

Dual isotopes tracing carbon and nitrogen dynamics during leguminous and non-leguminous litter decomposition under controlled precipitation

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Research Article

Keywords: Dual isotope tracing, Leaf litter decomposition, Precipitation, Leguminous, Non-leguminous, Nutrient cycling

Posted Date: June 8th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1672169/v1>

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Abstract

Background and aims Plant litter decomposition figures importantly in the cycling of C and N pools in terrestrial ecosystems. We investigated how C and N fluxes changed during the decomposition of leaf litters and how those processes respond to different precipitation regimes.

Methods We using dual isotope tracing and in situ decomposition methods, and investigated differences of leaf and soil C, N, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in northwest China.

Results $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were 3604‰ and 56‰ for *Robinia pseudoacacia* (Leguminosae) and 8115‰ and 452‰ for *Populus ningshanica* (Salicaceae) leaf litters. Through decomposition, $\delta^{13}\text{C}$ decreased in all litters, and the $\delta^{15}\text{N}$ of leguminous increased while non-leguminous litter decreased. In surface soil, the fractions of litter-derived C (20%) and N (14%) from leguminous were higher than non-leguminous litters after 16 months. C and N concentrations of both litters and soil were always coupled during decomposition, and the responses of N to C changes in soil were reduced by litter cover. Increased precipitation enhanced the litters' C and N coupling. The 600-mm precipitation treatment most benefited litter C transformation to SOC, and drought conditions promoted the transformation of legume litter N to soil TN, but inhibited non-legume litter N.

Conclusions C and N changes are always coupled in soil and litters. After 16 months, the proportion of C and N in soil from legumes was higher than non-legumes. Reduced precipitation can promote legumes N into soil. Our results provide a scientific basis for accurately predicting the C and N cycles in terrestrial ecosystems.

Highlights

1. Litter-derived C and N fractions were higher in leguminous than non-leguminous litters
2. Drought promoted leguminous, but inhibited non-leguminous, N infiltration to soil
3. Legume litter is more conducive to soil C and N storage, so it achieves C neutrality
4. Through decomposition, soil and litter C and N coupling decreased but never decoupled
5. $\delta^{13}\text{C}$ decreased in all litters; $\delta^{15}\text{N}$ increased in legume, decreased in non-legume litters

1. Introduction

As an important nutrient source, leaf litter is a link between plants and soil in the nutrient cycle, and its decomposition process is vital for the composition and circulation of C and N pools in terrestrial ecosystems (Bimüller et al. 2013). The return of C and N from leaf litter to soil cannot only change the existing morphological stability and availability of soil C and N but can also affect the structure and function of soil ecosystems and their responses to environmental changes (Cleveland et al. 2014).

Enhancing ecosystem C sink capacity is key for and an effective way to help mitigate atmospheric CO₂ increase and global warming (Yang et al. 2022). In semi-arid areas such as the Loess Plateau in China, the ecological significance of litter decomposition is also seen in the maintenance of ecosystem stability and soil texture improvement (Cornwell et al. 2008). Therefore, a global-scale database that contains information about litter decomposition and its return to soil can contribute to a useful and comprehensive understanding of biogeochemical cycling within ecosystems, as well as of the formation of soil organic matter (Berg and McClaugherty 2003). Currently, ecologists and environmentalists are establishing such a database.

The C and N released directly into the atmosphere and that released into the soil during litter decomposition have different but very important ecological and environmental significances that must each be identified and quantified. Isotopic labeling can identify, with great accuracy, the C and N paths in litter decomposition and can accurately describe each path's flux and dynamic characteristics (Qiu et al. 2015). Altering the isotopic content of a substrate by either enriching or depleting a stable isotope (i.e., labeling) allows researchers to trace nutrients through an ecosystem and make inferences about biogeochemical processes such as C flux (Fahey et al. 2011), soil organic-matter inputs, and turnover rates (Rubino et al. 2010). But that technique is rarely used because both the production of labeled residues and the ensuing long-term experiments are time and resource intensive (Bimüller et al. 2013). However, dual labeling of stable ¹³C and ¹⁵N isotopes can reveal both C and N circulation processes in litter decomposition, and thus provide an accurate, simultaneous picture of those circulation processes. Also, that procedure's research, analysis, and marking material costs, as well as research time, are lower than those of single-element labeling studies, so it has better prospects for broad applications (Rubino et al. 2010). Glaser et al. (2012) latest research results using the field labeling method indicated that low cost, isotopic labeling materials can achieve the same labeling effect and obtain a large number of labeled materials. In addition, a quantitative analysis of SOC dynamics under litter cover requires tracing both the SOC compounds' origins and their transformation pathways, and that can be done by stable isotope (e.g., ¹³C and/ or ¹⁵N) labeling (Flessa et al. 2000). However, most of the current, litter decomposition studies, such as that of Pei et al. (2019), used single isotope markers, and few such studies have used dual-isotope labeling (Glaser et al. 2012; Gautam et al. 2016). Therefore, by using dual labeling of ¹³C and ¹⁵N isotopes while conducting in situ litter decomposition studies in the field, we can obtain accurate C and N cycle pathways in the decomposition process, and that has much value for predicting C and N cycles in terrestrial ecosystems.

The climate and plant diversity of arid and semi-arid regions are major determinants of C and N dynamics in decomposing plant litters (Santonja et al. 2019), and those areas are sensitive to climate change (Sanaullah et al. 2012). Among the water and temperature factors, water more significantly influences litter decomposition (Gray and Fierer 2012). Additionally, water availability controls ecosystem structure and processes by affecting long-term balances between the input and output of elements and the cycling of carbon and nutrients within those ecosystems (Ma et al. 2012). Precipitation changes can influence litter decomposition directly by restricting the physical breakdown of litter and indirectly by

affecting decomposer activities (Salamanca et al. 2003). Paul et al. (2003) found that soil organic matter decomposition is governed by soil water conditions, with relatively weak decomposition in drier soils than in wetter soils, and Sanaullah et al. (2012) found that drought inhibited grassland litter decomposition by more than 50%. Many studies have shown significant decomposition reductions with reduced water availability (e.g., Chihuahuan Desert [Francois et al. 2017], Mediterranean area [Santonja et al. 2015]). Climate changes may have great impacts on the fragile ecosystems of the Loess Plateau of China, which is one of the most severely eroded regions in the world. Li et al. (2011) assessed the predicted 2010–2039 site-specific climate change impacts on hydrology, soil loss, and crop yields of the Loess Plateau and found that the predicted changes would significantly impact its ecosystems. To date, few studies have used C and N isotope labeling to examine common Loess Plateau leaf litter decomposition dynamics under different precipitation gradients, and the effect precipitation has on leaf litter entry into Loess Plateau soil is not clear. In general, such research can lay an important foundation for the study of C and N cycles during the litter decomposition.

In this study, we used the ^{13}C and ^{15}N tracing technique to investigate C and N flux pathways during litter decomposition in a simulated in situ decomposition experiment. We also examined how C and N circulation during litter decomposition responds to different precipitation regimes. Here we tested the following hypotheses: 1) Litter decomposition weakens C and N coupling in soil and such decoupling progresses as decomposition progresses, 2) Higher precipitation increases litter decomposition and C and N release rates and strengthens the C and N coupling relationship, and 3) Legume litter is more conducive to soil C and N storage.

