examine the overall effect of N addition on the concentration of litter N, C and P, ratios of C/N, C/P and N/P, the fraction of initial mass remaining, and the C, N and P content of the decomposing needles (values of X/Xo). One-way ANOVA with Tukey's HSD test was performed to test the difference in decomposition rate (values of X/Xo) among treatments for each sample date (Mo et al., 2006), decomposition constant (k) among treatments, and initial chemical quality of pine needles between nutrient-rich needles and nutrient-poor needles. One-way ANOVA with Tukey's HSD test was also used to examine the effect of time on litter N of decomposing needles (values of X/Xo) in the Control plots for each type of needle. Differences in the final litter mass remaining, final C, N and P content, and their decomposition constants in the Control plots between nutrient-rich needles and nutrient-poor needles were compared using a standard t-test. All analyses were conducted using SPSS 13.0 for windows. Statistical significant differences were set with p values < 0.05 unless otherwise stated.

3. RESULTS

3.1. Initial chemical quality of needles

At the beginning of the experiment, there was a significantly higher concentration of N and P in the nutrient-rich needles compared to the nutrient-poor needles ($p < 0.001$), but no significant difference in C concentration and N/P ratio ($p > 0.33$) (Tab. II). Conversely, there were significantly lower ratios of C/N and C/P in nutrient-rich needles than in nutrient-poor needles ($p < 0.001$). These results suggest that previous N addition (continued for 26 months) had increased the nutrient status of pine needles in the N treated plots relative to the Control plots.

3.1.1. Changes in mass, and C and P contents

The amount of litter mass decreased linearly with time in both types of needle (nutrient-poor and nutrient-rich) and in all three N treatments ($p < 0.001$, Figs. 1a–1b), whereas the C and P contents in decomposing needles followed a similar pattern, decreasing exponentially with time in both types of needle and in all treatments ($p < 0.05$, Figs. 1c–1f). The regression equations describing their changes over time were all significant ($p < 0.05$), with the coefficients of determination (r^2) ranging from 0.78 to 1.00 (Tab. III).

In Control plots with no experimental N input, losses of litter mass and litter C tended to be faster in nutrient-rich needles than in nutrient-poor needles (Tab. III), but the differences were not statistically significant ($p = 0.079$ for litter mass, $p = 0.062$ for litter C). However, the losses of litter P were significantly faster in nutrient-rich needles than in nutrient-poor needles ($p < 0.001$, Tab. III).

Nitrogen addition had no significant effect on the mass loss of nutrient-poor needles ($p = 0.5$) (Fig. 2a). However, both levels of N addition significantly decreased mass loss of nutrient-rich needles ($p < 0.002$) (Fig. 2a). These significant negative effects on the nutrient-rich needles were mostly found in the later stage of decomposition (Fig. 1b). The differences between the Control and Low/Medium-N plots, in terms of the fraction of initial mass remaining, became larger with the increase of decomposition time. At the end of the 12-month experiment, the mass remaining was on average, about 5% higher in N-treated plots than that in the Control plots (Fig. 1b).

The effect of N addition on C content varied depending on litter type, N treatment level and the length of decay (Figs. 1c and 1d). During early stage of decomposition, both levels of N addition significantly increased (i.e. negatively affected) C content in both types of needle relative to those in the Control plots ($p < 0.05$). This increase was more pronounced in nutrient-rich needles than in nutrient-poor needles (Figs. 1c and 1d). In nutrient-poor needles, this increase was only found in the first three months and was 4%–5% higher in N treated plots than in the Control plots (Fig. 1c). Whereas in nutrient-rich needles, this increase was found in the first nine months and was 4%–15% higher in N treated plots compared to the Control plots (Fig. 1d). Later in decomposition, N addition significantly decreased (positively affected) C content of both litter types ($p < 0.05$). These positive effects on C loss were less pronounced in nutrient-rich needles than in nutrient-poor needles (Figs. 1c and 1d). At the end of the experiment, the final

