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Original Research

Factors Controlling Decomposition Rates of Needle Litter Across a Chronosequence of Chinese Pine (*Pinus tabuliformis Carr.*) Forests

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Abstract

We investigated how factors underlying local spatial variations controlled needle litter decomposition across a chronosequence of Chinese pine (*Pinus tabuliformis Carr.*) forests. Litterbag methods were used to measure changes in litter chemistry and the mass loss of leaf litter, as well as selective biotic and abiotic factors during the growing seasons (May-October) in 2013 and 2014 in a set of fully replicated *P. tabuliformis Carr.* secondary forest stands that differ in age in northern China. During the two growing seasons the path analysis identified the litter lignin/N ratio, soil microbial metabolic quotient (qCO_2), soil diversity of fungal assemblages (SFD), and soil-water content (SWC) as dominant controlling factors in needle litter decomposition, collectively explaining 76.9% of the total variation in mass loss across the entire age sequence. Litter lignin/N and soil qCO_2 had the greatest negative effects on the k value, followed by weaker positive effects of SFD and SWC. Our findings indicate that forest stand age has a great influence on needle litter decomposition by determining litter quality, with soil microbial activity and local environmental factors being secondary drivers in needle litter decomposition across a chronosequence of Chinese pine (*Pinus tabuliformis Carr.*) forests.

Keywords: litter decomposition, litter quality, environmental factors, *Pinus tabuliformis Carr.*

Introduction

Leaf litter decomposition is an important ecological process that regulates the cycle of matter, such as the release of CO_2 into the atmosphere and nutrient mineralization in soil, providing the main source of nutrients for biological activity and playing a crucial role in the maintenance of soil fertility in forest ecosystems [1-2]. Generally, the rate of litter decomposition is positively correlated with the N content of initial litter, while it is negatively correlated with C/N and lignin/N ratios of initial litter over a wide range of ecosystems [3]. Moreover, environmental conditions, including soil fertility, microclimate, and

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faunal and microbial communities in the forest floor, can also indirectly alter decomposition rates [4]. Particularly, soil microorganisms are the animate component of soil organic matter, and they play essential roles in the transformation from litter to soil organic matter, as well as the formation and development of the soil structure [5]. An in-depth understanding of the determinants of decomposition rates of leaf litter will greatly contribute to understanding the functioning of forest ecosystems.

Currently, the importance of intraspecific variability in litter quality is receiving more attention [6]. Litter quality, which is controlled by species identity, also varies considerably within the same species due to differences in nutrient availability and climate conditions [7]. Although there are many studies on the intraspecific variation of litter quality through genetic variability and environmental heterogeneity, studies on forest stand age as a potential driving factor of intraspecific litter quality variability are scarce [8]. Thus, there is a need for further investigation concerning the forest stand age-associated variability of litter decomposition.

In addition, as a powerful predictor of ecosystem structure and function, forest stand age could affect soil physical and chemical properties and the qualitative and quantitative composition of decomposer communities [9-10] because soil decomposer organisms have species-specific feedback interactions with leaf litter quality based on forest stand age in the transformation of litter to soil organic matter, which contributes to the nutrient availability from different root exudates and the litter decomposition of the corresponding forest stand age type [11]. Consequently, the type and quantity of soil microbial communities are influenced by specific forest types at different ages [12]. Soil microbial indicators were applied in litter decomposition programmes because of their essential roles in leaf litter decomposition [5, 10]. Specifically, the major indicators of soil microbial activity are microbial biomass, microbial respiration, microbial diversity, etc. [13].

Additionally, forests of different ages are considered to have distinctive microclimates on the forest floor, such as different soil temperatures and moisture contents [14]. Many studies have described the importance of soil temperature and precipitation on the rates of litter decomposition [15-17]. Wang proposed that the temperature sensitivity of broadleaf litter decomposition was equivalent to that of coniferous needle litter, which varied with the extent of decomposition in a subtropical forest in China [18]. Berbeco observed that soil warming accelerated the decomposition rates of fine woody debris in temperate forests [15]. Cortez attributed the predominant control of litter decomposition to soil temperature and moisture limitations [17]. Therefore, it is necessary to observe whether or not the soil temperature and moisture content in the chronosequence stands influence needle litter decomposition.

As a result, variations in both litter quality and environmental conditions in forest stands may result in the spatial heterogeneity of decomposition rates of litter at different ages. However, the relative contributions of various factors in controlling the decomposition rates of needle litter across a chronosequence of Chinese pine forests are poorly understood.

In this study, we investigated needle litter decomposition across a chronosequence of Chinese pine forests in northern China, including young (≤30-year-old), middle-aged (40-year-old), immature (50-year-old), and mature (60-year-old) stands. Specifically, the objectives of the study were to identify the dominant controlling factors and their relative contributions to variations in the decomposition of needle litter across a chronosequence of Chinese pine forests.

