Postprint: Litter Decomposition and Soil C and N Stoichiometric Characteristics in Pinus massoniana Forests of Different Stand Ages under Simulated Nitrogen Deposition

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Abstract

Simulating the effects of N deposition on forest ecosystems represents a key issue in current global change ecology research. Soil carbon pools are highly sensitive to N deposition. N deposition increases exogenous N content during litter decomposition, indirectly affecting the chemical processes of litter decomposition and modifying litter decomposition rates. Therefore, investigating the relationship between litter decomposition and soil C-N under simulated N deposition is critical for predicting forest C sequestration. Using the in situ litterbag method, we examined the stoichiometric responses and relationships of litter-soil C and N during litter decomposition in Pinus massoniana forests of different stand ages in the Three Gorges Reservoir area under simulated N deposition. N deposition treatments consisted of control (CK, 0 g m ^{2} a ^{1}), low nitrogen (LN, 5 g m ^{2} a ^{1}), medium nitrogen (MN, 10 g m ^{2} a ^{1}), and high nitrogen (HN, 15 g m ^{2} a ^{1}). The results showed that after 540 days of decomposition, N deposition promoted litter decomposition in 20-year and 30-year-old Pinus massoniana forests. In the 46-year-old forest, only the low nitrogen treatment promoted litter decomposition. Among all treatments, decomposition was fastest in the 30-year-old stand, indicating that litter with low initial N content from the same tree species showed a positive response to N deposition. N deposition treatments promoted decomposition of litter with low initial N content, while litter with high initial N content easily reached "N saturation" during decomposition. N deposition inhibited C release from litter in the 20-year and 46-year-old stands (0.62%-6.69% lower than control), but promoted C release in the 30-year-old stand (0.28%-5.55% higher than control). N immobilization in the 30-year and 46-year-old stands was higher than the control (0.15%-21.34% higher than control), while in the 20-year-old stand it was lower than the control (5.70%-13.87% lower), indicating that simulated N deposition treatments promoted C release from litter with low initial C content and N immobilization in litter with low initial N content. Under N deposition treatments, only the 30-year-old Pinus massoniana forest exhibited increased soil organic carbon compared to the control. Soil organic matter was positively correlated with litter C, N, and decomposition rate, and significantly negatively correlated with litter C/N ratio. Soil total nitrogen was positively correlated with litter decomposition rate and litter N content, while the soil organic carbon/total nitrogen ratio was positively correlated with litter C and N contents. In the control treatment, the ranking of influence of litter decomposition indices on soil nutrients was decomposition rate < litter C content < litter C/N ratio < litter N content, while in the low, medium, and high nitrogen treatments, the ranking was litter C content < decomposition rate < litter N content < litter C/N ratio. The study indicates that Pinus massoniana forests with low soil nutrient content showed a positive response to N deposition. N deposition promoted litter decomposition and improved soil fertility in forests with low soil nutrients. Ecosystems with low litter quality and low soil nutrient content showed more significant soil C responses to N deposition.

Full Text

Preamble

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Relationship between leaf litter decomposition and soil C, N stoichiometry in different-aged *Pinus massoniana* stands exposed to simulated nitrogen deposition

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Abstract

The effects of simulated nitrogen (N) deposition on forest ecosystems represent a major research focus in global change ecology. Soil carbon storage is particularly sensitive to atmospheric N deposition, which increases exogenous N content during litter decomposition and indirectly affects the chemical processes and decomposition rates of leaf litter. Understanding the relationship between litter decomposition and soil carbon and nitrogen dynamics under N deposition is therefore critical for accurately predicting ecosystem carbon sequestration.

Using an *in situ* litterbag method, we investigated the relationship between leaf litter decomposition and soil C and N stoichiometry in different-aged *Pinus massoniana* stands in the Three Gorges Reservoir Region under simulated N deposition. Four N deposition treatments were established: control (CK, 0 g N m ^{2} yr ^{1}), low N (LN, 5 g N m ^{2} yr ^{1}), medium N (MN, 10 g N m ^{2} yr ^{1}), and high N (HN, 15 g N m ^{2} yr ^{1}).