2. Materials And Methods

2.1 Isotope labeling and leaf sample collection

The leaf litters of the leguminous black locust (*Robinia pseudoacacia*, RP) and a non-legume, poplar (*Populus ningshanica*, PN) were used as study materials. Since isotope labeling efficiency is greatly affected by water availability, we conducted the labeling during the rainy season (July 28, 2015–August 3, 2015), using the technique proposed by Glaser et al. (2012). During that process, tree stems were injected with $^{15}\text{NH}_4^{15}\text{NO}_3$ to effect ^{15}N uptake while the trees were simultaneously gassed with $^{13}\text{CO}_2$ to effect ^{13}C uptake via photosynthesis. Specifically, stems were injected with a 2% $^{15}\text{NH}_4^{15}\text{NO}_3$ solution for each of 2 consecutive days. After completing ^{15}N labeling, we performed ^{13}C labeling 1 day after balancing, so that ^{15}N was as evenly distributed as possible in the leaves. To begin, each tree was sealed in a large plastic bag with high light transmittivity (95%) and then we fixed a vessel containing sulfuric acid (5 mol L^{-1}) to the tree stem and mixed in a high-purity $\text{Na}_2^{13}\text{CO}_3$ solution, which then released $^{13}\text{CO}_2$ gas. A fan fixed to the tree stem below the vessel evenly distributed the $^{13}\text{CO}_2$ around the tree body. We performed labeling with the same concentrations of ^{13}C every 2 hours during the day (09:00–15:00 hours). After the ^{13}C labeling was completed, ^{15}N and ^{13}C labeled leaves were immediately harvested and

dried at 45 °C, their ^{15}N and ^{13}C abundances were measured, and then they were stored in dry shade for subsequent use as leaf litter in this experiment. Ultimately, we obtained 2.18 kg (fresh weight) of RP leaves and 2.06 kg (fresh weight) of PN leaves. The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for the labeled leaves were 3604.0‰ and 55.6‰ for RP and 8115.0‰ and 452.0‰ for PN, respectively. (The values for unlabeled leaves were 91.0‰ and -23.8.0‰ for RP and 940.0‰ and -9.6‰ for PN, respectively). The quantities and isotopic abundances of labeled materials met this study's requirements.

2.2 Study site and experimental design

This study was carried out at the Northwest A&F University field experimental site located in Yangling, Shaanxi, China (34°16'18"N, 108°04'59"E; 525 masl). Its mean annual precipitation and temperature are 580.5 mm and 15.6°C, respectively, and it is characterized by a warm temperate monsoon climate (Zhang et al. 2012). We used soil that was collected from farmland (pH = 7.8) that had been abandoned for at least 8 years. The soil's organic matter, total nitrogen, and extractable phosphorus and potassium concentrations were 22.04 g kg⁻¹, 1.63 g kg⁻¹, 48 mg kg⁻¹, and 490 mg kg⁻¹, respectively; its texture was clay loam, and it had a gravimetric moisture content at field capacity of 22%. The sand, silt, and clay contents were 27%, 41%, and 32%, respectively (Du et al. 2020). Given that all the soil used in this study was the same, the effects of the initial soil properties on litter decomposition were negligible.

In situ litter decomposition field experiments began by first sinking 20-cm diameter by 20-cm long PVC (polyvinyl chloride) tubes ($N=108$) 15 cm into soil that had been screened through a 2-mm mesh. Then, 10 g of leaf litter was placed on the surface of the soil inside each tube and a 2-mm mesh nylon net (20 cm diameter) was carefully placed on the litter to minimize loss due to wind and other factors. Also, 18 control tubes were set up identically to the experimental tubes except they received no litter. Finally, the experimental site was roofed over, and the experiment carried out in the shade to avoid photolysis of the litter (Fig. S1).

The experimental design included the decomposition stage, precipitation treatments, and litter types. Decomposition was measured at 2 (June 2016), 4 (August 2016), 6 (October 2016), 8 (December 2016), 12 (April 2017), and 16 (August 2017) months after the experiment began. The precipitation treatments were controlled by an artificial irrigation system and included 400, 600, and 800 mm of water per year, amounts that reflect actual ranges of average precipitation for the area. The artificial rainfall system was installed under the rooftop and each sprinkler head had a short, stable, 360° full-circle spray range. Also, the sprinkler's water droplets were small enough to simulate raindrops (Fig. S2). Each of the 2 types of leaf litter was tested in combinations of the 6 decomposition stages and 3 precipitation treatments (2 × 6 × 3) with 3 replicates, resulting in a total of 108 experimental plots.

2.3 Litter collection and soil sampling

Over the whole experiment period, the litter remaining in each container after 2, 4, 6, 8, 12, and 16 months of decomposition was collected with tweezers and placed in paper bags. A very small amount of soil particles was attached to the decomposed litter of some of the samples, so we used a soft brush to

carefully remove those particles. The litter was oven-dried at 60°C to a constant weight, the mass lost during decomposition was calculated, and then it was ground fine enough to pass through a 0.25-mm sieve for chemical analysis. Samples from the control (no litter) and litter-treated soils were collected in layers (0–2, 2–5, 5–10, and 10–15 cm) at each sampling time, naturally air dried, and ground through a 0.25-mm sieve for chemical analysis.

2.4 Chemical and isotopic analyses

Wearing gloves to prevent contamination, we packed each litter and soil sample separately in approximately 5×9-mm tin capsules (4 mg litter and 40 mg soil) and mailed them to the Stable Isotope Facility of the University of California, Davis. There, litter and soil samples were analyzed for total C and N contents and ¹³C and ¹⁵N isotopes using an Elementar Vario EL Cube (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

2.5 Calculations and statistical analysis

The rate of mass loss (%) during litter decomposition in each treatment was calculated as follows:

$$Mass\ loss = \frac{M_0 - M_t}{M_0} \times 100\% \quad (1)$$

where M_0 and M_t are the mass (g) of the litter before and after decomposition, respectively, for each stage.

The annual decay rate constants (k) of the litter types were calculated using the Olson (1963) negative exponential decay model:

$$k = \frac{-\ln\left(\frac{X_t}{X_0}\right)}{t} \quad (2)$$

where X_0 is the initial weight of litter and X_t is the weight of the residual litter after sampling at time t , and t is the sampling duration (in years).

The fraction of litter-derived C and N in the soil was obtained from the variation in $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ in soil and litter and by applying a two-source mixing model (Rubino et al. 2010):

$$f_{litter} = \frac{\delta_s(t) - \delta_c(t)}{\delta_l(t) - \delta_c(t)} \quad (3)$$

Where f_{litter} (%) is the fraction of litter-derived C or N in soil over the total soil C or N, $\delta_s(t)$ is the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ of soil at the end of incubation, $\delta_c(t)$ is the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ of control soil at the end of incubation (c = control) and $\delta_l(t)$ is the $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ of the litter sample.

A sample's provisional isotope ratio is measured relative to a reference gas peak analyzed with each sample. Those provisional values are finalized by correcting the values for the entire batch based on the known values of the included laboratory reference materials. The long-term standard deviation was 0.2‰ for ^{13}C and 0.3‰ for ^{15}N . The final delta values are expressed relative to the international standards, VPDB (Vienna Pee Dee Belemnite) and Air for C and N, respectively. All correlation analyses were calculated using Pearson correlation coefficients. Multiway analysis of variance examined the direct effects precipitation (400, 600, and 800 mm) had on the litter mass loss rates, C and N releases, and ^{13}C and ^{15}N isotope abundances. Linear regression and further statistical analyses were performed using SPSS version 19.0 (IBM, USA).

