Chemical Quality Changes of Pinus massoniana Litter and Its Relationship with Decomposition Rate under Experimental Manipulation: Postprint

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Abstract

Masson pine (Pinus massoniana) litter exhibits slow decomposition; thus, promoting its decomposition, enhancing nutrient return rates, and maintaining soil fertility stability have become critical issues in the sustainable management of Masson pine plantations. Accordingly, an orthogonal experimental design L9(3^4) was employed, selecting four artificial regulation factors—microbial agents, surfactants, different carbon-nitrogen nutrient solutions, and organic fertilizers—to conduct a litter decomposition regulation experiment under Masson pine stands, aiming to elucidate the effects and efficacy of different regulation combinations on litter decomposition rates and chemical quality. The results demonstrated that organic fertilizer and microbial agents significantly influenced the decomposition rate of Masson pine litter, with the combined application of decomposer agent 2 and chicken manure being particularly conducive to decomposition.

During natural decomposition of Masson pine litter under the forest canopy, chemical quality parameters shifted in a direction favorable to decomposition; N and P exhibited net accumulation, while C/N, C/P, L/N, and L/P ratios displayed decreasing trends. Artificial regulation measures accelerated this transformation process. The impacts of different regulation measures on litter chemical quality parameters varied. Organic fertilizer addition facilitated increased N and P contents in residual litter and decreased C/N, C/P, L/N, and L/P ratios. Microbial decomposer agent 2 promoted reductions in L/P and C/P ratios. Surfactant OP 10 contributed to decreased L/N ratios in litter.

Under artificial regulation, regulation factors could influence decomposition rates by altering litter chemical quality. N content and C/N ratio were the primary factors affecting Masson pine litter decomposition rate, whereas the

effects of P concentration, L/N, C/P, and L/P on decomposition rate were irregular or non-significant.

Full Text

Preamble

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Impact of Different Control Measures on Leaf Litter Chemical Quality Dynamics and Its Relations with Decomposition Rate Under Pure *Pinus massoniana* Forest

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Abstract

The slow decomposition rate of *Pinus massoniana* litter is an important problem that results in soil nutrient imbalance in forests. Accelerating litter decomposition to increase the nutrient return rate and maintain soil productivity stability is considered an efficient measure for sustainable management of *P. massoniana* plantations. This study employed orthogonal tests to examine four artificial regulation factors: microbial inoculants, surfactants, different carbon-nitrogen nutrient solutions, and organic fertilizers. The objective was to understand the effects of different regulation combinations on litter decomposition rate and chemical quality dynamics.

Field experiments conducted under the forest canopy revealed that organic fertilizer and microbial inoculants significantly affected the decomposition rate of *P. massoniana* litter, with the fastest decomposition occurring under the combined treatment of microorganisms 2 and chicken manure. Under natural conditions, nitrogen and phosphorus become relatively concentrated in residual litter during decomposition, while the ratios of C/N, C/P, lignin/N (L/N), and lignin/P (L/P) decrease over time—changes that accelerate decomposition. Additives can speed up this process according to the same principle.

The effects of different additives varied considerably. Organic fertilizer addition increased nitrogen and phosphorus concentrations while reducing C/N, C/P, L/N, and L/P ratios. Microbial inoculants resulted in lower C/P and L/P ratios, while surfactants helped reduce the L/N ratio of residual litter. Across all control treatments, litter decomposition rate correlated positively with nitrogen concentration and negatively with the C/N ratio. However, the decomposition rate showed no obvious relationship with phosphorus content, L/N ratio, C/P ratio, or L/P ratio.

Under artificial control, the decomposition rate can be affected by changing litter chemical quality, with nitrogen content and C/N ratio being the main factors involved in regulating litter decomposition. Artificial regulation measures accelerate the change process of C/N, C/P, L/N, and L/P ratios. Under artificial control, regulation factors can influence decomposition rate by altering litter chemical quality, with C/N ratio being the primary factor affecting *P. massoniana* litter decomposition rate.

Keywords: different control measures; decomposition; litter chemical quality; *Pinus massoniana*

Introduction

Forest litter represents a critical component of material cycling and energy flow in forest ecosystems. The rate of litter decomposition and its associated nutrient release determines the characteristics of ecosystem nutrient processes and the supply of available soil nutrients, which subsequently affects plant nutrient uptake. At smaller spatial scales, the physical and chemical properties of fresh forest litter play a primary role, exhibiting differences in decomposition characteristics and rates.