Our results showed that simulated N deposition accelerated leaf litter decomposition in 20- and 30-year-old stands. In 46-year-old stands, however, only the LN treatment promoted decomposition over the 540-day study period. The decomposition rate was highest in 30-year-old stands across all treatments, indicating that leaf litter with lower initial N content from the same species responded positively to N deposition. Specifically, N deposition promoted decomposition of litter with low initial N content, while litter with high initial N content more readily reached N saturation.

Nitrogen deposition inhibited carbon release from litter in 20- and 46-year-old stands (0.62%–6.69% lower than control), but promoted carbon release in 30-year-old stands (0.28%–5.55% higher than control). The amount of immobilized N in leaf litter was 0.15%–21.34% higher in N deposition treatments compared to control in 30- and 46-year-old stands, but 5.70%–13.87% lower in 20-year-old stands. This suggests that simulated N deposition accelerated carbon release from litter with low initial carbon content and promoted nitrogen immobilization in litter with low initial N content.

Soil organic carbon increased in 30-year-old stands under N deposition compared to control and was positively correlated with leaf litter C and N content and decomposition rate, while negatively correlated with the C/N ratio. Soil total nitrogen was positively correlated with leaf litter decomposition rate and N content, and the soil organic carbon to total nitrogen ratio was positively correlated with leaf litter C and N content.

In the control treatment, leaf litter N content had the strongest effect on soil nutrients, followed by litter C:N ratio and C content, while decomposition rate had the weakest effect. In LN, MN, and HN treatments, however, the litter C:N ratio showed the strongest effect on soil nutrients, followed by litter N content and decomposition rate, while litter C content had the weakest effect.

Our study demonstrates that *P. massoniana* stands with low soil nutrient con-

centrations responded positively to N deposition, which accelerated leaf litter decomposition and improved soil fertility. These results suggest that ecosystems with low-quality leaf litter and low soil nutrient concentrations would respond more strongly to N amendment than other ecosystems.

Keywords: *Pinus massoniana*; simulated N deposition; different-aged stands; leaf litter decomposition; stoichiometry

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1. Study Area Overview

The study area is located in Zigui County, Yichang City, Hubei Province (30°38 14 -31°11 31 N, 110°00 14 -111°18 41 E). The region has a subtropical continental monsoon climate with an average annual temperature of 17-19°C. Extreme temperatures range from -2.5°C (1989) to 44.2°C (1998). Annual precipitation is 1000–1250 mm, concentrated in May to September. Relative humidity ranges from 60% to 80%.

Elevation in Zigui County varies from 40 to 2057 m. Below 500 m, the landscape is dominated by tea plantations, citrus orchards, and farmland, while areas above 500 m are primarily shrubland and forest. The study sites are located in Maoping Town, Zigui County, above 500 m elevation. All *P. massoniana* stands originated from aerial seeding in the late 1970s. Understory shrubs are predominantly *Camellia oleifera*, with herbaceous species including *Dicranopteris gigantea*, *Miscanthus sinensis*, and *Echinochloa crusgalli*. Lianas such as *Ficus martini* and *Smilax china* are common in the interlayer. The basic characteristics of the study sites are shown in .

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2. Experimental Design and Sample Collection

2.1 Plot Establishment

Three *P. massoniana* stands of different ages (20, 30, and 46 years) were selected. In each stand, we established a 20 m × 30 m plot, which was divided into twelve 3 m × 3 m subplots spaced at least 10 m apart to prevent interference. Before placing litterbags, the existing litter layer was removed from each subplot.

