

Original Research

# Effects of Precipitation on Forestry Soil Microorganisms

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## Abstract

**Background:** Since the future climate is predicted to be more extreme even in the case of semi-arid conditions, data on such conditions will be more important in the global context.

**Aims:** The current work focuses on the effects of precipitation control on soil microorganisms in deciduous wild forest ecosystems in northeast China.

**Methods:** To study the amount of rainwater reaching forest litter and determine the effect of rainfall on leaf mass loss, respiration rate, and microbial biomass, three regimes with six repetitions were applied: 1) full coverage (100% reduction in precipitation); 2) partially covered areas (50% reduction); and 3) completely open areas.

**Results:** Rainwater did not reach entirely covered areas. Similarly, coverage did not always impact soil respiration and microbial biomass. Massive losses of fully open and partially covered litter were 20-35% larger than those of closed litter. Mass loss of the five litter types was in the following order: *Ulmus japonica* > *Quercus mongolica* > *Fraxinus mandshurica* > *Juglans mandshurica* > *Tilia amurensis*. Respiration intensity from closed litter decreased in all species six months after planting. For one year, a significant effect of the closed plot was observed only on *U. japonica* and *Q. mongolica* litter. An exception was noted for *T. amurensis* since three months after planting, microbial biomass values were comparable, irrespective of the extent of cover closure. According to the results of the experiment, precipitation on partially covered plots of the five litter types was reduced by approximately 45-50%.

**Conclusion:** The absence of precipitation had an adverse effect on some biological processes in the litter but had a sporadic effect on soil processes. The lack of precipitation, although soil moisture was maintained, may have had an impact on the organic matter cycle in forest litter.

**Keywords:** soil microorganisms, forest soil moisture, broad-leaved forest litter in China, forest area

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## Introduction

Since the early part of the last century, there have been global changes in rainfall, temperature, and climate extremes [1]. At the same time, it has been found that heavy rainfall has become less frequent and more extreme [2]. In China, there is variability in monsoon rainfall, which will pose a threat to soils, including forests [3]. Since the future climate is predicted to be more extreme even in the case of semi-arid conditions, data on such conditions will be more important. To anticipate the future of the carbon cycle and its capacity for carbon sequestration in a dynamic world, it is essential to comprehend the seasonal variations in key processes that play a pivotal role in the carbon cycle.

Soil is recognized as an important factor in the fertility and stability of forest ecosystems [4]. Soil microorganisms are responsible for the decomposition and transformation of organic matter for vegetative development and plant growth [5]. Soil microbial biomass can act as a source or consumer of available nutrients [6], and changes in microbial biomass also affect soil organic matter cycling [5]. A major component of soil microbial biomass is carbon, which is responsible for controlling carbon and nutrient fluxes in ecological systems [7]. Live microbial biomass carbon and dead microbial biomass contribute to the microbial biomass carbon pool [8]. Dead microbial necromass represents an enormous quantity of carbon in the soil and can act as a readily available carbon source for living microorganisms [9]. Soil structure is also heavily influenced by rainfall, which can be traced by indicators of soil biological activity because it influences soil moisture, temperature, and particle size composition. In the decomposition of waste, the leaching effect of precipitation increases the mass loss at the initial stage of the decomposition process by microorganisms [10].

Microbial biomass in the litter-soil system serves as a source and sink for available plant nutrients and regulates the breakdown of plant waste [11]. Microbial biomass is believed to facilitate more sensitive changes in organic matter than total organic matter content. Besides, it can detect changes over even a few years rather than decades [1]. Depending on the purpose of the experiment, microbial biomass in decaying forest litter is studied through the incubation of laboratory microcosms of time series [12] or by collecting organic material from different layers in forest litter [13]. The first approach may distort important biophysical variables, whereas the second approach does not accurately reflect decay states per unit of time.

Soil respiration, especially forestry soil respiration, is the largest potential source of atmospheric carbon. Consequently, even small changes in soil respiration can increase or decrease atmospheric carbon dioxide levels [14]. Soil respiration refers to the release of carbon dioxide to the soil surface, which controls the primary carbon cycle in ecosystems [15]. Terrestrial respiration consists of two components: autotrophic respiration

and heterotrophic respiration. While autotrophic respiration is carbon dioxide released by plant roots, heterotrophic respiration is defined as carbon dioxide released as a result of microbial activity. It is associated with the decomposition of soil organic matter, which constitutes 60% of the total respiration in the forest [16]. Photosynthesis and heterotrophic respiration are key processes for regulating carbon balance on Earth [17]. Soil enzymes catalyze many important biological processes associated with increased soil metabolism and contribute to the movement of nutrients [6].