Materials and Methods

Site Description

The study was carried out in the Liao River Source Nature Reserve (LRSNR, 41°01′-41°21′N, 118°22′-118°37′E), Pingquan County, Hebei Province, China. This nature reserve occupies an area of 3.356×10^4 hm^2 with elevation ranging from 625 m to 1,738 m. The region is in the transition area between the temperate to cold-temperate zones. Long-term mean annual precipitation is 550 mm, occurring mainly in June to September. The mean annual temperature is 7.3°C with monthly average temperature ranging from -10.8°C (January) to 22.9°C (July). The annual frost-free period is 115-130 days, with an early frost in October and late frost in April. Soil type is brown soil and cinnamon soil classified as Eutriccambisol [19].

Experimental Design

This work was conducted based on the Forestry Standards "Observation Methodology for Long-term Forest Ecosystem Research" of the People's Republic of China (LY/T 1952-2011) [20].

Pinus tabuliformis Carr., which is an endemic and widespread native conifer species in northern China, spans from 31°13'N to 43°33'N and from 103°20'E to 124°45'E, covering almost 228.10×10^4 ha of forestland in China. Moreover, *P. tabuliformis Carr.* is a woody perennial and primarily pioneer species of secondary succession [5]. In the study sites, there are four age groups of P. *tabuliformis Carr*, which were natural regeneration, including ≤30, 40, 50, and 60-year-old stands, representing young, middle-aged, immature, and mature stands, respectively. For each forest stand age group we established three 20 × 30 m permanent replicate plots 50 m apart, which have similar landscape position, topographic features, elevation, and exposure to ensure comparability between test results. Detailed information for these four sites is shown in Table 1.

In 2012 we collected freshly senesced leaves of *P. tabuliformis Carr* from the forest floor under four forest stand age stands. All of the litter was air dried until

Table 1. Site characteristics and physiochemical properties of the top soil layer (0-5 cm) of *Pinus tabuliformis Carr*. natural secondary forests (means ±standard error, $n = 3$).

	Forests age classes			
	Young	Middle-aged	Immature	Mature
Stand characteristics				
Tree density (ha^{-1})	1,900	1,050	930	430
Slope angle (°)	27	23	25	19
Aspect	East	East	East	East
Elevation (m)	1,018	985	1,006	992
Mean DBH (cm)	11.75±1.05a	17.16±1.96b	23.17±6.69c	34.05±9.10d
Tree height (m)	10.2±2.7a	14.9±4.2b	17.3±4.4c	19.8±3.5d
Litter layer depth (cm)	1.87±0.45a	2.23±0.55a	3.51±0.52b	4.78±0.48c
Soil characteristics				
SOC ($g \cdot kg^{-1}$)	23.72±0.57a	24.97±0.41b	27.48±0.24c	29.15±0.31d
TN ($g \cdot kg^{-1}$)	0.91±0.05a	1.22±0.05b	1.51±0.07c	1.64±0.04d
TP ($g \cdot kg^{-1}$)	0.23±0.05a	0.38±0.04b	0.59±0.04d	0.48±0.03c
TK ($g \cdot kg^{-1}$)	4.23±0.07b	3.98±0.08a	4.25±0.05b	4.48±0.04c
Soil bulk density ($g \cdot cm^{-3}$)	1.49±0.15c	1.41±0.14b	1.40±0.11b	1.33±0.26a
pH value	6.01±0.05a	6.45±0.05c	6.32±0.04bc	6.22±0.03b

Values designated by the different letters within a variable are significantly different at $p<0.05$; DBH, diameter at breast height, SOC, soil organic carbon content, TN, total nitrogen content, TP, total phosphorus, TK, total potassium, pH, soil pH value

constant weight after collection and stored for the litter decomposition test. In order to calculate the correction factor from air-dried to oven-dried weight, five sub-samples of the litter were dried to a constant weight at 70°C.

A litterbag method was used for estimating rates of leaf litter decomposition, a widely used technique to determine litter mass loss, during a 24-month period. Each litterbag (20 × 30 cm) made from polyethylene netting with a 1.0 × 1.5 mm mesh size. Each bag was filled with 10 g of air-dried litter with a weight accuracy of 10^{-3} g, labeled and sealed with rust-proof staples.

On 19 October, 2012, 288 litterbags (4 forest stand ages × 3 litter-bag replicates × 3 replicate plots × 8 harvests) were deployed between the litter layer and the soil horizon in a random arrangement and fastened to the ground with non-corrosive nails in their corresponding forest aging plots.

Litterbag Sampling and Analysis

Litterbags of each litter at four forest-stand ages were randomly retrieved after 144, 216, 288, 360, 504, 576, 648, and 720 days of decomposition. The litter remaining in each bag was cleaned from extraneous matter, such as attached soil particles, in-growth plant materials and small animals, using tweezers and a brush. In the end, the litter-samples were oven-dried at 70ºC for 72 h to reach a constant mass and then weighed to determine the remaining dry leaf mass.