2.2 Nitrogen Deposition Treatments

Based on the substrate quality of *P. massoniana* leaf litter and referencing international N manipulation studies, four treatments were established: control (CK, 0 g N m ^{2} yr ^{1}), low N (LN, 5 g N m ^{2} yr ^{1}), medium N (MN, 10 g N m ^{2} yr ^{1}), and high N (HN, 15 g N m ^{2} yr ^{1}). NH NO was dissolved in deionized water and applied monthly using a 1.5 L sprayer from January to December 2010. Control plots received equivalent amounts of water only. The total water added annually was equivalent to 1.0–1.5 mm of precipitation, minimizing differences between treatments.

2.3 Litter Decomposition Experiment

Freshly fallen leaf litter was collected from each stand in October 2010, mixed thoroughly, and air-dried. Fifteen grams of air-dried litter were placed in 20 cm × 20 cm litterbags with 0.5 mm mesh size. In November 2010, litterbags were placed on the mineral soil surface after removing the litter layer. Five litterbags per treatment were randomly retrieved at 90, 180, 270, 360, 450, and 540 days after placement. Retrieved samples were cleaned of roots and soil, oven-dried at 105°C for 12 hours, and weighed to calculate dry mass remaining.

2.4 Soil Sampling

In October 2010, background soil samples were collected from the 0–5 cm layer using a 5 cm diameter soil auger along diagonal transects in each plot. Additional soil samples were collected beneath litterbags at each retrieval date. All samples were stored in sealed bags for analysis of soil organic carbon (SOC) and total nitrogen (TN).

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3. Litter and Soil Sample Analysis

Litter samples were oven-dried, ground, and analyzed for total carbon (C) using the potassium dichromate oxidation method, and total nitrogen (N) using the Kjeldahl digestion method. Initial lignin and cellulose contents were determined by acid detergent and neutral detergent fiber methods, respectively.

Soil samples were analyzed according to the Forestry Industry Standards of the People's Republic of China [22]. Soil organic carbon was measured by potassium dichromate oxidation with external heating, and total nitrogen by sulfuric acid digestion. Fresh soil samples were oven-dried at 105°C for 12 hours to determine moisture content and calculate air-dry to oven-dry conversion coefficients.

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4. Data Processing

The dry mass remaining rate (R) of leaf litter was calculated as:

$$R = \frac{M_t \times k}{M_0} \times 100\%$$

where M_0 is the initial air-dry mass, M_t is the oven-dry mass at time t, and k is the conversion coefficient between air-dry and oven-dry mass.

The decomposition rate constant (L) was calculated using the negative exponential model:

$$L = -\ln\left(\frac{R}{100}\right)$$

Statistical analyses were performed using Excel 2007 and SPSS 16.0. One-way ANOVA and least significant difference (LSD) tests were used to examine treatment effects at = 0.05. Canonical correspondence analysis (CCA) was conducted using Canoco 4.5 to analyze relationships between litter decomposition indices and soil nutrients.

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5. Results

5.1 Litter Decomposition Rates Under Simulated N Deposition

The decomposition patterns were similar across N treatments, showing an initial slow phase followed by accelerated decomposition. In 20-year-old stands, the dry mass remaining at 540 days was significantly lower in the HN treatment (64.25 ± 4.34%) compared to CK (65.29 ± 1.14%) ($P < 0.05$). At 360 days, the MN treatment showed significantly lower remaining mass than other treatments ($P < 0.05$), indicating N deposition promoted decomposition.

In 30-year-old stands, decomposition was fastest among all stand ages. At 540 days, dry mass remaining followed the order: MN (58.70 ± 2.11%) < HN (60.44 ± 0.80%) < LN (60.67 ± 4.40%) < CK (60.89 ± 2.79%). The HN treatment significantly promoted decomposition at 450 days ($P < 0.05$).

In 46-year-old stands, only the LN treatment enhanced decomposition. At 540 days, dry mass remaining was lowest in LN (62.74 ± 4.99%), which was significantly lower than CK (64.38 ± 2.79%) ($P < 0.05$). The MN and HN treatments showed higher remaining mass than CK, indicating inhibition of decomposition at higher N loads.