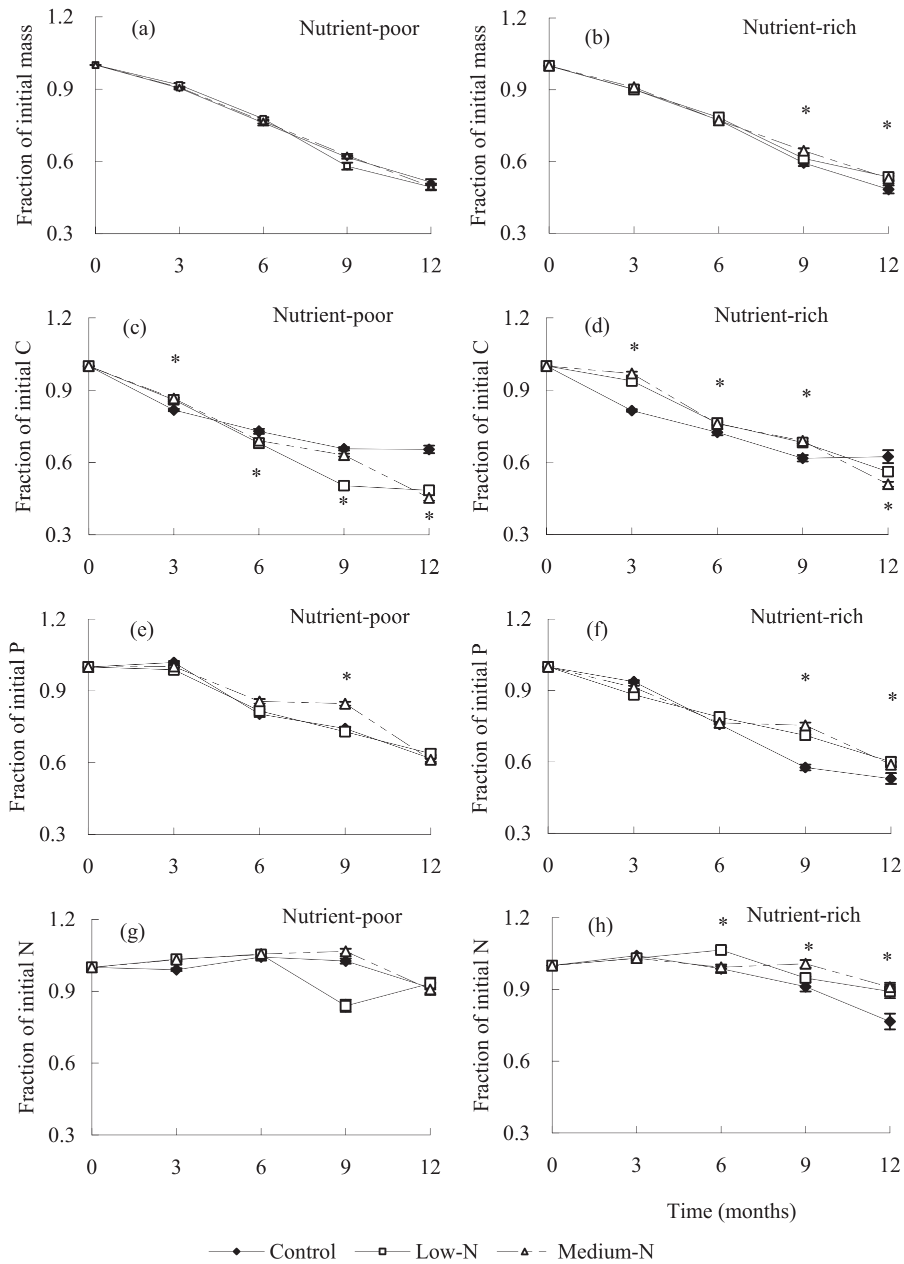

Figure 1. Changes in mass and contents of C, N and P in decomposing pine (*Pinus massoniana*) needles with two different nutrient-status in a tropical pine plantation in southern China. Asterisk (*) indicates significant different at least between two treatments at $p < 0.05$ ($n = 3$). Nutrient-poor pine needles were collected from the Control plots and areas without experimental N inputs; Nutrient-rich pine needles were collected from the plots receiving experimental N inputs continuously in the previous 26 months (including Low-N and Medium-N plots).

C content was, on average, 19% lower in the Low/Medium-N plots than that in the Control plots for nutrient-poor needles, whereas for nutrient-rich needles, the final C content was, on average, 9% lower in the Low/Medium-N plots than that in the Control plots (Figs. 1c and 1d).

The effect of N addition on the change of P content during decomposition was more pronounced in nutrient-rich needles than in nutrient-poor needles (Figs. 1e and 1f). Both levels of N addition significantly reduced the loss of P over the entire decomposition period in nutrient-rich needles ($p < 0.05$) (Fig. 2c), whereas in nutrient-poor needles, only Medium-N addition had a significant effect on the P loss ($p < 0.05$).