It is well known that storing and decomposing organic carbon in soil impact carbon storage in terrestrial ecosystems and the global carbon balance [7]. Consequently, soil microbial properties such as soil respiration, enzyme activity, and microbial biomass are considered as important for predicting dynamics in many recent studies [18]. Various studies have shown seasonal variations in soil respiration [19, 20], soil enzyme activity [21], and soil microbial biomass carbon [7] in different forest ecosystems. Previous studies by Zhu et al. [20], Lepcha and Devi [22], and Zhang et al. [23] reported seasonal variations in microbial biomass in some forest ecosystems. However, studies describing the same phenomena in semi-arid forests are still lacking. Moreover, the seasonal effect of the relationship between soil respiration and enzyme activity and soil microbial biomass is uncertain and poorly understood.

Soil organic carbon decomposition processes are dominated by the soil microbial community, consisting mainly of bacteria and fungi. The formation of microbial biomass can be influenced directly by rainfall through stimulating litter carbon dioxide release or indirectly through stimulating high carbon dioxide pulses by delivering large amounts of dissolved organic carbon from litter to the topsoil [3]. Studying the interplay between rain-induced changes in soil carbon dioxide flux, microbial activity, and community composition can provide a clearer understanding of the underlying sub-microbial mechanisms responsible for the soil carbon pulse triggered by rainfall. For example, rains usually stimulate the fast growth of soil bacteria because bacteria need a film of water for the mobility and diffusion of the substrate [2].

Most research on the effects of precipitation on forest litter and soils is carried out in the laboratory by soaking the litter for some period [12] or simulating heavy rainfall with the help of irrigation water [24], determining then the mass loss. Other workers leach litter in the lab before installing it in the field [25] or suspend tethered litter in the field so that it can be washed out by rain [10]. These approaches can either exclude the synergy effects of microbes and wildlife. A long soaking period can alter the physical structure under the stick, resulting in deformed results. They can also exclude the regulatory effect of disintegration by-products on the microbial stock, especially at the early stages of the disintegration process [21]. That inspired the authors to conduct studies under natural conditions.

Microbial biomass in the bedding-soil system acts as a source and sink of nutrients available for plants and regulates the decomposition of plant waste. During its decomposition, the leaching effect of rainfall enhances the loss of soil mass at the initial stage of the decomposition, and also significantly affects the metabolic processes in the forest substrate. In arid and semiarid ecosystems, abundant seasonal rainfall, and irregular drying and wetting cycles prevail. The uneven distribution of rainfall in semiarid regions can create drying and wetting constraints on forest soils, negatively affecting their quality. In such ecosystems, soil microbes are very sensitive to the pulse of water and precipitation. Consequently, it initiates the decomposition of organic carbon in the soil, leading to a cascade of different reactions.

In studies investigating the effects of precipitation on litter decomposition rates and soil microorganism abundance in forests, the following gaps may exist:

1. Insufficient research quantity: There is a limited number of conducted studies, resulting in an inadequate database and limited information regarding the influence of precipitation on the mentioned processes.

2. Ecosystem diversity: Forest ecosystems vary in their characteristics, such as soil type, vegetation composition, climatic conditions, and more. Studies may be restricted to specific geographic regions or forest types, hindering the generalization of results to other regions.

3. Study duration: Precipitation can have short-term and long-term effects on litter decomposition and soil microorganisms. Insufficient long-term studies can impede the understanding of long-term effects.

4. Measurement complexity: Assessing litter decomposition rates and soil microorganism abundance is a complex process that requires precise methodologies and measurements. Different methods can yield divergent results, making comparisons across different studies challenging.

5. Interaction with other factors: Precipitation interacts with other factors such as temperature, light availability, soil composition, and the presence of other organisms. Studies may be limited to analyzing only one factor, disregarding the comprehensive ecosystem context.