Chemical Analyses of Litter

Subsamples of the initial leaf litter at four forest stand ages and each litter sample harvested during decomposition were ground to fine powder with a micro-plant mill. All analyses were carried out on three sub-samples. Total C content was determined using the H_2SO_4–$K_2Cr_2O_7$ oxidation method [21]. Total N concentration was determined with an azotometer (Kjeltec 2300, Foss, Sweden) after 0.5 g sub-samples had been digested in 10 ml of concentrated H_2SO_4 mixed with 5 ml of H_2O_2. The concentration of total P was measured using the ammonium molybdate method followed by a colorimetric analysis at 880 nm after acid digestion [22]. Lignin concentration was determined using the acid-detergent fibre procedure [23].

Soil Sampling and Analysis

Soil temperatures were measured with the temperature sensor attached to an automated soil CO_2 flux system (LI-8100, Li-Cor Inc., Lincoln, NE, USA), and the gravimetric soil water content (%SWC) was calculated from the mass loss after drying soil samples to a constant weight (105ºC, 48 h) periodically at each sampling time.

Due to the majority of microorganism distribution between soil depths of 0 cm and 20 cm [24], soil samples were sampled between 0 cm and 5 cm. In each replicate plot, three soil samples were collected randomly between 0 cm and 5 cm respectively after the removal of the upper three litterbags, using a stainless steel soil auger (3 cm in diameter), which were mixed thoroughly to obtain homogeneous composite soil samples.

Soil samples were sieved moist through a 2-mm sieve and stored at 4ºC until soil microbial parameters were performed. All microbial parameters were performed within 7 days of sampling [25].

Soil Microbial Biomass Carbon

The microbial biomass carbon (MBC) of soil samples was determined by the chloroform fumigation and extraction methods, using 0.38 as a conversion factor to convert extracted carbon by 0.5 M K_2SO_4 to MBC [26].

Soil Microbial Respiration

Soil microbial respiration (MR) was measured by the alkali absorption method to quantify CO_2 production at 28ºC in the dark for a 6-day period of incubation [27].

Soil Microbial Metabolic Quotient

The soil microbial metabolic quotient (qCO_2) was calculated by dividing soil microbial respiration (MR) by the corresponding soil microbial biomass carbon (MBC) [28].

All analyses of the indicators of soil microbial activity were carried out in triplicate with one control, and the values were calculated on the basis of the oven-dry (105ºC, 48 h) weight of soil.

Soil Diversity of Fungal Assemblages

The soil diversity of fungal assemblages (SFD) was measured with DNA Extraction and PCR-DGGE, following the experimental procedure in Gao et al. [29].

Calculations

The remaining litter mass (RM) within each litterbag was calculated by dividing litter dry weight (X_i) at each sampling time i by the initial litter dry weight (X_o) using the formula:

$$\%RM = X_i/X_O \times 100$$

The remaining litter nutrient (RN) within each litterbag was calculated using the formula:

$$\%RN = (C_i/X_i)/(C_o/X_o) \times 100$$

Specifically, C_i was the nutrient concentration at each sampling time i and C_o was the initial nutrient concentration.

To quantify the dynamics of mass loss, we fitted change in the amount of litter over time as a negative exponential decay function, developed by Olson [30] and further refined by Barlocher [31], i.e., $M_t = M_0 \times e^{-kt}$, where M_t is the remaining mass at time t, M_o the initial mass of the litter, k the rate of decomposition, and t the incubation time of the litterbags. The time required for 50% and 95% mass loss was calculated as $t_{50\%} = -\ln 0.5/k$ and $t_{95\%} = -\ln 0.5/k$, respectively [30].

Each detected band was identified as a ribotype, and the number of bands (S) reflected the genotypic richness of each sample [32]. The relative abundance of each band (p_i) was expressed as the pixel intensity of the band [33]. The Shannon-Wiener index of richness (H) [34] was calculated with the following equation:

$$H = -\sum_{i=1}^{N} p_i \ln p_i$$

…where $p_i = n_i/N$, n_i is the pixel intensity of each band and N is the total intensity of the sample in a lane.

Statistical Analysis

A one-way ANOVA was conducted to analyze the significance of the differences in environmental factors (soil temperature, soil water content and soil biophysical factors) among the four stand-age classes, followed by Tukey's test during the sampling time. Repeated ANOVA measurements were applied to examine differences in the remaining mass and chemical dynamics of litter among four stand-age classes. The statistical analyses were performed using SPSS version 13.0.1 statistical software (SPSS, Chicago, IL, USA), with the level of significance set as 0.05 in all cases.

A path analysis was conducted to examine the controls of dominant biotic and abiotic factors on litter decomposition. A path model is an advanced, multivariate statistical technique based on hypothesis testing of complex path-relation networks [35-36]. In the most appropriate path model, correlation analyses were used to examine the relationships of the decomposition rate (k) with all the monitored factors. Among the 11 variables significantly related to litter decomposition (i.e., SMC, T, C/N_{litter}, %N, %C, lignin/N, %lignin, MBC, SMR, qCO_2, and SFD), four were chosen in the path analysis due to the autocorrelation among variables, including SMC, lignin/N, qCO_2, and SFD. Specifically, lignin/N was retained to represent plant trait, qCO_2 and SFD as indicators of soil biotic properties, and SMC was considered representative of habitat environmental factors. The analyses were conducted using the maximum likelihood estimation procedures of Amos 17.0 (SPSS, Chicago, IL, USA).