3.1.2. Changes in N contents

In Control plots, the N content in nutrient-poor needles slightly increased (net immobilization) during the first

Table III. Decomposition constants (k) for the pine (*Pinus massoniana*) needles with two different nutrient-status in a tropical pine plantation in southern China.

Item/needle type	Control	Low-N	Medium-N
Litter mass*			
Nutrient-poor	0.50(0.01)aA	0.54(0.02)aA	0.52(0.01)aA
Nutrient-rich	0.54(0.01)aA	0.49(0.01)bB	0.48(0.01)bB
C content**			
Nutrient-poor	0.43(0.02)bA	0.72(0.02)aA	0.76(0.02)aA
Nutrient-rich	0.51(0.03)bA	0.58(0.02)bB	0.68(0.02)aB
P content**			
Nutrient-poor	0.51(0.02)aB	0.48(0.02)aA	0.46(0.02)aA
Nutrient-rich	0.71(0.03)aA	0.49(0.02)bA	0.50(0.02)bA

Values are means with 1 SE in parentheses, $n = 3$; * values are obtained from linear regressions, ** values are obtained from exponential regressions. The regressions showed in the table are all significant at $p < 0.05$ level, with the coefficients of determination (r^2) ranging from 0.78 to 1.00; Different capital letter within a single column indicate significant difference ($p < 0.05$) in a given item; Different lowercase letter within a single row indicate significant difference ($p < 0.05$).

9 months decomposing, followed by a decline over the next three months with 91% remaining at the end of the study (Fig. 1g). The N content in the first three sampling dates was not significant different compared to the initial N content, whereas it was significantly lower (9%) in the last sampling date ($p < 0.001$). The N content in nutrient-rich needles slightly increased during the first 3 months of decomposition, followed by a decline over the next 9 months with 77% remaining at the end of the study (Fig. 1h). The N content in the first two sampling dates was not significantly different compared to the initial N content, whereas it was significantly lower in the third (9%) and the last sampling date (23%) ($p < 0.001$). The loss in N content during decomposition was significantly faster in nutrient-rich needles than in nutrient-poor needles ($p < 0.01$).

Similar to P content, both level of N addition significantly increased N content over the entire decomposition period in nutrient-rich needles relative to the Control plots ($p < 0.05$), but no such significant effect was found in nutrient-poor needles (Fig. 2d).

3.2. Nutrient concentration and their ratios

Nitrogen addition had no significant effect on the measured nutrient indices (concentration of C, N and P, and ratios of C/N, C/P and N/P) in nutrient-rich needles over the entire decomposition period (Figs. 2e–2j). Nitrogen addition had no effect on the concentration of N and P, and ratios of N/P in decomposing nutrient-poor needles (Figs. 2f, 2g and 2j). However, N addition significantly decreased the concentration of C, and ratios of C/N and C/P in nutrient-poor needles over the entire decomposition period ($p < 0.05$, Figs. 2e, 2h and 2i).

4. DISCUSSION

In a previous paper, we have reported that decomposition constant (k) of pine needles in the unfertilized pine plantation (control plots) was 0.40 during the period from July 2003 to June 2004 (Mo et al., 2007). That value was slightly lower than the corresponding value (nutrient-poor needle collected from the Control plots and areas without experimental N inputs, and decomposed in the Control plots) observed in this study. The reasons for the relative higher decomposition rate here could be explained by the seasonal and yearly variations in litter decomposition. The litter for this experiment was incubated in October 2005, whereas the litter for the previous experiment was incubated in July 2003 (Mo et al., 2007).

Our results were consistent with many other studies reporting that litter with high initial N concentration exhibits relatively faster decomposition rate (Berg and Matzner, 1997; Fog, 1988; Sundarapandian and Swamy, 1999; Xu, 2006). In Control plots with no additional experimental N input, rates of litter mass loss and litter C loss tended to be faster in nutrient-rich needles than in nutrient-poor needles. Moreover, rates of N and P loss were significantly faster in nutrient-rich needles than in nutrient-poor needles during the decomposition.

We have previously reported that N addition had no significant effect on mass loss rate of decomposing needles in the 1st year of this N fertilization experiment ($p = 0.279$, from Mo et al., 2007). Results from this study showed that, after 26 months of continuous N fertilization, further N addition had no significant effect on the mass loss of nutrient-poor needles (pine needles grown in the Control plots and areas without experimental N inputs), indicating that cumulative N addition did not change the effect on mass loss of decomposing needles in this pine plantation.