To address these gaps, further research is required in diverse ecosystems, encompassing long-term temporal coverage and considering the interaction of multiple factors. This will enable a more comprehensive understanding of the effects of precipitation on litter decomposition and soil microorganisms.

The objective of this study is to analyze the effect of rainwater on litter decomposition rate and soil microorganism number in the broadleaf forestland of northeastern China. It constitutes the novelty of this research, as no similar investigations have been conducted in this region. Research investigating the effects of precipitation on litter decomposition rates and soil microorganism abundance in forests

holds rationality and significance for several reasons:

1. Ecological importance: Forest ecosystems play a vital role in biodiversity and the functioning of the planet. Understanding the influence of precipitation on litter decomposition processes and soil microbiology helps elucidate the mechanisms regulating these processes and their link to nutrient cycles within the ecosystem.

2. Climate change implications: Climate change, including alterations in rainfall patterns, can have a substantial impact on forest ecosystem functioning. Studies in this field aid in assessing how changes in precipitation regimes may affect decomposition processes and soil biology, which is crucial for developing strategies for sustainable forest management.

3. Connection to the carbon cycle: Forests play a role in carbon sequestration and climate change mitigation. The rate of litter decomposition and the activity of soil microorganisms influence the amount of carbon retained in the soil or released into the atmosphere. Investigating the influence of precipitation on these processes helps improve our understanding of how precipitation changes can affect the carbon balance in forest ecosystems.

4. Ecosystem management: Research in this area can assist in identifying optimal strategies for managing forest ecosystems to enhance productivity and resilience while preserving their integrity. By understanding how precipitation impacts litter decomposition and soil microbiology, better forest management practices can be developed, mitigating negative impacts on the ecosystem.

The authors suggest that the detected reductions in soil mass within the fully exposed and partially covered litter layers may be associated with leaching processes and microbial activity. In contrast, the losses observed in the covered litter layer appear to be influenced by a combination of microbial action and the presence of soil fauna.

The fulfillment of the set goal included the following tasks:

- identifying the most diverse terrain among the species in the deciduous forest and delineating survey sites;
- identifying the species composition of forest vegetation in the selected plots;
- identifying the features of the soil litter and classifying it into types;
- analyzing the effect of rainfall on microbial biomass, the degradability of five types of litter, and soil respiration in a forested area of China.

## Material and Methods

### Region of Study

Studies were conducted from 2016 to 2021 in a broad-leaved forest (Misty Forest) in the northeastern



Fig. 1. Topographic map of the study area in broad-leaved forests of China during 2016–2021.

People's Republic of China (PRC) in Jilin Province (43°53'03" N, 125°18'30" E) (Fig. 1).

The province has a northern continental monsoon climate with a dry and windy spring, warm rainy summer, sunny cool autumn with large temperature variations, and long dry and cold winter. The research was carried out year-round over six years.

#### Composition of Forest Vegetation and Soil Treatments

Soil samples with a volume of 150 cm<sup>3</sup> were collected using an automated soil sampler, the Duoprob-60-UP, from the soil surface and at a depth of 5 cm. Between sample collection sessions, the sampler was thoroughly cleaned, particularly the parts that directly contacted the soil. The cleaning procedure involved: 1) removing adhered material particles using a diluted soapy solution or detergent solution and a brush; 2) thoroughly rinsing with distilled water. The experiment consisted of two parts: initially, the authors investigated three litter cover regimes and their impact on microbial biomass quantity, followed by the assessment of litter decomposition degree on the forest floor.

To study the effect of precipitation on decomposition and the amount of microbial biomass, three regimens with six replicates were used:

- 1) complete coverage (100% reduction of rainfall) - this was done using a transparent plastic vinyl sheet attached to a steel arch frame up to 1.5 m high in order to prevent rainwater from falling on an area of

25 m<sup>2</sup>. An area of approximately 20 cm between the forest floor and the edge of the plastic sheet was left open to allow free air circulation;

- 2) partially covered (reduced by 50%) - for this purpose, used strips of transparent roofing 10 cm wide, spaced 10 cm apart, which reduced the amount of precipitation by 50%;
- 3) open areas (control group).

Rainfall and ground temperature were recorded using a stand-alone TMC6-HD recorder (Onset Computer Corporation, Bourne, MA, United States).