Table 2. Initial chemical properties of needle litter in *Pinus tabuliformis Carr*. natural secondary forests (means ±standard error, $n = 3$; mg/g).

Litter quality attributes	Forest stand age classes			
	Young	Middle-aged	Immature	Mature
C	529.19±6.16c	551.82±4.13b	564.82±5.55a	570.18±7.54a
N	4.82±0.12c	5.72±0.15b	6.12±0.11a	6.36±0.10a
C/N	109.79±3.9a	96.47±2.6b	92.29±2.7bc	89.65±3.2c
Lignin	217.31±5.03c	231.33±5.14b	239.17±4.48a	244.63±4.65a
Lignin/N	45.09±1.01a	40.44±1.11b	39.08±0.95b	38.46±0.92b

Different letters within the same row indicate significantly different means ($p < 0.05$, Tukey's test) among the stands.

Results

Variations in Soil Characteristics and Initial Litter Chemistry Across Forest Stand Age Classes

Among the chronosequence of Chinese pine forests, the mature forest stands had the highest values of SOC and TN, which decreased in the order of mature > immature > middle-aged > young forest stands. TP varied in the order of immature > mature > middle-aged > young forest stands. The highest values of TK occurred in mature forest stands, followed by young/immature forest and middle-aged forest stands. The pH values of all soil samples in this pine age sequence ranged from 6.01 to 6.45, meaning the litter was acidic. Soil bulk density showed a decreasing trend with increasing stand age, with litter variation between middle-aged and immature forest stands (Table 1).

Pinus tabuliformis Carr. litter across the chronosequence stands significantly differed in nutrient concentrations and in nutrient stoichiometry. The detailed descriptions are shown in Table 2.

Temporal Patterns of Changes in Soil Temperature and Soil water Content Across Forest Stand Age Classes

Temporal patterns of changes in soil temperature exhibited a distinct seasonal variation consistent with that in the air temperature observed in the study plots (Fig. 1a). The soil temperature ranged from 7.4ºC to 20.6ºC during the 2013 growing season, and from 8.5ºC to 21.9ºC during the 2014 growing season.

Temporal patterns of changes in soil water content initially showed a fluctuating uptrend during both growing seasons, reaching a peak in August, followed by a rapidly linear decrease to reach the minimum in October (Fig. 1b). There were significant differences

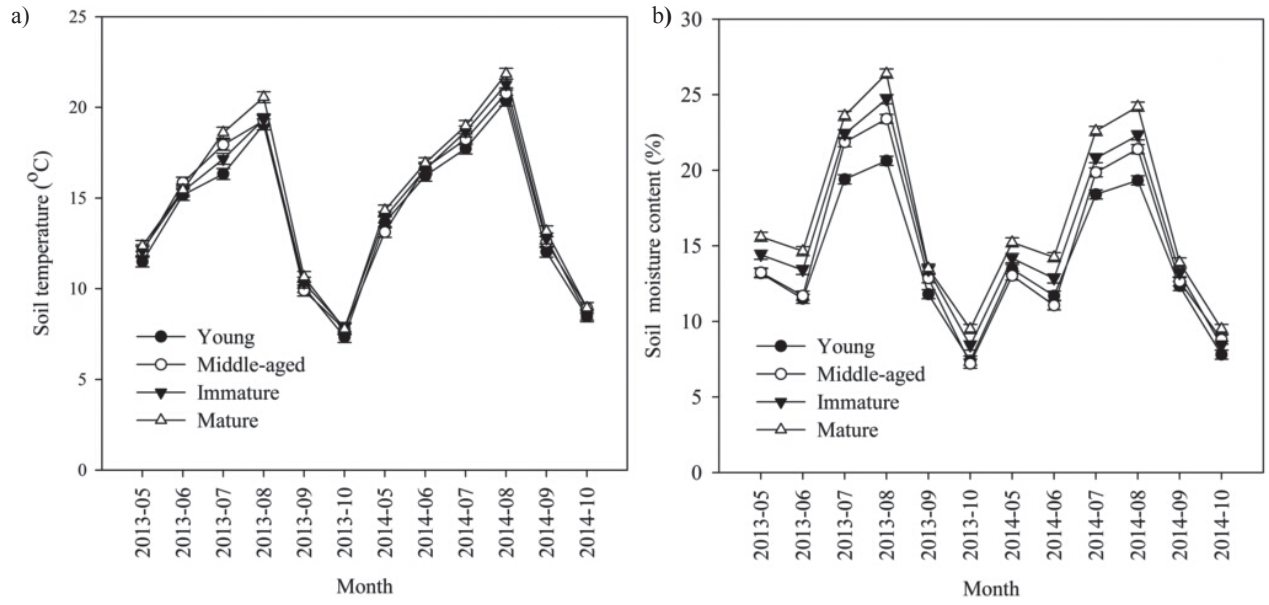

Fig. 1. Seasonal variation in a) soil temperature, *T* (ºC), and b) soil moisture, *M* (%), of all four age classes of forest stands during the 2013 and 2014 growing seasons

Table 3. Decomposition parameters *a*, coefficient *k*, and time (*t*) required for different levels ($t_{50\%}$ and $t_{95\%}$ mass loss) of decay of leaf litter in *Pinus tabuliformis Carr*. forests (means ±standard error, $n = 3$).