With deepening understanding of the relationship between litter chemical attributes and decomposition, parameters such as C/N, C/P, lignin/N, and lignin/P have become common indicators of litter chemical quality. Some scholars have attempted to influence litter decomposition through artificial measures that alter substrate quality, including nutrient regulation by adding nitrogen and phosphorus, inoculating functional fungi or microbial agents, and mixing litters with different substrate qualities. While most previous research has employed single regulation methods, studies using multi-factor comprehensive regulation remain scarce.

Pinus massoniana is one of the most important timber species in southern China, characterized by wide distribution and high utilization. Masson pine needles are mostly thick and coriaceous with well-developed cuticles, containing high levels of lignin and tannins that resist microbial decomposition and impede leaching and mechanical breakdown by soil fauna, resulting in slow decomposition. Accelerating the decomposition of *P. massoniana* litter to increase nutrient return and maintain soil fertility stability has become a key issue in sustainable plantation management. This study selected different regulation factors—microbial inoculants, surfactants, carbon-nitrogen nutrient solutions, and organic fertilizers—to compare their effects on litter decomposition rate and chemical quality changes.

1 Study Site Overview

The experimental site was located in a *Pinus massoniana* forest on Songlin Slope, South Campus of Guizhou University (26°34 N, 106°42 E, 1110 m). The area has a subtropical monsoon humid climate with mean annual precipitation of 1178.1 mm, mean annual temperature of 15°C, 1354 annual sunshine hours, and a frost-free period of 270 days. The soil developed from Quaternary red clay. The stand age was 31.6 m (dominant height) with an average DBH of 42.3 cm.

The shrub layer mainly included *Camellia oleifera*, *Lindera nacusua*, *Ilex intermedia*, *Viburnum fordiae*, and *Rhododendron simsii.* The herbaceous layer was dominated by *Dryopteris erythrosora*, *Carex* sp., *Ophiopogon bodinieri*, *Rubus burgei*, and *Carex tristachya.*

2 Experimental Materials

The pine needles used in the experiment were freshly fallen, collected from the same middle-aged *P. massoniana* forest in Longli Forest Farm, Guizhou. The collected needles were brought to the laboratory, where intact, undecomposed needles were retained, rinsed quickly with tap water, drained, and oven-dried for sealed storage.

3 Experimental Design and Methods

3.1 Orthogonal Test Design

Based on the main factors influencing and limiting *P. massoniana* litter decomposition, four regulation factors were selected: (A) microbial inoculants, (B) surfactants, (C) carbon-nitrogen nutrient solution, and (D) organic fertilizer. An orthogonal test design was employed with three levels for each factor (Table 1).

Table 1 Orthogonal test design of leaf litter decomposition

Test number	Microbial inoculants	Surfactant	Carbon and nitrogen nutrient solution	Organic fertilizer
1	A1	B1 (Tween 80)	C1 (NaNO3)	D1
2	A1	B2 (OP-10)	C2	D2
3	A1	B3	C3 (NH4Cl)	D3
4	A2	B1	C2	D3
5	A2	B2	C3	D1
6	A2	B3	C1	D2
7	A3	B1	C3	D2
8	A3	B2	C1	D3
9	A3	B3	C2	D1

3.2 Treatment Methods

Microbial inoculants: Microorganisms are the primary participants in litter decomposition. Two types of functional microbial agents were inoculated: (1) *Purpureocillium lilacinum* isolated from the litter layer under *P. massoniana* stands, and (2) a crude fiber-degrading microbial agent produced by Guangzhou Weiyuan Biological Technology Co., Ltd.

Surfactants: Surfactants have wax-softening effects. Given the well-developed cuticle of pine needles that impedes decomposition, surfactants (Tween 80 or OP-10) were used at 4 g/L to pretreat litter, aiming to disrupt the waxy layer. Needles were soaked for 4 hours, then rinsed repeatedly with tap water, drained, and oven-dried.

Carbon-nitrogen nutrient solution: To ensure microbial growth and reproduction, different forms of nitrogen nutrient solution were sprayed monthly (30 mL per bag). The solution contained glucose at 20 g/L with C/N ratio of 85.37, prepared with NaNO3, NH4Cl, or urea to compare different nitrogen forms.

Organic fertilizer: Two types of organic fertilizer (chicken manure with C/N 6.25 and oil cake with C/N 7.13) were purchased from Guiyang Flower and Bird Market and mixed with pine needles at a 1:1 ratio.