The leaf litter was air-dried for three weeks according to the methodology outlined by Taylor [26] before placing 20 g of each litter type into 20 cm × 20 cm garbage bags with a mesh size of 1 mm. Following the experimental treatments described above, a total of 156 bags containing soil samples were prepared by the end of the leaf litterfall in 2021. This number of bags allowed for the extraction of six samples at intervals of three months, six months, and one year in a single processing session. The soil sample bags were placed in the centre of 3 m × 3 m plots and secured to the ground using plastic-coated stakes. During the sample collection, the bags were individually packaged in polyethylene bags and transported to the laboratory. The litter samples were transferred to a sealed plastic container with a volume of 500 ml and left to stabilize in a laboratory incubator at 24°C for 24 hours. Soil moisture content was determined based on dry weight (dried at 105°C for 24 hours). The following parameters were assessed in the soil samples: pH in a water-salt



suspension (1:3) and basal soil respiration (mg CO<sub>2</sub>/h/g dry litter) [27]. Microbial biomass was determined using the fumigation method [28]. Respiration and microbial biomass data were expressed on a dry mass basis (dried in a muffle furnace at 105°C for 24 hours), and mass loss was calculated in an ash-free state (550°C for 8 hours) [29]. The degree of decomposition of the forest floor litter was assessed following the method by Liu [29]. The decomposition intensity of plant biomass was calculated as grams per square meter per year.

Leaf litter from Mongolian oak (*Quercus mongolica* Fisch. ex Ledeb.) (*Fagaceae*), Manchurian ash (*Fraxinus mandshurica* Rupr.) (*Oleaceae*), Manchurian walnut (*Juglans mandshurica* Maxim.) (*Juglandaceae*), Japanese elm (*Ulmus japonica* (Rehder) Sarg.) (*Ulmaceae*) and Amur linden (*Tilia amurensis* Rupr.) (*Tiliaceae*) were collected by shaking the trees or collecting just fallen leaves at the peak of leaf-fall in November 2016-2021. Following the methodology described in reference [26], the leaf fall was allowed to undergo natural air drying for three weeks.

### Statistical Analysis

Forest vegetation analysis data at all sites were tabulated and calculated. The results were processed using a one-factor ANOVA analysis of variance using Microsoft Excel and Statistica 10 software [30]. Differences in the results obtained are probable at a significance level of  $P \leq 0.05$  according to Student's test.

## Results and Discussion

### Characteristics of Forest Soil and Village Vegetation of the Study Area

The area of the forest massif, where the research was conducted, is a regulated system with installed

covering devices to correct the amount of precipitation falling on the forest floor and affecting the moisture and temperature of the soil (Fig. 2).

This system was constructed to observe the effect of rainfall on forest soil transformations, taking into account the microorganisms that inhabit it. The experimental plan allowed us to study the effect of rain manipulation on the decomposition of forest litter while keeping most of the decomposition medium and its components intact. Despite the presence of a cover, the surface of the litter-soil interface became moist and remained within the microbial threshold. This was because no devices were installed to prevent seepage (lateral movement of water in the soil). Consequently, the litter-soil interface became moist even in the covered litter, which allowed the reducing organisms to survive and remain active in soil biochemical processes. Although temperatures were higher in the covered areas, its effect on decomposition rates was probably overshadowed by the effects of rain. Based on soil moisture data, rainfall did not decrease significantly and affected the water potential of the soil in fully open and partially covered plots, so soil moisture does not cause differences in soil mass loss and respiration rate.

Broad-leaved tree species were found in the study area, with the following dominant species: Mongolian oak, Manchurian ash, Manchurian walnut, Japanese elm, and Amur linden. According to the results of the experiment, rainfall on partially covered plots was reduced by about 45%, while on fully covered plots, rainwater did not reach these plots. Similarly, litter moisture content (Fig. 3) was markedly reduced in covered plots compared to fully open and partially covered litter, which were comparable on all sampling dates.

Consequently, the decomposition rate of all five litter species studied was reduced in the covered plots, while in the fully open and partially covered plots, it was comparable on all sampling dates.