3. Results

3.1 Mass loss of litters

When averaged across the 2 leaf types and 3 precipitation treatments, the mass loss of leaf litters increased linearly with time during the 16-month decomposition period, with rapid decomposition in the first 4 months ($35.93 \pm 1.64\%$) and slowed changes after 8 months (40.31% to 43.37%) (Fig. 1). Additionally, mass loss varied with leaf type, with decay rate constants (k) of 0.41 yr^{-1} and 0.48 yr^{-1} for RP and PN leaves, respectively. During the 16-month decomposition period, PN leaf mass loss ($45.85 \pm 2.48\%$) was significantly higher than that of RP leaves ($34.93 \pm 1.47\%$) ($P = 0.027$).

The mass loss of both leaf types was significantly affected by precipitation regime during litter decomposition ($P < 0.01$), and the effects varied with leaf type ($P = 0.028$ for the interaction). After 16 months of decomposition, PN leaf mass loss was 34.47%, 49.24%, and 38.97% in the 400-, 600-, and 800-mm treatments, respectively, while the k values were $0.33 \pm 0.03 \text{ yr}^{-1}$, $0.56 \pm 0.01 \text{ yr}^{-1}$, and $0.58 \pm 0.04 \text{ yr}^{-1}$, respectively, and the mass loss for RP leaves was 27.26%, 36.55%, and 39.99% in the 400-, 600-, and 800-mm treatments, respectively, after 16 months of decomposition, while the k values were 0.37 ± 0.01 , 0.41 ± 0.02 , and $0.45 \pm 0.08 \text{ yr}^{-1}$, respectively.

3.2 Leaf litter C and N dynamics

The C concentrations of both leaf litters were similar, while that of N was significantly higher in RP than in PN litter (Table 1). Also, C and N concentrations decreased as decomposition progressed, and decreases were greater for PN than for RP, while C decreased more overall than N did. For instance, the C and N in RP litter decreased by 102.49 g kg^{-1} and 7.20 g kg^{-1} , respectively, while that in PN litter decreased by 141.89 g kg^{-1} and 10.35 g kg^{-1} , respectively.

Leaf litter C and N dynamics during decomposition were not significantly affected by precipitation regime ($P > 0.05$) (Table 1). In the 400-, 600-, and 800-mm precipitation treatments, the mean RP leaf C content was 450 g kg^{-1} , 436 g kg^{-1} , and 431 g kg^{-1} , respectively, and mean N concentrations were largely unaffected by precipitation (40.15 g kg^{-1} , 39.84 g kg^{-1} , 35.87 g kg^{-1} , respectively). For PN leaf litter, the mean C contents were 445 g kg^{-1} , 421 g kg^{-1} , and 428 g kg^{-1} , respectively, and the mean N concentrations were 29.36 g kg^{-1} , 29.46 g kg^{-1} , and 31.04 g kg^{-1} , respectively.

During litter decomposition, the C:N ratio was significantly lower in RP (11.10–10.74) than in PN (14.75–14.43) leaves (Table 1) primarily because the RP leaves began with significantly higher N concentrations. The average C:N ratios of both leaf litters during decomposition decreased with increasing precipitation, with C:N ratios of 11.20, 10.93, and 10.79 for RP and 15.23, 14.29, and 13.81 for PN in the 400-, 600-, and 800-mm treatments, respectively.

3.3 Leaf litter $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ dynamics

The initial $\delta^{13}\text{C}$ of PN leaf litter was significantly higher (452.00‰) than that of RP leaf litter (55.60‰) regardless of precipitation regime and decomposition time (Fig. 2). As decomposition progressed, the $\delta^{13}\text{C}$ decreased and those decreases were greater for PN than for RP. The drop occurred mainly in the first 2 months: RP litter $\delta^{13}\text{C}$ decreased to 16.28‰ and PN litter decreased to 38.50‰ . For RP litter, the $\delta^{13}\text{C}$ was significantly higher in the 400-mm treatment than in the 600- and 800-mm treatments ($P < 0.05$), but there was no significant difference for PN litter. While the $\delta^{13}\text{C}$ in RP leaf litters were relatively stable (16.28‰ to 23.44‰), they were significantly lower than the initial value, while that in PN leaf litters decreased over the decomposition time from 133.84‰ to 38.50‰ .

The $\delta^{15}\text{N}$ was also significantly higher in PN leaf litters than that in RP leaf litters across the 3 precipitation regimes. The RP leaf litter $\delta^{15}\text{N}$ increased from 3604‰ to 4552‰ as decomposition progressed, while that of PN leaf litter decreased from 8115‰ to 4118‰ . Additionally, the average $\delta^{15}\text{N}$ showed no significant effect for both litters (Fig. 2).

3.4 Soil C and N dynamics

As precipitation increased from 400- to 800-mm, soil moisture increased from 14.97% to 32.00%, conductivity increased from 7.57 to 19.14 dS m^{-1} , but soil temperature decreased from 19.27 to 17.74 °C (Fig. 3).

Soil C concentrations were significantly higher in the 0–2-cm (3.76 g kg^{-1}) than in the 2–5-, 5–10-, and 10–15-cm soil layers (3.48 , 3.43 , and 3.41 g kg^{-1} , respectively) ($P < 0.001$, Fig. 4), but there were no significant differences in N concentrations between the different soil layers (0.52 , 0.55 , 0.53 , and 0.53 g kg^{-1} , respectively; Fig. 4). Previous research results showed that litter decomposition mainly changed surface soil C and N contents. Therefore, we only analyzed the 0–2-cm soil layers' large variations. While all leaf litter-treated soils had similar C concentrations, RP soil had significantly higher N concentrations than PN soil had. Over time, C concentration peaked at 8 months for RP soil and at 12 months for PN soil. For N concentrations, the peak values appeared at 6 months for both RP and PN soils.

Precipitation regimes did not significantly affect soil C and N dynamics during decomposition, and such influences were independent of leaf type (Fig. 4). In the 0–2-cm soil layer, C and N concentrations increased slightly as precipitation increased. In the 400-, 600-, and 800-mm precipitation treatments, the RP soil C concentrations were 3.55 , 3.81 , and 3.93 g kg^{-1} , respectively, and the N concentrations were 0.54 , 0.56 , and 0.58 g kg^{-1} , respectively. For PN soil, the C concentration was almost unaffected by precipitation, and N concentrations were 0.58 , 0.61 , and 0.63 g kg^{-1} , respectively.

3.5 Soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ dynamics

As soil depth increased, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values gradually decreased (Fig. 5), and those values in the 0–2-cm soil layer were significantly higher than those in the 2–15-cm soil layers. Also, the $\delta^{15}\text{N}$ decrease was significantly greater than that of $\delta^{13}\text{C}$'s. In the 0–2-cm soil layer, mean $\delta^{13}\text{C}$ was significantly higher in PN soil (-10.49‰) than in RP soil (-13.34‰), while the reverse was true for $\delta^{15}\text{N}$ (445.75‰ in RP soil and 126.75‰ in PN soil). The $\delta^{13}\text{C}$ peak values of RP and PN soils appeared at 6 months (-10.82‰) and 2 months (-8.14‰) of decomposition, respectively, and $\delta^{15}\text{N}$ peaked at 8 months for both RP and PN soils (557.83‰ and 414.89‰ , respectively) (Fig. 5). In the 0–2-cm soil layer, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were not significantly affected by the different precipitation treatments, but PN's $\delta^{13}\text{C}$ value in the 600-mm treatment was higher than those in the 400- and 800-mm treatments.