Forest stand age classes	Parameter *a*	Coefficient *k*	$T_{50\%}$(year)	$T_{95\%}$(year)
Young	101.02±0.13a	0.24±0.01a	2.81±0.04c	12.13±0.23c
Middle-aged	101.68±0.11b	0.27±0.01b	2.56±0.03b	11.10±0.25b
Immature	101.72±0.14b	0.28±0.01b	2.48±0.05b	10.70±0.21b
Mature	102.18±0.15c	0.32±0.01c	2.16±0.03a	9.36±0.22a

Values followed by different lower letters in the same line indicate a significant difference between means ($p<0.05$)

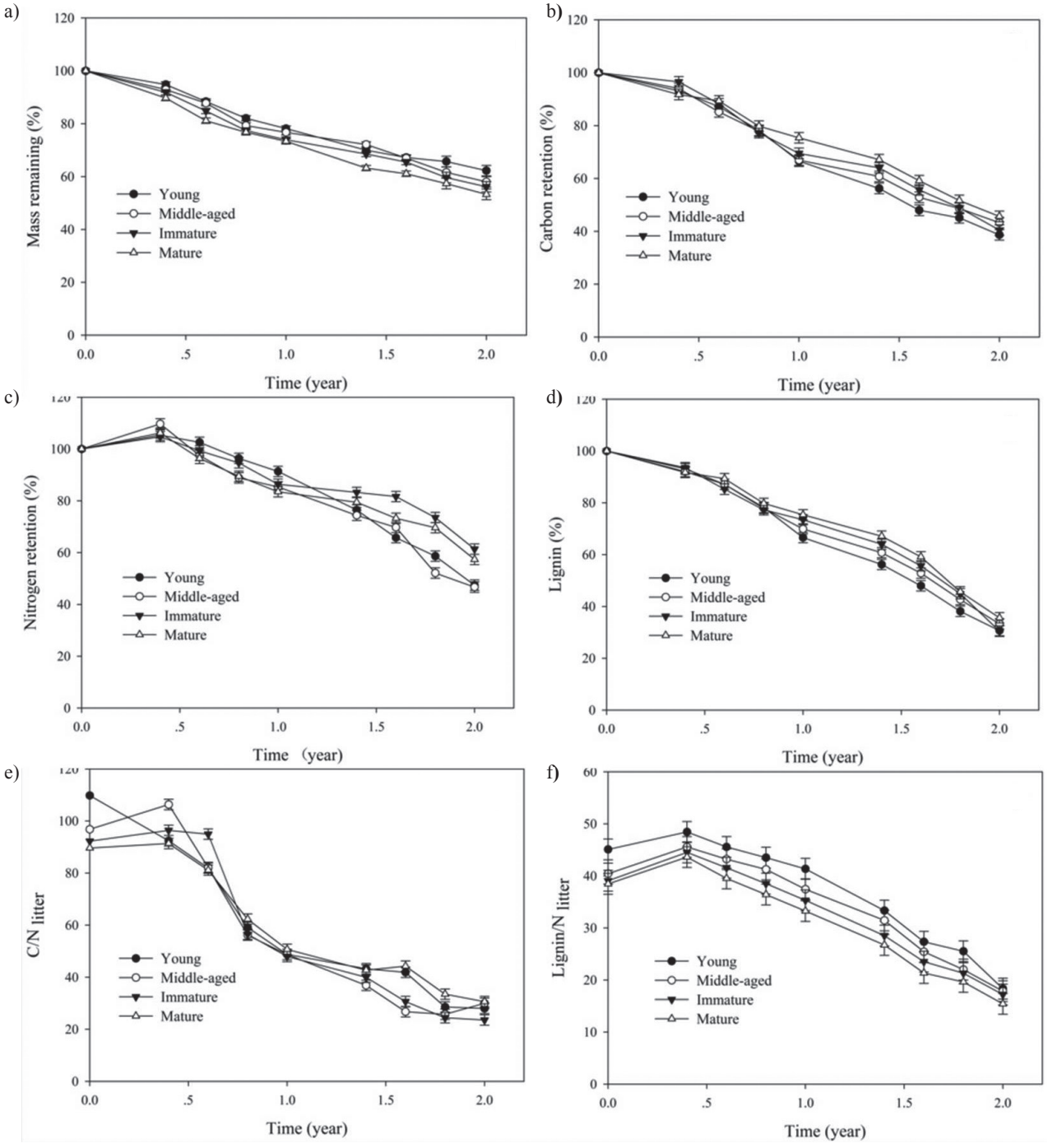

Fig. 2. Temporal changes in litter mass remaining, C retention, N retention, lignin retention, C/N_{litter} ratio, and lignin/N_{litter} ratio during litter decomposition across stand age classes in *Pinus tabuliformis Carr*. Forests; vertical bars indicate standard errors of means ($n = 3$).

in the mean monthly soil water content among the four forest stand-age classes during the two growing seasons (p<0.05), especially between May and August.