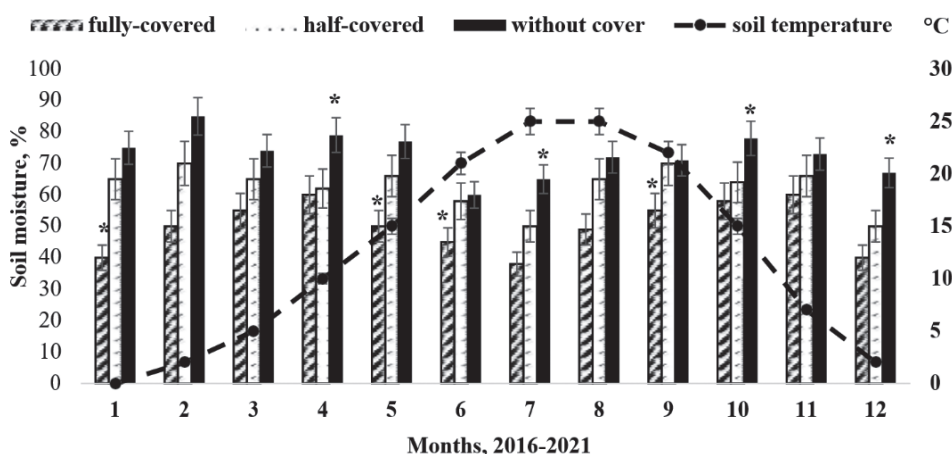


Fig. 2. Dynamics of soil moisture (%) depending on the applied coverage and soil temperature (°C) in the studied forest area in 2016-2021.

Notes: \*Values are statistically significant at  $P \leq 0.05$ .