Our finding that N addition significantly decreased litter mass loss in nutrient-rich needles supports the hypothesis that decomposition of nutrient-rich needles would be more sensitive to N deposition than the decomposition of nutrient-poor needles. The similar negative effects of experimental N addition on mass loss of decomposing litter were also found in a "sister" decay project of broadleaf litter (Fang et al., 2007). In that study, the initial N concentrations of the broadleaf litter (14.8 mg kg^{-1} for *Schima superba* litter and 13.8 mg kg^{-1} for *Castanopsis chinensis* litter) were much higher than the concentration of nutrient-poor needles, and after 26 months of continuous N addition, further N addition significantly decreased mass loss of decomposing litter for the above broadleaf species in this plantation (Fang et al., 2007).

The negative effect of N addition on litter mass loss may be related to the negative effect of N input on the decay of older and more humified material in the later stages of decomposition (Berg and Matzner, 1997). In this study, N addition significantly increased N content of decomposing nutrient-rich needles in the later stage of decomposition relative to the Control plots. This corresponded to the negative effect of N addition on the loss of litter mass during that stage. However, no such significant increase of N content was found in nutrient-poor needles. It is proposed that during later stages, lignolytic enzyme efficiency maybe limited by high N content or by high

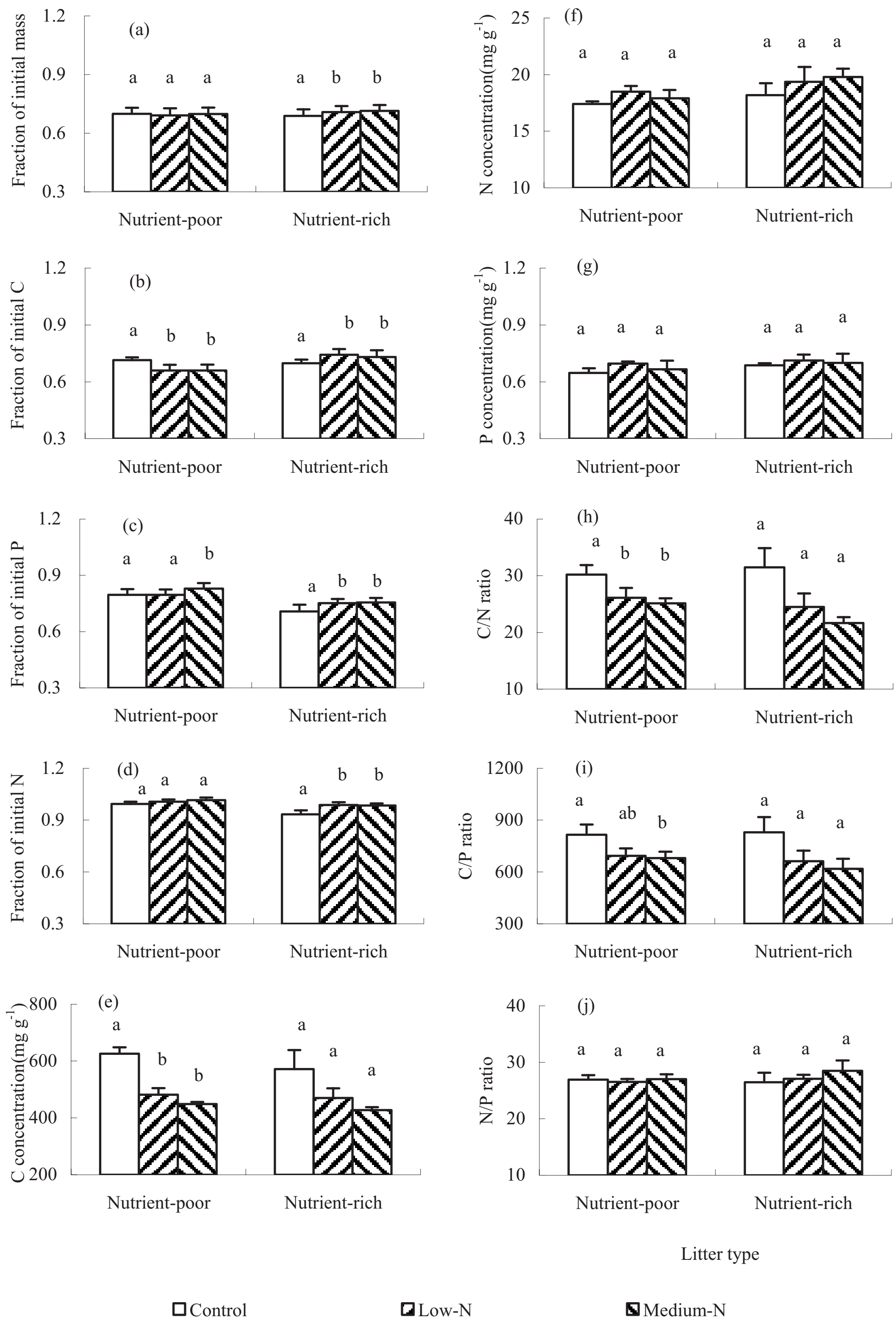

Figure 2. Overall effects of N addition on the measured indices (litter mass, C, N, P, e.g.) of decomposing pine (*Pinus massoniana*) needles with two different nutrient-status in a tropical pine plantation in southern China. Each column represents the mean value across all sampling dates in a given treatment. Mean values sharing the same letter are not significantly different among treatments at $p < 0.05$ (repeated measure ANOVA test, $n = 3$).

availability of inorganic N (Ågren et al., 2001; Berg, 1986; Berg et al., 1998; Fog, 1988; Hagedorn et al., 2003; Magill and Aber, 1998; Micks et al., 2004). This is also supported by the results found in laboratory experiments (Craine et al., 2007).

The depressed effect of N addition on mass loss of litter in this study is consistent with those found in a long-term litter bag study, in which N addition was found to significantly decrease the rate of litter mass loss (Magill and Aber, 1998; Prescott et al., 1999; Ågren et al., 2001), and similar to the results found in our previous study in a N saturated old-growth forest (Mo et al., 2006; Fang et al., 2007). Our results are also similar to the results found in an NH_4NO_3 addition experiment in an oak forest and a red pine plantation in North America (Micks et al., 2004), and the results that N addition suppressed mineralization of soil organic matter in Europe (Hagedorn et al., 2003).