Temporal Patterns of Litter Decomposition and Changes in Litter Chemistry Across Forest Stand Age Classes

After 360 days, the rate of decomposition of the four forest stand age classes decreased in the following order: mature > immature > middle-aged > young (p<0.05, Table 3). Specifically, cumulative mass losses of litter in young, middle-aged, immature, and mature stands were 21.86%, 23.23%, 26.07%, and 26.67%, respectively (Fig. 2a). Litter C retention rapidly declined in the first year of development and then decreased at a relatively slower rate during the remaining period of study. Moreover, there was a significantly (p<0.05) lower rate of decline in litter C retention in mature stands than in other forest stand age groups (Fig. 2b).

The change in litter N retention during decomposition initially (0-210 days) exhibited a slight increase in all age groups and decreased thereafter. At the end of the study, the N retention in immature and mature forest stands was significantly greater than that in young and middle-aged forests stands (Fig. 2c).

Contrary to the patterns of changes in litter C retention, the patterns of changes in litter lignin retention experienced a slight decline during the first growing season, followed by a relatively faster and linear decrease at approximately the same level at the end of the study (Fig. 2d).

The patterns of changes in C/N_{litter} during decomposition varied among all forest stand age groups (Fig. 2e). Specifically, the values of litter C/N experienced an initial phase with a great increase in middle-aged forest stands, as well as a slight increase in immature and mature forest stands in the first year of deployment, and then settled down to become stable approximately within the range of 25-35 at the end of the study.

Changes in litter lignin/N during decomposition displayed an initial phase of rapid increase across the entire age sequence and then exhibited an almost linear decreasing function during the remaining period of study (Fig. 2f). Specifically, the order of litter lignin/N was always as follows: mature < immature < middle-aged < young (p<0.05, Fig. 2f) throughout the study period.

Temporal Patterns of Changes in Soil Microbial Biomass Carbon, Basal Respiration, and Microbial Metabolic Quotient Across Forest Stand Age Classes

Fig. 3 shows the variation in soil microbial indicators across the chronosequence of the forests during two growing seasons. In particular, the microbial biomass carbon (MBC) across the chronosequence sharply increased with the duration of decomposition up to its peak in August and drastically decreased thereafter (Fig. 3a). On the whole, MBC exhibited a steady increase from young to mature stands. However, there was no significant difference in MBC between the young and middle-aged forest stands sampled in May and October, as well as the MBC between the immature and mature forest stands sampled in July 2013. In 2014 there was