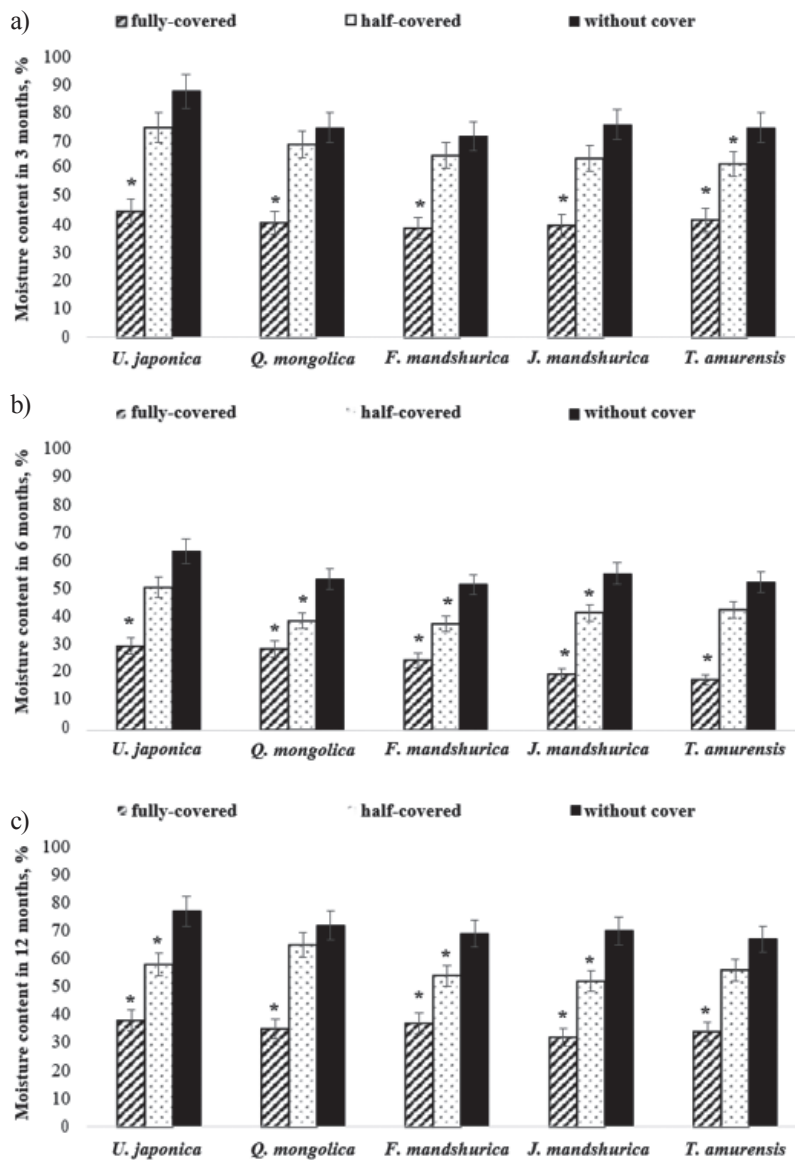


Fig. 3. Dynamics of moisture content (%) in a different litter (*U. japonica*, *Q. mongolica*, *F. mandshurica*, *J. mandshurica*, *T. amurensis*) depending on the applied coverage in the studied forest area after 3 a), 6 b) and 12 c) months in 2016-2021.

Notes: \*Values are statistically significant at  $P \leq 0.05$ .

### Analysis of Microbial Biomass and Soil Respiration

Mass losses of completely open and partially closed litter were 20-35% higher than those of closed litter. Mass losses of the five litter types were in the following order: *U. japonica* > *Q. mongolica* > *F. mandshurica* > *J. mandshurica* > *T. amurensis*. The intensity of closed litter respiration was reduced in all species six months after planting, but at one year a significant effect of the closed site was observed only in *U. japonica* and *Q. mongolica* litter. Energy flow through microbial biomass is the driving force behind residue and detritus decomposition, so there is a good correlation between mass loss and microbial biomass.

The amount of microbial biomass C (Table 1) in the residual litter was consistently decreasing in a covered

litter, except for *F. mandshurica* and *J. mandshurica*, while fully open and partially open litter were comparable.

No significant difference was observed in microbial biomass values for *T. amurensis* three months after planting, irrespective of the level of cover closure. In most cases, litter microbial biomass increased with decomposition, so that a positive linear relationship between litter microbial biomass and mass loss was observed using data for all litter types and all cover regimes. In terms of soil respiration rates, closed plots differed from partially and completely open plots in May and October, and the trend appeared to follow increases and decreases in soil temperature (Fig. 2). Microbial biomass is known to play a major role in regulating the rate of decomposition and nutrient cycling [11], as evidenced by the close correlation between mass loss

Table 1. Microbial biomass (mg C / g dry litter) depending on the applied coverage in the studied forest area after 3 (A), 6 (B) and 12 (C) months in 2016-2021.

Month	Cover	Forest litter				
		<i>U. japonica</i>	<i>Q. mongolica</i>	<i>F. mandshurica</i>	<i>J. mandshurica</i>	<i>T. amurensis</i>
3	Without	1.8±0.16	1.9±0.17	1.7±0.12	2.0±0.19	2.0±0.18
	Half	1.6±0.14	1.7±0.09	1.5±0.13	1.9±0.16	1.9±0.11
	Full	1.4±0.10	1.5±0.12	1.9±0.11*	1.9±0.20*	1.8±0.18
6	Without	6.5±0.48	6.8±0.50	6.2±0.44	6.8±0.58	6.6±0.49
	Half	6.1±0.52	5.8±0.59	5.9±0.60	6.2±0.54	6.0±0.57
	Full	2.6±0.35*	3.9±0.50*	3.9±0.41*	3.1±0.30*	3.9±0.58*
12	Without	11.2±0.98	10.9±0.97	10.8±1.14	11.0±0.95	10.7±0.99
	Half	9.8±0.14	9.7±0.91	9.5±1.05	9.9±1.16	8.9±1.13
	Full	3.9±0.30*	3.5±0.22*	3.9±0.41*	3.9±0.40*	2.7±0.28*

and microbial biomass index [6]. Decomposing litter represents an area of the active microbial pool due to the presence of carbon that provides ecophysiological support, which serves as an energy source for them [17].