As N addition significantly increased soil available N (NH_4^+-N and NO_3^--N), that would lead to a significant increase of N concentration in pine needles. The negative effect of N addition on litter mass loss in nutrient-rich needles suggests that future N deposition would decrease the decomposition in this plantation, enhancing C sequestration in the soils. This interpretation is also supported by the results on C release from decomposing needles.

While cumulative N input did not change the effect on mass loss, it did alter the effect on nutrient release from decomposing needles in this plantation. We have also previously reported that N addition significantly decreased release of N from decomposing needles in the 1st year of this N fertilization experiment ($p = 0.006$, from Mo et al., 2007). Results from this study indicated that N addition had no significant effect on the release of N from decomposing nutrient-poor needles. These results indicate the cumulative N input had increased soil available N in this plantation. It is generally accepted that microbes will immobilize N from soil solutions during decomposition in N-poor systems. Similar responses were also found for the release of P from decomposing nutrient-poor needles during these two experimental periods (Mo et al., 2007). Our results are similar to other studies in tropical forests reporting that fertilization can induce microbial nutrient retention without simultaneous effects on mass loss of decomposing litter (McGroddy et al., 2004; Cleveland et al., 2006).

5. CONCLUSIONS

After 26 months of continuous N addition, further N addition had no significant effect on the rate of litter mass loss in nutrient-poor needles (pine needles collected from the Control plots and areas without experimental N inputs) and significantly decreased litter mass loss in nutrient-rich needles (pine needles collected from the plots receiving experimental N inputs).

The induced C loss by N addition during decomposition was more pronounced in nutrient-poor needles than in nutrient-rich needles. Nitrogen addition also significantly reduced P release in decomposing needles, but this suppressed effect was more pronounced in nutrient-rich needles than in nutrient-poor needles. Similarly, N addition significantly reduced N release in the decomposition of nutrient-rich needles, but no such effect was found in the decomposition of nutrient-poor needles, thus, after 26 months of continuous N addition, the effect of continued N deposition on litter decomposition would be more sensitive due to the change of litter chemistry.

Our results, combined with the results from previous litter decomposition experiment in the same site, indicate the importance of long-term measures of litter decomposition in response to N deposition (measured at different stages of N saturation) and suggest that: (1) cumulative N deposition could change the effect of N availability on litter decomposition; (2) this effect of N varies depending on the nutrient status of litter; (3) future continued atmospheric N deposition in reforested ecosystems in southern China could affect forest carbon balance and nutrient cycling due to its shifting effect on litter decomposition.

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