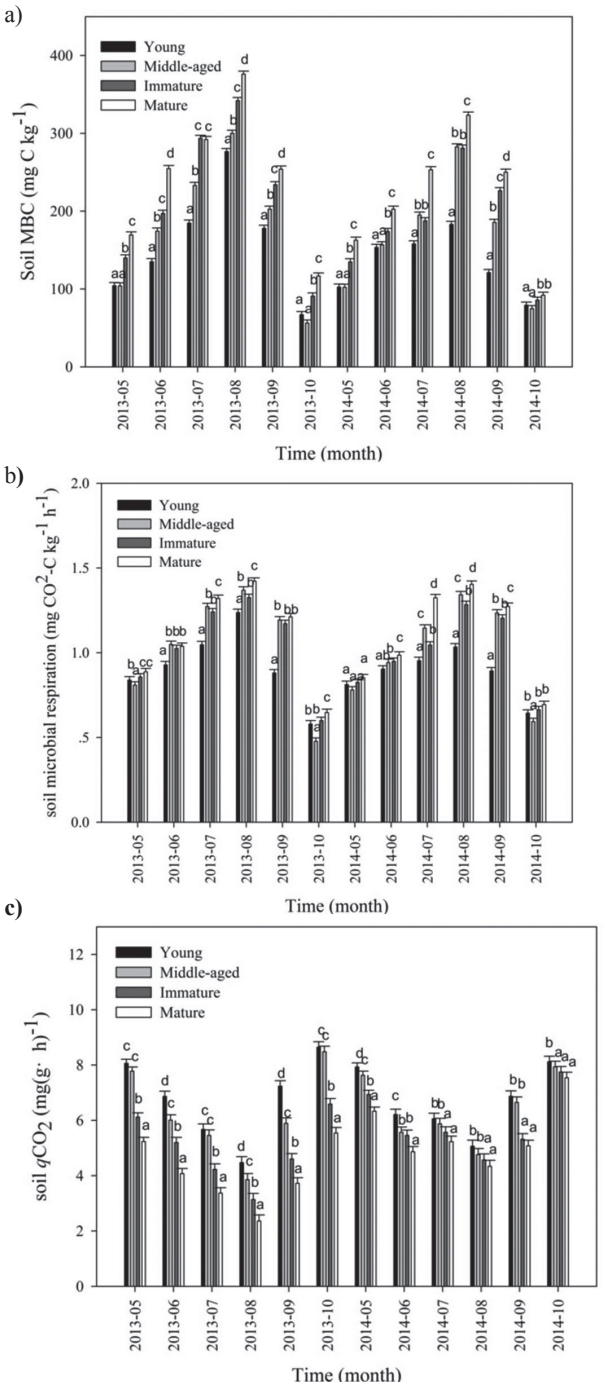

Fig. 3. Seasonal variation in soil microbial biomass carbon a), soil microbial respiration b), and soil microbial metabolic quotient c) of the four age classes in the *Pinus tabuliformis Carr*. forests during the growing season; different lowercase letters indicate a significant difference between age groups in the same depth at the same sampling time (p<0.05); *Error bars* are SE of the mean (n = 3).

Table 4. Shannon-Wiener index (*H*), the richness of the DGGE bands (*S*), and mass loss (*M*) (%) during the decomposition of *Pinus tabuliformis Carr*. natural secondary forests.

Forest stand		Sampling time(day)			
age classes		180	240	300	360
Young	*H*	2.7	3.1	3.5	3.1
	S	17	25	28	25
	M	11.6	17.9	21.8	29.5
Middle-aged	*H*	2.8	3.2	3.7	2.9
	S	19	28	29	21
	M	12.2	18.7	23.3	27.9
Immature	*H*	2.9	3.1	3.9	3.3
	S	22	27	31	26
	M	13.2	19.6	26.1	31.5
Mature	*H*	3.8	3.9	4.1	3.3
	S	29	30	32	28
	M	13.9	21.2	26.7	36.8

no significant difference in MBC between the young and middle-aged forest stands sampled in May, June, and October, as well as the MBC between the middle-aged and immature forest stands sampled in July, August, and October.

Similar to the trend of MBC, the soil basal respiration across the chronosequence steadily increased from May to August and then drastically decreased thereafter, with the minimum values in October. Moreover, soil basal respiration across all four age classes decreased in the following order: mature > middle-aged > immature > young during the two growing seasons (Fig. 3b). However, there was no significant difference in soil basal respiration among the chronosequence stands in May and October over the two growing seasons.

However, the metabolic quotient (qCO_2) pattern exhibited the opposite trend toward the soil MBC and basal respiration, which prominently decreased in the first four months, with the minimum values in August, but drastically increased in the latter two months of the experiment (Fig. 3c). Moreover, the qCO_2 significantly decreased as the stand age increased during the first growing season.

Temporal Patterns of Soil Diversity of Fungal Assemblages Across Forest Stand Age Classes

Fingerprinting analysis of the fungal assemblages based on the PCR-DGGE technique indicated significant differences between the numbers of DGGE bands across forest stand age classes during decomposition (Table 4). The initial numbers of DGGE bands were low and increased to reach the maximum values at day 300, after which they decreased dramatically on day 360 (Table 4). In addition, the numbers of DGGE bands increased from young to mature stands along the chronosequence. In addition, the diversity reflected by the Shannon-Wiener indices exhibited similar patterns to the variations in the DGGE bands.

Biotic and Abiotic Factor Controls on Litter Decomposition

The best-fit path model identified Lignin/N, qCO_2, SFD, and SMC as the most prominent factors controlling the rate of litter decomposition (Fig. 4). During the two growing seasons, the path analysis identified the litter lignin/N ratio, soil microbial metabolic quotient (qCO_2),

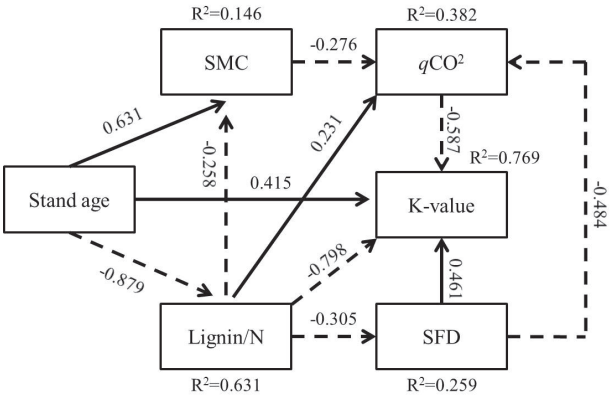

Fig. 4. Path model illustrates direct and indirect effects of abiotic and biotic factors on litter decomposition; values on arrows are standardized direct path coefficient, solid arrow lines represent positive effects, and dash arrow lines indicate negative effects; R^2 value represents the proportion of total variance explained for the specific dependent variable.

soil diversity of fungal assemblages (SFD), and soil water content (SWC) as dominant controlling factors in needle litter decomposition, collectively explaining 76.9% of the total variation in mass loss across the entire age sequence. Specifically, the model explained more than 60% of the variance in k and Lignin/N and 38.2%, 25.9% and 14.6% of the variance in qCO_2, SFD, and SMC, respectively. Among the four explanatory variables, Lignin/N and qCO_2 had the greatest negative effect on the k value, followed by positive effects of SFD. In addition, Lignin/N also had strong negative effects on SMC and SFD. Apart from the strong negative effect of Lignin/N, SFD was also strongly and positively affected by qCO_2.