3.6 The fractions of litter-derived C and N in the soil

The fractions of litter-derived C and N gradually decreased as soil depth increased, and the 0–2-cm soil layer had significantly higher fractions than the 2–15-cm soil layers had (Fig. 6). Moreover, in the 0–2-cm soil layer the fractions of litter-derived C and N from RP leaf litters (20.79% and 14.13%) was higher than those from PN leaf litters (13.90% and 3.27% , respectively). Also, the fraction of litter-derived C (17.34%) was significantly higher than that of litter-derived N (8.7%).

After 16 months of decomposition, the fractions of litter-derived C and N were also related to precipitation regimes. In the 0–2-cm soil layers, the fractions of litter-derived C (12.72% , 24.22% , and 25.44%) and N (17.34% , 9.38% , and 15.67%) from RP did not increase with increases in precipitation regimes (400, 600, and 800 mm), but the fractions of litter-derived C (20.01%) and N (4.61%) from PN

were highest in the 600–mm precipitation treatment (Fig. 6, $P < 0.05$). In addition, with the deepening of soil layers, the differences among the 3 precipitation regimes were smaller.

4. Discussion

4.1 Mass loss of litters

The leaf litters rapidly lost mass in the first 4 months (simulated rainy season) of irrigation (Fig. 1), indicating that many unstable water-soluble compounds were lost. Similarly, Rubino et al. (2010) demonstrated that mass loss had stabilized after 100 days of incubation and Gautam et al. (2016) reported an initial phase (up to 60 days) that was characterized by a rapid decline in litter weight. Peak litter decomposition rates usually occur at the beginning of the rainy season, mainly because a large number of unstable water-soluble compounds are lost due to precipitation (Xu and Hirata 2005). Afterward, decomposition rates reduce during later decomposition phases because of a predominance of recalcitrant metabolites (Gautam et al. 2016).

The least amount of mass lost in our study was in the 400-mm precipitation treatment, and loss did not increase in all treatments as precipitation increased, possibly because drought limited conditions do not favor microbial decomposition of litter. For instance, both Hawai'ian and Mediterranean studies found that decomposition reduced significantly as water availability reduced (Austin and Vitousek 2000; Santonja et al. 2015). Moreover, higher precipitation (i.e., 800 mm) in humid soil hinders the oxygen supply to microorganisms and inhibits the biological activity of decomposers (Garcia-Pausas et al. 2004). So not surprisingly, precipitation regimes significantly affected mass loss during litter decomposition.

The mass loss and k value of non-legume leaf litter were higher than those of legume (higher N content and lower C:N ratio) leaf litter in our study. In contrast, other studies have shown that decomposition is rapid when N contents are high and C:N ratios are low (Osono et al. 2014). This may be because the C:N ratios (11 and 15) of the 2 trees in our study are similar, or because the poplar leaves we selected for convenient ^{13}C isotope labeling were too tender and leaves is broadleaf and has a large surface area, leading to the decomposition of lignin and phenolic components in the early stage of decomposition of easily by microorganisms. Alternatively, low tannin content may have aided decomposition, as Dreschel et al. (1991) suggested when they found that the low decomposition rate of *Acacia* was caused by its high tannin content. A complex of factors, such as precipitation changes, microbes, arthropods, litter quality and physical properties of litters also affect litter decomposition rates, and such factors likely interact to produce variable effects (Lensing and Wise 2007).

4.2 Leaf litter C and N dynamics

Both C and N decreased in the leaf litters during decomposition, with C having greater decreases than N, thus indicating that litter C and N were always released. Similarly, Du et al. (2020) found that the C content in plant litter gradually reduced after 1 year, and Connin et al. (2001) reported that plant litter N

contents decreased 60% during the first 120 days of decomposition. However, Parton et al. (2007) reported a simultaneous net increase of N content in decomposing litter, perhaps because N may be immobilized by bacteria and/or nutrients may transfer to the decomposing litter from humus or mineral soil via fungal hyphae (Rubino et al. 2010). In our study, the leaf litter N content was $> 24 \text{ g kg}^{-1}$ and the C:N ratio was < 17 , and relatively higher N concentrations and lower C:N ratios (i.e., N content $> 20 \text{ mg g}^{-1}$ and C:N < 25) favor organic matter decomposition and thus larger N loss (Wei et al. 2013). Therefore, the dynamic characteristics of C and N are related to litter quality.

In our study, litter C and N losses were not significantly affected by precipitation, (Table 1) thus indicating that increases in precipitation did not increase the loss of litter C and N. However, Austin and Vitousek (2000) reported that N mineralization generally increased with increasing precipitation on the island of Hawai'i (500 to 5500 mm). The differences between our results and theirs may be due to the relatively smaller range of precipitation regimes in our study (400 to 800 mm). Also, the extent of the effects of reduced rainfall may depend on the type of ecosystem, and also on the amount of rainfall that is removed (Santonja et al. 2019).

4.3 Leaf litter $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ dynamics

All $\delta^{13}\text{C}$ values decreased gradually, while the $\delta^{15}\text{N}$ value for RP leaf litter showed an enrichment and that for PN leaf litter decreased. These findings indicate that different types of leaf litters may have greatly different isotope distributions. Other studies have found that $\delta^{13}\text{C}$ decreased by 75.83% after 11 months (Rubino et al. 2010), enriched over time (Gautam et al. 2016), and shifted negligibly in residual litters (Ngao and Cotrufo 2011). Connin et al. (2001) also reported 2 different isotopic shifts within the same geographic region for 2 different species—one shift led to enrichment and the other to depletion. Other studies found that the $\delta^{15}\text{N}$ was depleted (Bimüller et al. 2013) and ^{15}N enriched (Xu et al. 2011) in litters. The rapid $\delta^{13}\text{C}$ change was caused mainly by decreased tannin content and the destruction of aromatic C structures in the initial stage of decomposition (Ono et al. 2011). The $\delta^{15}\text{N}$ enrichment may be explained by the preferential retention of ^{15}N enriched substrates (Connin et al. 2001), and/or by fungal-dominant decay processes (Hobbie EA, and Hobbie JE, 2008). However, $\delta^{15}\text{N}$ decreases may reflect decomposition processes dominated by bacteria (Bragazza et al. 2010).

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of our leaf litters were not significantly different among precipitation gradients, except for RP's $\delta^{13}\text{C}$ (Fig. 2). This indicates that the increase of precipitation in this study did not change the isotope distributions in the litters. Water availability is arguably the most influential factor affecting leaf $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in semi-arid and arid environments (Ma et al. 2012). Also, a large body of literature has reported negative correlations between plants' $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and the availability of precipitation (Murphy and Bowman 2009). The conflicting research results between those studies and ours may indicate that the differences in our precipitation regimes were too small and future research should employ larger differences.