3.3 Experimental Setup

According to the experimental design, 10 g of treated needles were placed in 25 cm × 15 cm decomposition bags (0.5 mm mesh). Each treatment group had 30 bags placed flat on the semi-decomposed layer in the experimental site, with 2-3 cm spacing between bags and larger gaps between different treatment groups. A control (CK) was established without any additives, receiving only 30 mL of water sprayed monthly.

4 Chemical Analysis

Total nitrogen and phosphorus concentrations were determined using the vario MACRO cube elemental analyzer and molybdenum-antimony colorimetry after perchloric acid digestion (LY/T1270—1999). Lignin content was analyzed using a cellulose analyzer based on the Van Soest method.

5 Data Processing and Statistical Analysis

Litter mass loss rate (Li) was calculated as:

$$Li = 100 \times (Mi - M_t)/Mi$$

where Mi is the initial mass (g) and M_t is the residual mass at different sampling times (g).

The improved Olson exponential model was used to fit decomposition dynamics:

$$y = a \times e^{-kt}$$

where y is the residual rate, t is time, k is the decomposition constant, and a is a fitting parameter.

SPSS 19.0 software was used for orthogonal test ANOVA and intuitive analysis to compare effects of different control measures on decomposition coefficients. Multi-factor ANOVA and LSD multiple comparisons were performed to examine effects on residual litter quality. Linear regression analysis explored relationships between mass loss rate and residual litter quality dynamics. Significance level was set at P = 0.05.

6 Results and Analysis

6.1 Litter Decomposition Dynamics and Differences

Mass loss rates of all treatment groups increased gradually over time (Figure 1). After 360 days, the cumulative mass loss rate ranking was: Group 7 > 9 > 1 > 6 > CK > 2 > 8 > 4 > 3 > 5. Group 7 showed the highest cumulative mass loss rate at 52.8%.

Table 2 Olson equations and decomposition parameters for different treatment groups

Test number	Olson equation	Correlation coefficient	Significance	Decomposition constant k	Half-life (a)	95% decomposition time (a)
1	y = 0.88e^(-0.46t)	0.8577	**	0.46a	1.51	6.52
2	y = 0.92e^(-0.74t)	0.9198	**	0.74a	0.94	4.05
…	…	…	…	…	…	…

Decomposition constant k values ranked as: 7 > 9 > 6 > 2 > 8 > CK > 5 > 1 > 4 > 3. Groups 1, 2, and 7 required less time for 50% decomposition than CK, while groups 2, 6, 7, 8, and 9 required less time for 95% decomposition. Group 7 consistently showed the shortest decomposition times, reducing 50% and 95% decomposition times by 2.14 and 0.88 years compared to CK, respectively.

Table 3 Variance analysis of decomposition constants based on different control measures

Indicator	Factors	Statistics	Optimal solution
Decomposition constant	Microbial inoculants	F = 75.00**	A3
	Surfactant	F = 81.50**	D2

Note: F0.05(2,2) = 19.00, F0.01(2,2) = 99.00; Ki represents the sum of results for level i.

Both microbial inoculants and organic fertilizer had significant effects on litter decomposition coefficients.

6.2 Residual Litter Chemical Quality Dynamics and Differences

Under natural decomposition (CK), nitrogen and phosphorus concentrations showed overall increasing trends, though still higher than initial values after 120 days. In contrast, C/N, C/P, L/N, and L/P ratios decreased below initial values, with fluctuations over time. Most treatment groups showed higher nitrogen and phosphorus concentrations than CK, particularly at 120 days.

Table 4 Multi-factor ANOVA of residual litter quality parameters based on different control measures

Litter quality	Factors	Statistics	Litter quality	Factors	Statistics
Nitrogen content	Microbial inoculants	F = 0.035	C/N ratio	Microbial inoculants	F = 0.139
	Surfactant	F = 0.460**		Surfactant	F = 0.001
	Nutrient solution	F = 12.46a		Nutrient solution	F = 37.86a
	Organic fertilizer	F = 11.28b		Organic fertilizer	F = 40.08a

Organic fertilizer and microbial inoculants significantly affected residual litter nitrogen content, with chicken manure showing stronger effects than oil cake. Organic fertilizer significantly reduced C/N, C/P, L/N, and L/P ratios, with chicken manure more effective than oil cake. Surfactants significantly affected L/P ratio, with OP-10 showing stronger effects than Tween 80.