Both N deposition and decomposition time had significant effects on litter decomposition ($P < 0.05$). The influence of N deposition became more pronounced over time, with positive effects in early stages but inhibitory effects later compared to control. The positive response was most evident in 30-year-old stands .

5.2 C, N, and C/N Dynamics During Decomposition

Nitrogen deposition significantly affected carbon release during decomposition. In 20-year-old stands, all N treatments reduced C release compared to CK (1.98%–7.84% lower). In 30-year-old stands, N treatments promoted C release (5.66%–11.21% higher than CK), with significant differences between HN and

CK at 540 days ($P < 0.05$). In 46-year-old stands, N treatments inhibited C release (-0.19% to -0.52% compared to CK), with HN showing significant inhibition at 540 days ($P < 0.05$).

Nitrogen immobilization patterns varied by stand age. In 20-year-old stands, N immobilization was lower in N treatments (28.60%–42.47% less than CK). In 30-year-old stands, N immobilization was higher in N treatments (34.51%–55.85% more than CK), with significant differences between treatments at 360 and 540 days ($P < 0.05$). In 46-year-old stands, N immobilization was also higher in N treatments (1.19%–4.19% more than CK).

The C/N ratio decreased during decomposition in all stands. In 20-year-old stands, the reduction was greatest in MN (39.68%) and smallest in CK (35.90%). In 30-year-old stands, the pattern was similar, with MN showing the largest decrease (34.62%). In 46-year-old stands, CK showed the greatest C/N reduction (32.95%) [Figure 2: see original paper].

5.3 Soil Organic Carbon and Total Nitrogen Dynamics

Seasonal variation in soil organic carbon (SOC) differed among stand ages and treatments. In 20-year-old stands, SOC was highest in CK during the growing season (May–September), while HN showed the highest values in other months. In 30-year-old stands, HN treatment had significantly higher SOC than other treatments in most months ($P < 0.05$). In 46-year-old stands, SOC was relatively stable across seasons.

Soil total nitrogen (TN) also showed distinct seasonal patterns. In 20-year-old stands, TN was highest in CK during the growing season. In 30-year-old stands, HN treatment significantly increased TN during the growing season ($P < 0.05$). In 46-year-old stands, TN was highest in CK and lowest in HN treatment.

The soil C/N ratio varied seasonally, with higher values in May–September and lower values in other months. The variation was most pronounced in 20-year-old stands and least in 46-year-old stands [Figure 3: see original paper].

5.4 Relationships Between Litter Decomposition and Soil Nutrients

Canonical correspondence analysis revealed significant relationships between litter decomposition indices and soil nutrients under N deposition. In CK treatment, litter N content had the strongest influence on soil nutrients, followed by C/N ratio and C content, while decomposition rate had the weakest effect. In LN, MN, and HN treatments, the litter C/N ratio showed the strongest influence, followed by litter N content and decomposition rate, while litter C content had the weakest effect.

Correlation analysis showed that soil organic carbon was positively correlated with litter C and N content and decomposition rate, but negatively correlated with litter C/N ratio. Soil total nitrogen was positively correlated with litter decomposition rate and N content. The soil C/N ratio was positively correlated with litter C and N content .

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6. Discussion

6.1 Effects of N Deposition on Litter Decomposition Rates

Simulated N deposition partially satisfied microbial N demands, thereby promoting litter decomposition [23]. When N input matches the biological and soil absorption capacity of the ecosystem, the degree of N limitation becomes a key factor controlling decomposition. In this 540-day study, N deposition accelerated decomposition in 20- and 30-year-old stands, consistent with previous research [14].