4.4 Soil C and N dynamics

Compared with control (no litter), the C and N contents in the 0–2-cm layer of litter-treated soil was significantly greater than those in the 2–15-cm layer, and the profile difference was larger. This suggests that litter decomposition markedly increased the soil C and N contents in the surface layer. Garten et al. (2000) also found that mean soil C contents declined with soil depth during litter decomposition. If soil surface moisture and temperature are suitable, and there are ample microorganisms, litter decomposes quickly. Litter cover can change the microenvironment, thereby affecting the soil C and N contents in the topsoil layer (Schmidt et al. 2011).

In litter-treated soil, surface soil C and N contents did not change significantly as precipitation increased, and they reached their maximums at 6 months (Fig. 4). These findings indicate that the organic C and N leaching from litters to soil reached their maximums after 6 months of decomposition (Fahey et al. 2011). A previous study showed rainfall positively correlated with litter decomposition rate, which in turn affected surface soil C and N dynamics (Tuo et al. 2018). This difference may be because our precipitation gradient was too small to reflect the impacts of precipitation differences on soil surface C and N, but for the annual precipitation pattern of the Loess Plateau, our settings were reasonable. This indicates that in the Loess Plateau (semi-arid region), a small amount of additional precipitation does not significantly change the amount of litter C and N entering the soil.

4.5 Soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ dynamics

Compared with the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the control soil, those values were significantly enriched in the 0–5-cm layers of the litter-treated soils ($P < 0.01$) (Fig. 5). These results indicate that litter addition significantly increased ^{13}C and ^{15}N enrichment in the soil surface. Similarly, litter-treated soils were significantly enriched in $\delta^{13}\text{C}$ (Rubino et al. 2010) and $\delta^{15}\text{N}$ (Bimüller et al. 2013) in the 0–5-cm soil layers in other studies. Altogether, these results show that litter decomposition significantly changes the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ profile distribution characteristics in soil.

As the $\delta^{15}\text{N}$ gradually enriched, the $\delta^{13}\text{C}$ remained unchanged in 0–2-cm soil layers during decomposition (Fig. 5), and overall were higher than those values in the control soil. These results indicate that the C and N isotope changes in litter are inconsistent and are gradually transferred to different soil layers as decomposition progresses. Consistent with our study, Bimüller et al. (2013) found that the $\delta^{15}\text{N}$ increased after 140 days of decomposition and Díaz-Raviña et al. (2005) observed a 4.4‰ enrichment in the 0–5-cm layer after 12 years. Our results show that ^{15}N peaks in winter, with most of it as dissolved organic N strongly retained in the upper 5 cm of soil (Fahey et al. 2011).

4.6 The fractions of litter-derived C and N in soil

The fractions of litter-derived C (17.35–1.51%) and N (7.89–0.93%) gradually decreased as soil layers deepened (Fig. 6). This indicates that litter-derived C and N accumulate mainly in topsoil, and part of the C and N remain in the soil as microbial by-products (Rubino et al. 2010). Studies have shown that the fraction of litter-derived C accounts for 5% of all soil C (Rubino et al. 2010); however, the fraction of litter-derived N is 16–23% after 2.25 years of decomposition (Pei et al. 2019) and it can even reach about 90%

at the soil surface (Hatton et al. 2012). Changes in aboveground litter inputs can cause significant changes in the soil organic carbon pool in the upper soil layer (Cao et al. 2020), so litter-derived C and N accumulate mainly in the top mineral soil, progressively increasing the topsoil's ^{13}C and ^{15}N signatures (Bimüller et al. 2013). In addition, we found that soil particles wrapped in humified leaf litter formed on the soil surface (Fig. S3). Zeller and Dambrine (2011) found that the ^{15}N released from decaying litter was recovered mainly in the coarse particulate organic matter fraction ($> 200 \mu\text{m}$). Future measurements should clarify the proportion of litter-derived C incorporated into a range of SOM fractions, thus forming a more holistic understanding of the litter-soil biogeochemical continuum (Rubino et al. 2010).

The fractions of litter-derived C and N from RP were higher than those from PN leaf litter, indicating that leguminous leaf litter contributed more to soil C and N than non-leguminous litter did. After investigating 130 cases, Berg (2000) found that the higher the N concentration of fresh litter (and the lower the C:N ratio) the more organic matter left the decomposing litter to become SOM. Litter with a higher N content tends to contain more labile complexes (e.g., phenolic acids and flavonoids) that are usually easily accessed by decomposers and can thus be quickly decomposed (Garcia-Palacios et al. 2016). Therefore, due to their litter's large N content, leguminous plants can boost soil N content faster than other plants (Horodecki and Jagodziński 2017).

We found that different precipitation gradients significantly affected the percentages of soil C and N from litter sources ($P < 0.05$). For instance, after litter C entered the surface soil (0–2-cm layer), the 600-mm precipitation treatment was most beneficial for C's transformation to soil organic carbon. In addition, drought conditions promoted the transformation of legume litter N to soil total nitrogen, but inhibited the transformation of non-legume litter N.

4.7 Linear C and N coupling

The concentrations of C and N in litter and soil, and the fractions of litter-derived C and N were coupled. As decomposition time extended, the coupling degree gradually decreased but never decoupled (Table 2). This does not support our first hypothesis that C and N would eventually decouple as decomposition progressed. Marichal et al. (2011) also reported that soil and litter C and N are highly coupled in tropical forest plantations, and Fan et al. (2014) reported that N and C release from maize litter were coupled in the first year. These findings suggest that microbes preferentially decomposed litter C and N simultaneously when the labile organic compounds were composed of both C and N during the early decomposition stage (Prescott 2010). Compared to control soil, litter-treated soils experienced significantly reduced soil N intensity in response to C change, and that may in turn alter SOC dynamics by changing plant litter inputs and decomposition rates (Cao et al. 2020). Our results also show that the slopes of the linear relationships between C and N contents were highest under the highest precipitation treatment (Table 2). This shows that the response N has to C change becomes stronger as precipitation increases. The study of C and N coupled during litter decomposition provides an important basis for the modeling and prediction of the materials cycles of ecosystems (Zhang et al. 2014).

5. Conclusion

Using dual isotope tracing and in situ controlled, experimental methods, we investigated how the C and N fluxes during both leguminous and non-leguminous leaf litter decomposition responded to changes in precipitation. The results showed that the dual-isotope tracer method can be used to study both C and N fluxes and how C and N pathways diverge during litter decomposition. While the $\delta^{15}\text{N}$ value of leguminous leaf litter increased gradually, that of non-leguminous leaf litter decreased. In the surface soil, the overall fractions of litter-derived C and N from leguminous leaf litters were higher than those from non-leguminous leaf litters, and the fraction of litter-derived C was greater than that of N. Furthermore, after litter C entered the soil, the 600-mm precipitation treatment best benefited C's transformation to soil organic carbon, and drought conditions promoted the transformation of legume litter N to soil total nitrogen, but inhibited the transformation of non-legume litter N. The C and N concentrations of both litters and soils were always coupled during decomposition and entering the soil did not change that coupling. Also, N responses to C changes in the soil were reduced by litter cover. However, increased precipitation enhanced C and N coupling in the litters. These results have implications for how C and N fluxes respond to precipitation changes, and thus provides new insights for further research on litter decomposition and its ecological significance under various global change scenarios.