Discussion

In this study, we found significant differences in decomposition rates among needle litter across a chronosequence of Chinese pine forests in northern China. Biotic and abiotic factors greatly contributed to the variations in litter decomposition.

Initial litter qualities such as N, C/N, and lignin concentrations were the best direct predictors of needle litter decomposition in various ecosystems [37-39]. Specifically, litter from older forests contained relatively higher levels of N concentration and lower C/N and lignin/N ratios than litter from younger forests [40]. Moreover, Jacob found that the rate of litter decomposition was positively associated with initial N concentrations but negatively associated with lignin/N and C/N ratios [41]. Therefore, the litter decomposition rates exhibited an increasing trend with increasing stand age. A possible explanation for the observed difference in decomposition rates across the age sequence could be the nutrients obtained from plant roots and leaf photosynthesis [8, 42]. Zhao et al. found that with the increase in the forest stand age, the number and size of Pinus tabul*i*formis *Carr.* roots gradually increased, and the biodiversity of shrub and herb undergrowth became more abundant, which could absorb huge amounts of nutrients from the soil for leaf litter growth [43]. In turn, more litter fall and decomposition accumulated more organic matter in soils [44]. Therefore, such a positive cycle accelerated the accumulation of nutrients in leaf litter with the increase in the forest stand age. Leaf litter in mature stands had the highest quality and decreased with decreasing forest stand age, which facilitated the abundance of microflora and microbial activities to efficiently decompose litter [36, 45-46].

Our path analysis identified a direct and significant ($P<0.05$) control of soil qCO_2 and SFD on litter decomposition. Specifically, the microbial metabolic quotient (qCO_2) is a sensitive index that reflects the influence of environmental factors on the microbial carbon pool, combining microbial activity with microbial biomass carbon and microbial respiration to indirectly evaluate microbial activities associated with litter decomposition [47-50]. In addition, Liu et al. found that the lower the qCO_2 value, the smaller the amount of carbon consumed by microbial respiration, while the proportion of carbon used by the construction of microbial cells relatively increased, which controlled the composition and diversity of microbial communities [48, 51-52]. In this study, the indices of soil biological activities increased with increasing stand age, and this was attributed to the following reasons. First, soil biological activities were regulated by leaf litter quality due to the differences in initial nutrient concentrations and rates of decomposition [53-54]. With the forest growth, litter would have more organic C, total N, and any other nutrients, which may contribute to the increase of abundance and activity of decomposers [52, 55-56]. Second, this could be because the older stand developed long enough to accumulate higher contents of nutrient resources and create more favourable habitat heterogeneity [12]. However, most of all, soil microbes act as the only vital organism playing important roles in biogeochemical cycles, energy transfer, and soil formation [57]. In turn, soil quality would influence the quantity and composition of the decomposer community. Thus, older forest stands have more microbial numbers and more microbial activity to efficiently decompose litter [58]. Third, with the increasing stand age, the canopy density of the forest stand could decrease due to the self-thinning rule, which greatly improved the temperature, moisture content in soil, and light intensity in the forest, making it more suitable for microbial growth and reproduction [59].

All of the soil biological activities exhibited significant temporal variation (Fig. 3). The results suggested that higher temperature accelerated microbial growth and activities to fix more carbon, intensifying microbial respiration [60]. Additionally, precipitation during the rainy season could accelerate the leaching of water-soluble substances from litter to soil, which provides nutrients available for microbial growth. These, in turn, might have had positive effects on soil biological activities [61]. Therefore, the temporal patterns of soil biological activities reflected the availability and rate of utilization of nutrient resources, as well as good adaptation to favourable environmental conditions [62].

Our findings suggest that forest stand age plays a primary role in controlling litter decomposition by determining the leaf litter quality, with soil biotic and abiotic conditions being secondary factors contributing to the variations in leaf litter decomposition across a chronosequence of Chinese pine forests. In addition, forest stand age could improve soil fertility through the input of nutrient elements, which indirectly control litter decomposition through soil microbial community diversity and microbial activity. Therefore, understanding the dominant factors driving litter decomposition is critically important for exploring nutrient cycling and sustaining the site productivity of forest ecosystems.

Conclusions

Forest stand age was confirmed to be a crucial driver of intraspecific variation in litter quality. In addition, decomposition rates and all soil biological parameters increased with increasing stand age, and the soil ecosystem tended to be stable. Our findings indicated that forest stand age had a great influence on needle litter decomposition by determining the litter quality, with soil microbial activity and local environmental factors being secondary drivers in needle litter decomposition across a chronosequence of Chinese pine forests. Therefore, consideration of forest stand age is critically important in assessing the needle litter decomposition of temperate Chinese pine forest ecosystems.

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Conflict of Interest

The authors declare no conflict of interest.

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