However, the response varied by stand age and initial litter quality. The 46-year-old stands showed positive response only to low N addition, suggesting that litter with different initial N contents responds differently to N deposition. This supports the hypothesis that N deposition promotes decomposition of low-N litter, while high-N litter more readily reaches N saturation. High N treatments may inhibit decomposition by accumulating inorganic N that impedes lignin and cellulose breakdown [25], while low N addition increases decomposition rates by improving substrate quality [26].

The temporal dynamics were also important. Both N deposition and decomposition time had significant effects ($P < 0.05$), with N deposition promoting decomposition in early stages but showing inhibitory effects later. This pattern supports findings that N deposition effects vary with decomposition stage [29,30]. The positive response was most evident in 30-year-old stands, which had lower initial soil and litter N contents, suggesting that N-limited ecosystems are more responsive to N addition [11].

6.2 Effects of N Deposition on Litter Decomposition Stoichiometry

Nitrogen deposition significantly affected carbon and nitrogen dynamics during decomposition. In 20- and 46-year-old stands, N treatments reduced carbon release compared to control, while in 30-year-old stands, N treatments enhanced carbon release. This indicates that N deposition accelerates carbon loss from low-carbon litter but inhibits it from high-carbon litter.

Nitrogen immobilization patterns revealed that low-N litter showed greater N retention under N deposition, while high-N litter showed less. This reflects the stoichiometric requirements of decomposer communities [34]. The C/N ratio decreased during decomposition, with the magnitude of reduction varying by treatment and stand age. The greater C/N reduction in N treatments suggests that N addition alleviated N limitation and stimulated microbial activity, particularly in 30-year-old stands.

6.3 Effects on Soil Organic Carbon and Total Nitrogen

Simulated N deposition increased soil organic carbon in 30-year-old stands, consistent with findings that N addition can enhance soil carbon storage in N-limited systems [29]. The positive correlation between SOC and litter decomposition rate indicates that faster decomposition contributes to soil carbon accumulation. However, the effect varied seasonally, with N deposition showing stronger effects during the growing season.

Soil total nitrogen dynamics reflected the balance between N input, immobilization, and plant uptake. In 30-year-old stands, HN treatment significantly increased TN during the growing season, suggesting that N addition enhanced soil N availability. In contrast, 46-year-old stands showed decreased TN under N deposition, possibly due to rapid uptake and leaching of available N [13].

The soil C/N ratio was negatively correlated with litter decomposition rate, indicating that faster decomposition leads to lower C/N ratios in soil. This relationship was most pronounced in N deposition treatments, where N addition directly altered soil stoichiometry.

6.4 Interactions Between Litter Decomposition and Soil Nutrients

The CCA analysis revealed that N deposition changed the relative importance of litter quality parameters in influencing soil nutrients. While litter N content was most important in control plots, the litter C/N ratio became the dominant factor under N deposition. This shift indicates that N addition alters the stoichiometric constraints on decomposition [37].

The significant correlations between litter and soil parameters demonstrate tight coupling between aboveground and belowground processes. Soil organic carbon and total nitrogen were positively correlated with litter C and N content, confirming that litter quality directly influences soil nutrient status. The negative correlation between soil C/N ratio and litter decomposition rate suggests that stoichiometric imbalances can regulate decomposition rates [38].

Our findings indicate that ecosystems with low-quality litter and low soil nutrient concentrations respond more strongly to N deposition [39]. The 30-year-old stands, which had lower initial nutrient status, showed the most positive response to N addition, with accelerated decomposition and improved soil fertility. This has important implications for forest management in the Three Gorges region, where *P. massoniana* plantations on nutrient-poor soils may benefit from moderate N deposition.

However, the inhibitory effects observed at high N loads and in older stands caution against excessive N input. High N deposition can alter microbial community structure, reduce enzyme activity, and disrupt ecosystem nutrient balance [40]. The transition from positive to negative effects with increasing N load and stand age highlights the importance of considering ecosystem-specific characteristics when predicting responses to N deposition.

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Note: Figure translations are in progress. See original paper for figures.