6.3 Relationship Between Litter Decomposition Rate and Chemical Quality Dynamics

Linear regression analysis revealed that mass loss rates correlated positively with nitrogen content (significant in groups 2, 3, 4, 6, 7, 9) and negatively with

C/N ratio (significant in groups 2, 3, 4, 6, 7, 9). Relationships with phosphorus content, L/N, C/P, and L/P ratios were inconsistent or non-significant across groups.

Table 5 Relationships between litter mass loss rate and residual litter chemical quality dynamics

Test number	Nitrogen content	Phosphorus content	C/N ratio	C/P ratio	L/N ratio	L/P ratio
1	r = 0.427	r = -27.673	r = -0.209	r = 0.007	r = 0.255	r = 0.012
2	r = 0.399*	r = 15.336	r = -0.798**	r = -0.023	r = 0.309	r = -0.020
…	…	…	…	…	…	…

7 Discussion and Conclusions

7.1 Effects of Different Control Measures on Litter Decomposition Rate

Initial litter chemical composition determines whether microbial communities can effectively obtain required energy and nutrients, significantly affecting decomposition rate. Adding exogenous nutrients to nutrient-poor litter can improve initial chemical quality and supplement microbial nutritional requirements, theoretically promoting decomposition. Previous studies on exogenous nitrogen and phosphorus additions have shown varied effects depending on litter type, environmental conditions, and treatment methods.

Organic fertilizer significantly affected litter decomposition coefficients in this study, with chicken manure showing better performance than oil cake. This indicates that organic fertilizers, rich in organic matter and various nutrients, can improve litter quality and affect decomposition rate. Microbial inoculants also significantly influenced decomposition coefficients, with mixed microbial agents performing better than single fungi. This demonstrates that adding appropriate functional microbial agents based on litter compound composition can increase decomposer populations and enhance decomposition rates.

Treatment group 7, containing optimal levels of both microbial inoculants and organic fertilizer, showed the best decomposition parameters. The combination compensated for nutrient deficiencies, improved litter chemical quality, and met microbial nutritional requirements, while enriching decomposer diversity and abundance. This suggests that multi-factor collaborative regulation based on litter characteristics is an effective approach to accelerate decomposition in *P. massoniana* forests.

7.2 Effects of Different Control Measures on Litter Quality Parameter Changes

During litter decomposition, release of non-organic nutrients occurs only when carbon-to-nutrient ratios fall below certain thresholds. If ratios exceed these values at decomposition onset, elements are immobilized until minimum values are reached. The initial C/N (85.37) and C/P (1774.6) ratios of *P. massoniana* litter were high, causing nitrogen and phosphorus to accumulate during natural decomposition, consistent with previous studies.

Under natural decomposition (CK), nitrogen and phosphorus concentrations increased while C/N, C/P, L/N, and L/P ratios generally decreased, indicating that residual litter quality parameters changed in directions favorable for nutrient release. All treatment groups showed more pronounced changes than CK, demonstrating that artificial regulation accelerated these processes.

Different regulation factors had distinct effects on quality parameters. Organic fertilizer, rich in nutrients, significantly increased nitrogen and phosphorus concentrations while reducing C/N, C/P, L/N, and L/P ratios. Microbial inoculants significantly affected C/P and L/P ratios by decomposing organic carbon to meet their growth requirements. Surfactant OP-10 significantly reduced L/N ratio, likely by softening the waxy cuticle and facilitating microbial colonization.

7.3 Influence of Litter Chemical Quality Dynamics on Decomposition Rate

As decomposition proceeds, litter chemical quality changes continuously. While most previous research focused on relationships between initial chemical quality and decomposition rate, the chemical quality of residual litter from previous stages inevitably affects subsequent decomposition. Examining this dynamic relationship provides more realistic insights into decomposition processes.

Under natural decomposition (CK), mass loss rate correlated significantly and positively with nitrogen content dynamics and negatively with C/N ratio dynamics. Under artificial regulation, these relationships became more pronounced, indicating that regulation measures enhanced the influence of these parameters on decomposition rate. Nitrogen content and C/N ratio were the main factors regulating decomposition in this study.

The relationships between mass loss rate and L/N, C/P, or L/P dynamics were inconsistent or non-significant, suggesting these parameters were not primary determinants of decomposition rate under artificial control. This study demonstrates that selecting appropriate regulation factors can influence litter decomposition rate by altering litter chemical quality, with C/N ratio being the key factor. Multi-factor collaborative regulation represents a promising approach for managing *P. massoniana* litter decomposition, though further research with expanded factor sets and replicated field plots is needed to validate these findings and elucidate underlying mechanisms.

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