Declarations

Acknowledgments

This study was supported by the National Natural Science Foundation of China (41471244 and 41977105).

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Tables

Tables 1 and 2 are available in the Supplementary Files section

Figures

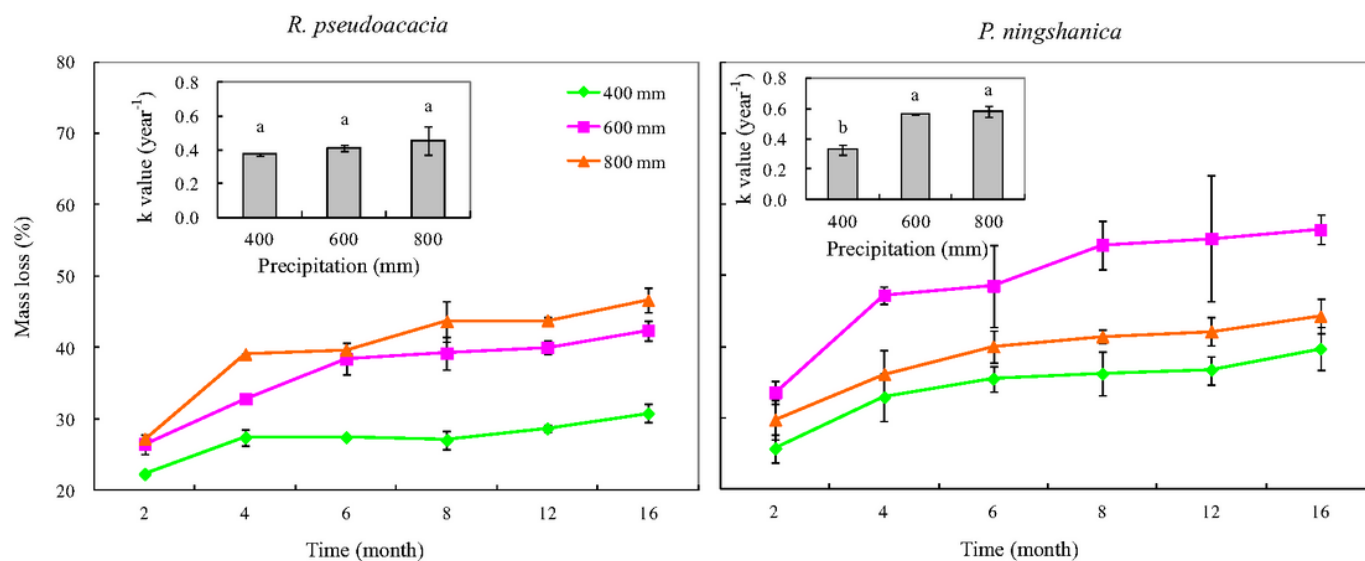


Figure 1

Leaf litter mass loss and annual decay rate constants (k value mean, SE) for 400-, 600-, and 800-mm precipitation treatments during decomposition (time) of

Robinia pseudoacacia (legume) and *Populus ningshanica* (non-legume). Data are means (SD) and means with different letters were significantly different at $p < 0.05$.

0.05.

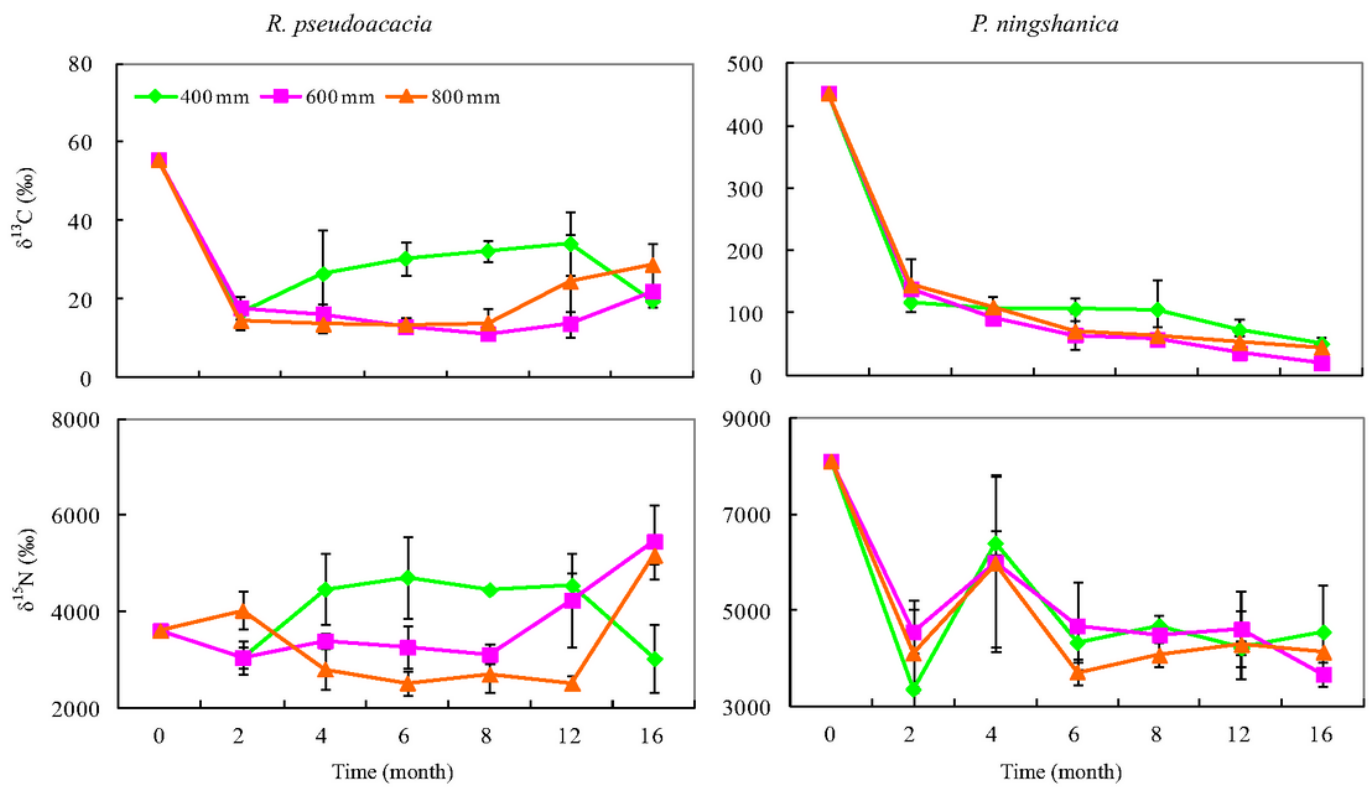


Figure 2

Leaf litter $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the 400-, 600-, and 800-mm precipitation treatments during decomposition (time) of *Robinia pseudoacacia* (legume) and

Populus ningshanica (non-legume).