Our results are consistent with the theory that rainwater increases the rate of mass loss of various fallen leaves due to the leaching of labile materials [25]. Washing efficiency is known to be affected by the intensity and amount of rainwater [10]. This is supported by the findings of Cong and Brady [18], who observed that the amount of rainfall correlates better than the total amount of rainfall. Thus, in this case, the impact of precipitation explains the acceleration of mass loss of fully and partially exposed litter. Conversely, the low rate of decomposition in fully covered areas is explained by the lack of leaching effects of rainfall along with drier conditions. The difference in mass loss between fully and partially open litter and closed litter persisted over time due to initial losses due to leaching [25]. Decomposition products at some stage play a crucial role in the decomposition process because they become resources for subsequent processes [21], hence initial losses due to leaching affect subsequent decomposition rates. This concept is best illustrated by the observation that microbial decomposition in leached litter tends to be higher because rainwater leaches out tannins that reduce litter quality [31]. Given the conditions of the decomposition environment, we can assume that the observed mass losses of fully and partially exposed litter were due to leaching, catabolism, and pulverization. Maximum leaching due to rain did not occur in closed areas because rain never reached the litter. Consequently, catabolism and shredding likely caused the observed mass loss in covered areas [32].

Soil respiration rate assesses the overall biological activity in the litter and soil layer and is known to depend on substrate availability, nutrients, moisture and

temperature [33]. Litter moisture, which is significantly lower in covered areas (Fig. 3), may have affected microbial abundance and activity. Soil respiration measurements have been reported to be closely related to soil temperature and moisture [18]. Thus, the respiration rate tends to increase when the soil temperature is high, and this occurs in summer. Soil microbial activity has been reported to withstand critical levels of moisture. Jiménez-Mejía et al. [34] noted that bacteria are primarily active in soils with high water potential.

Thus, the water potential even in closed litter-soil systems, where humidity was reduced, could maintain microbial activity. In open systems (without cover pads), where the litter-soil system was alternately moistened and dried, carbon dioxide production tended to be higher [9], probably due to microbial cell decomposition [8]. Carbon input largely determines the size of the microbial biomass [6]. In addition to carbon input, other factors that regulate microbial biomass growth and activity are nitrogen, phosphorus, and sulfur, as well as climate, soil moisture or water potential, substrate quality, quantity and distribution, clay type, and soil water osmotic pressure [21, 35-39]. Given the microbial biomass determinants listed above, open and partially closed litter-soil systems were expected to produce higher microbial biomass than closed systems. During the observation period, there is a positive correlation between the microbial biomass and the decomposition rates of the uncovered litter. However, microbial biomass of litter residues is predicted to have an inverse relationship to mass loss later in the decomposition process, as carbon will steadily decrease over time [7]. Our subsequent work will focus on the effect of soil moisture on other types of forest litter, in particular coniferous forests. In addition, the results of our experiments will be used to optimize the processes of microbial degradation of forest soils.

## Conclusions

Broad-leaved tree species were found in the forest area of northeastern China under study, with the following dominant species: Mongolian oak (*Q. mongolica*), Manchurian ash (*F. mandshurica*), Manchurian walnut (*J. mandshurica*), Japanese elm (*U. japonica*) and Amur lime (*T. amurensis*). Mass weight loss of the five litter types was in the following order: *U. japonica*>*Q. mongolica*>*F. mandshurica*>*J. mandshurica*>*T. amurensis*. The intensity of closed litter respiration was reduced in all species six months after planting, but at one year only *U. japonica* and *Q. mongolica* were observed to have a significant effect on closed litter. An exception was noted for *T. amurensis* because three months after planting, microbial biomass values were comparable regardless of the degree of cover closure. According to the results of the experiment, the amount of precipitation on partially covered plots of the 5 mentioned litter types was reduced by about 45-50%, while on fully covered plots rainwater did not reach these plots. The results of the observations showed that the soil mass losses observed in the completely open and partially covered litter were related to leaching and microbial action, while losses in the covered litter were probably limited to the combined action of microbes and soil fauna. In most cases, litter microbial biomass increased with decomposition, so that a positive linear relationship between litter microbial biomass and mass loss was observed using data for all litter types and all cover regimes. The presence of rainwater cover had a significant effect on litter mass loss and some biological activity as evidenced by the microbial biomass data. However, manipulating rainfall (by the partial cover) did not produce a consistently significant difference in soil biological parameters. In general, the absence of rain, even for a short period, can slow down the turnover of organic matter in forest litter. The findings we acquire will be utilized in the future to enhance the processes by which microorganisms break down forest soils. Our future research will specifically concentrate on investigating how soil moisture affects different types of forest litter, with a particular focus on coniferous forests.

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## Conflict of Interest

The authors declare no conflict of interest.

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