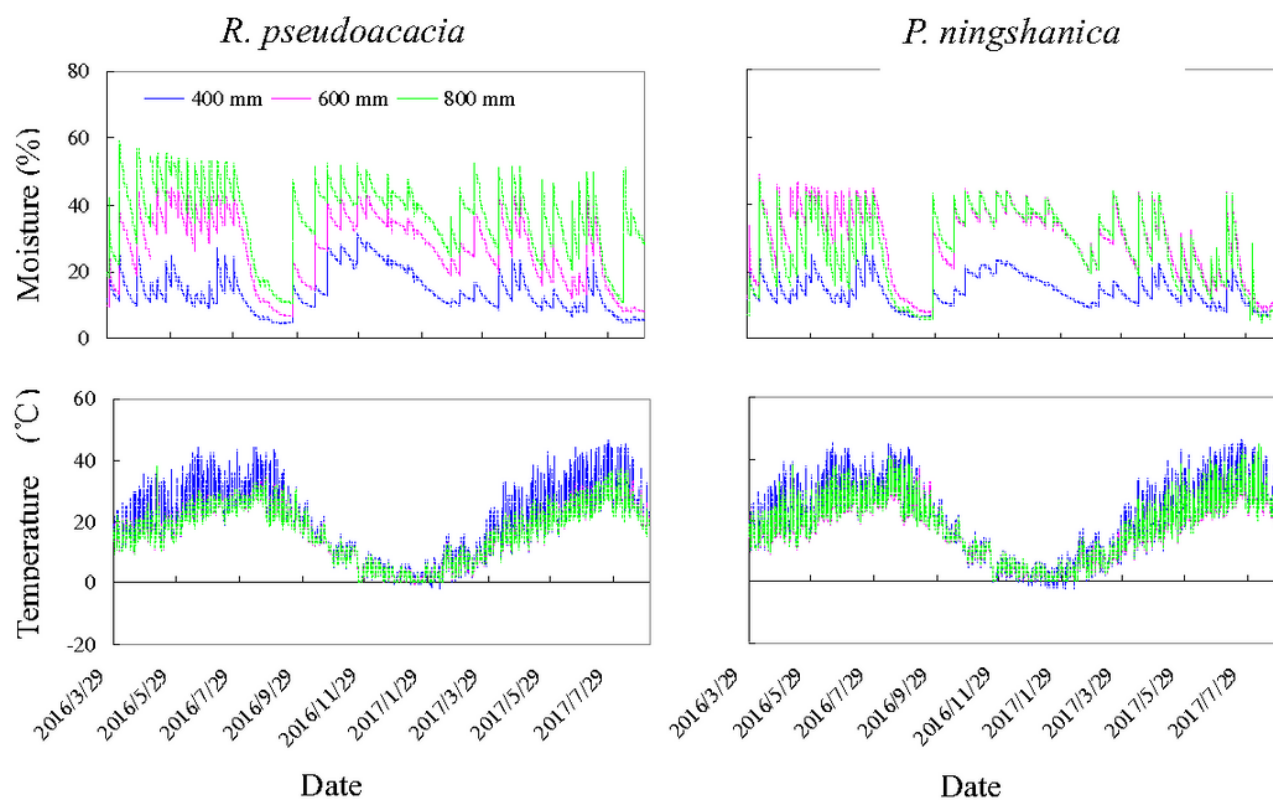


Figure 3

Soil temperature and moisture in the 400-, 600-, and 800-mm precipitation treatments during decomposition (Date).

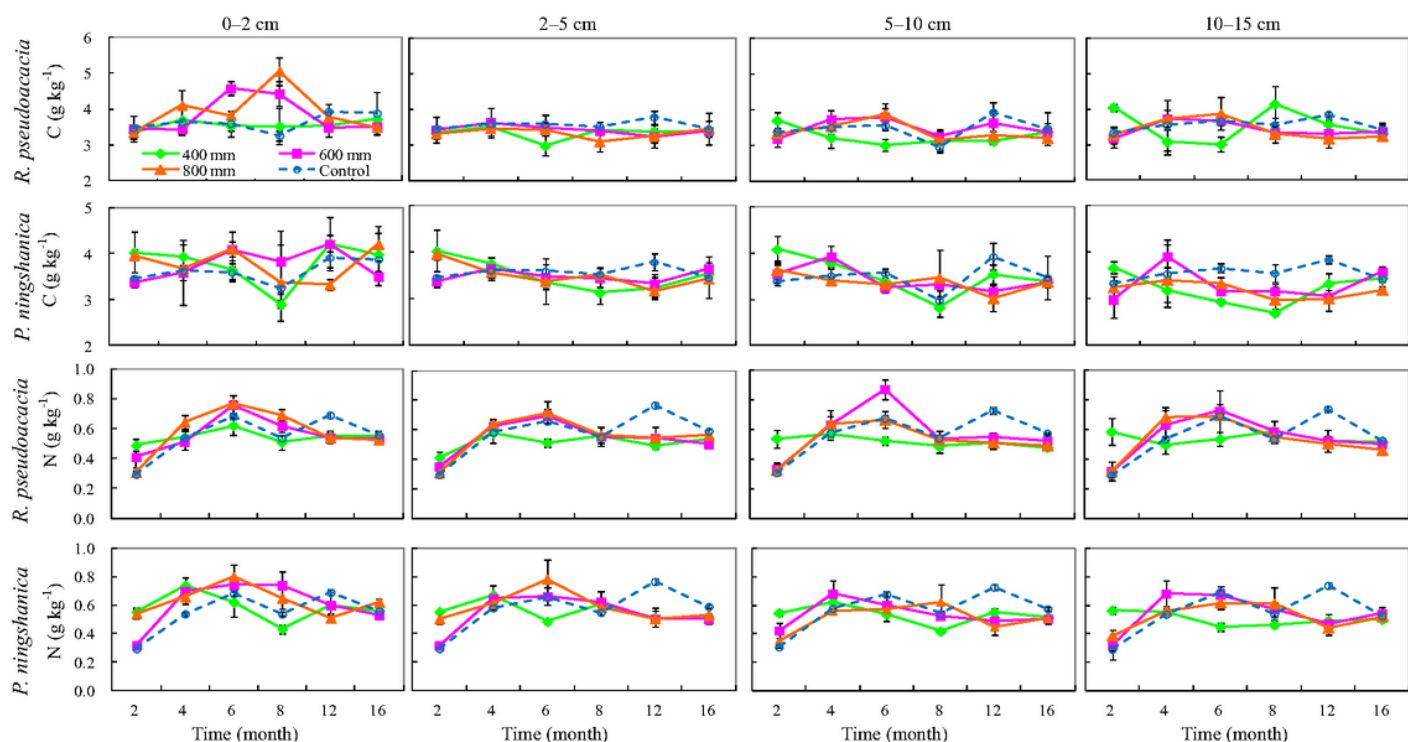


Figure 4

Soil C and N contents for the 400-, 600-, and 800-mm precipitation treatments during decomposition (time) of *Robinia pseudoacacia* (legume) and *Populus ningshanica* (non-legume) leaf litter. The control had no litter. Data are means (SD).

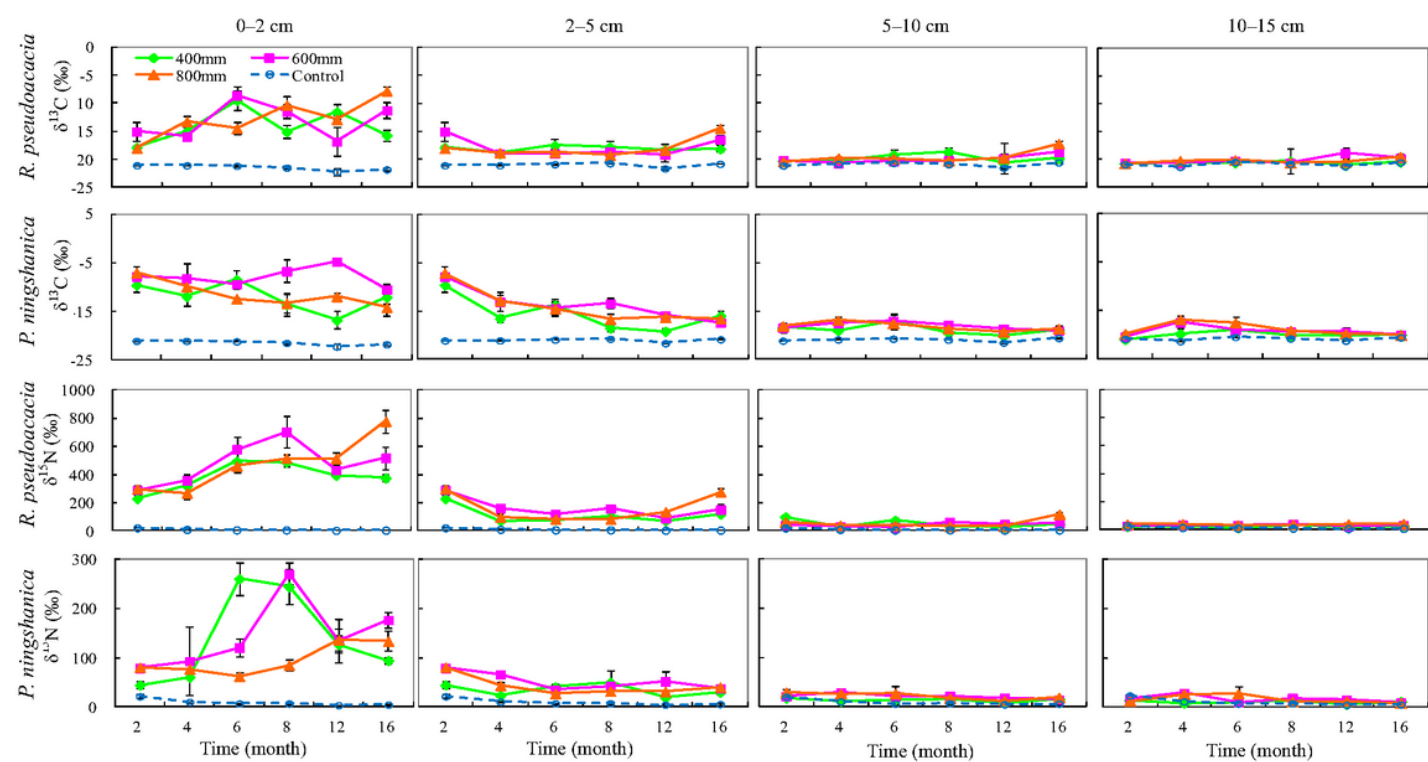


Figure 5

Soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for the 400-, 600-, and 800-mm precipitation treatments during decomposition (time) of *Robinia pseudoacacia* (legume) and *Populus ningshanica* (non-legume) leaf litter. The control had no litter. Data are means (SD).

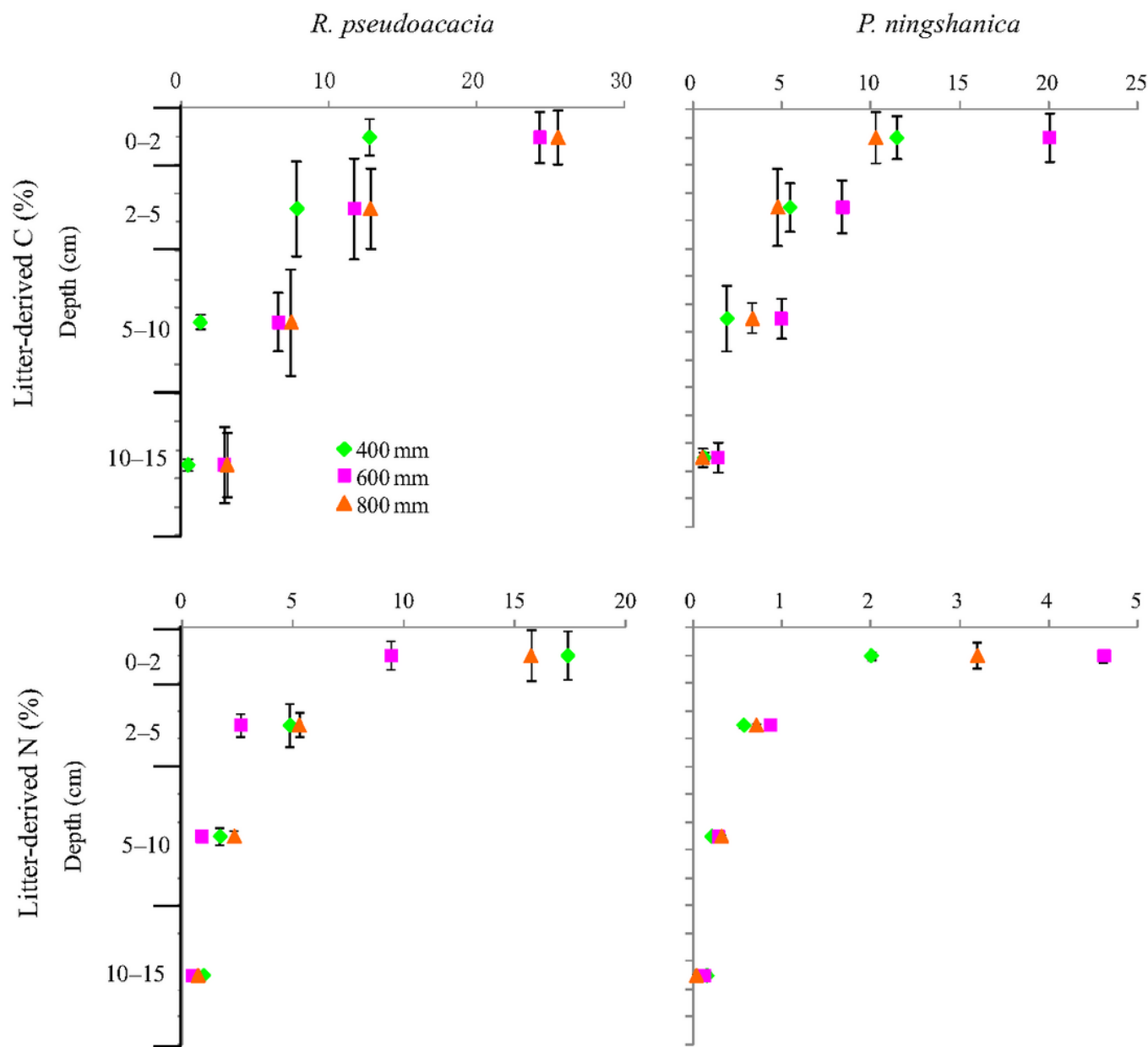


Figure 6

The fractions (%) of litter-derived C and N in various soil layers (0–2, 2–5, 5–10, 10–15 cm depths) and under the 3 precipitation regimes (mm) after 16 months of *Robinia pseudoacacia*

(legume) and *Populus ningshanica* (non-legume) leaf litter decomposition. Data are means (SD).

Supplementary Files

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