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Dual Isotopes Tracing Carbon and Nitrogen Dynamics during Leguminous and Non-Leguminous Litter Decomposition under Controlled Precipitation

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Abstract: 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 leguminous and non-leguminous leaf litters, and how these processes responded to different precipitation regimes. We used the dual-isotope tracing method to investigate differences in leaf and soil C and N, along with δ^{13}C and δ^{15}N, in the soil of the Loess Plateau in China. The δ^{15}N and δ^{13}C values were 3604‰ and 56‰ for *Robinia pseudoacacia* (Leguminosae) and 8115‰ and 452‰ for *Populus tomentosa* (Salicaceae) leaf litters. Through decomposition, δ^{13}C decreased in all litters, and δ^{15}N in the leguminous litter increased while it decreased in the non-leguminous litter. In the surface soil, the fraction of litter-derived N (14%) from the leguminous litter was significantly higher than that of the non-leguminous litter after 16 months. The C and N concentrations of both litters and soil always had a positive correlation during decomposition, and the responses of N to C changes in soil were reduced by the litter cover. Increased precipitation enhanced the litters' C and N correlation. The 600 mm precipitation treatment most benefited litter C's transformation to SOC, and drought conditions promoted the transformation of legume litter N to soil TN, but inhibited non-legume litter N. In the soil and both litters, C and N changes always had a positive correlation. After 16 months, the proportion of soil N from legumes was higher than that from non-legumes. Reduced precipitation could promote leguminous N in soil. Our results provide a scientific basis for accurately predicting the C and N cycles in terrestrial ecosystems.

Keywords: dual-isotope tracing; leaf litter decomposition; precipitation; leguminous; non-leguminous; nutrient cycling

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1. Introduction

Leaf litter is both a link between plants and soil in the nutrient cycle and an important nutrient source, and its decomposition process is vital for the composition and circulation of C and N pools in terrestrial ecosystems [1]. The return of C and N from leaf litter to soil can change not only the existing morphological stability and availability of soil C and N, but it can also affect the structure and function of soil ecosystems and their responses to environmental changes [2]. C and N released directly into the atmosphere and those released into soil during litter decomposition have different but very important ecological and environmental significances, and each must be identified and quantified. Isotopic labeling can accurately identify the C and N paths in litter decomposition and describe each path's flux and dynamic characteristics [3]. Most of the current litter decomposition studies, such as that of Pei et al. [4], have used single isotope markers, and few such studies have used dual-isotope labeling [5,6]. However, dual labeling of stable ^{13}C and ^{15}N isotopes can reveal both C and N circulation processes in litter decomposition and, thus, simultaneously provide an accurate picture of both circulation processes. Additionally, this procedure's research, analysis, and material costs, as well as research time, are lower than those of single-element labeling studies, so it is better suited for broad applications [7]. Glaser et al. [5] used the field labeling method to show that low-cost, dual-isotope labeling materials can achieve the same labeling effect and obtain a large number of labeling materials, compared to the results using single-element and indoor labeling. In addition, a quantitative analysis of soil organic carbon (SOC) dynamics under litter cover requires tracing both the SOC compounds' origins and their transformation pathways, and this can be performed by stable-isotope (e.g., ^{13}C and/ or ^{15}N) labeling [8]. Therefore, using ^{13}C and ^{15}N isotope dual labeling while conducting in situ litter decomposition studies in the field can reveal how litter decomposition and return to soil contribute to a comprehensive understanding of the biogeochemical cycles within ecosystems and the formation of soil organic matter (SOM) [9].

Because of reduced vegetation coverage, China's Loess Plateau has become one of the most severely eroded regions in the world [10]. To restore the forest cover and prevent soil erosion on sloped croplands in this region, the Chinese government has established 5.2 million hectares of tree plantations there [11]. A leguminous black locust (*Robinia pseudoacacia*, RP) and a non-leguminous poplar (*Populus tomentosa*, PT) are two important forest species that have been widely planted in the Plateau for soil and water conservation. Showing promise for reforestation, RP grows quickly and is able to fix atmospheric N, so it can supplement soil N pools, increase N return in litter fall, and enhance soil N mineralization rates when it is grown in a nutrient-poor ecosystem [12,13]. Ma et al. [14] suggested that PT can effectively control soil erosion and has the potential to maintain water. Therefore, RP and PT planted in the Loess Plateau importantly improve the regional ecological environment in northwest China. Xia et al. [15] reported that litter cover can reduce surface runoff and soil erosion. In semi-arid areas such as the Loess Plateau, litter decomposition is ecologically significant because it helps maintain ecosystem stability and improve soil texture [16]. Among many studies on leaf litters on the Loess Plateau, Tateno et al. [17] studied litter yield, forest floor organic matter quantity, and litter decomposition rate of nitrogen-fixing RP and non-N-fixing oak (*Quercus liaotungensis*) forests. Zhang et al. [18] conducted a 345-day controlled, laboratory decomposition experiment using RP litter mixed with 10 other species litters to investigate the impacts of mixed leaf litter decomposition on the release of eight main nutrients and to assess interspecific relationships. Xia et al. [15] used a rainfall simulation on a loess hillslope to examine the effects of forest litter cover on water interception, runoff, and infiltration and soil erosion. When Zhou et al. [19] examined the soil respiration and biophysical factors of PT and four other forest tree species, they found that 48% of the variation in annual soil CO_2 efflux was explained by the combined C stocks in the litter and topsoil. Li et al. [20] placed ground leaf litters of PT and 11 other tree species into soil, both separately and as a mixture, and incubated the samples in a laboratory to analyze the effects of leaf litter decomposition on soil properties and the interactions between the litters' decompositions. In addition, previous studies have surveyed biomass and C storage, soil organic C, and soil chemical and microbiological properties of RP plantations to understand their ecological restoration process after afforestation on the Loess Plateau [3,13,17,21,22], and some of these studies have used isotopes. For example, Liu and Wang [23] discussed the potential relationship between the $\delta^{15}N$ values of a plant–soil system and climate factors, such as temperature and precipitation, and they noted that the N isotope values of plant litter and soil both declined as mean annual precipitation increased and with summer precipitation. However, there are few studies using dual-isotope labeling on the Loess Plateau, and studies on the impact of precipitation on the fractions of litter-derived C and N have not been reported, but this is crucial for assessing carbon sequestration and sustainable development of forestland in this region.

The climate and plant diversity of arid and semi-arid regions are major determinants of C and N dynamics in decomposing plant litters [24], and these areas are sensitive to climate changes [25]. Therefore, climate changes may have great impacts on the Loess Plateau's fragile ecosystems. The Loess Plateau, a semi-arid region with scarce water and large variations in inter-annual rainfall, is subject to frequent dry and wet cycles and severe drought during the dry season [26]. This is important because water more significantly influences litter decomposition than does temperature [27]. Water availability controls ecosystem structure and processes by affecting long-term balances between the input and output of elements and the cycling of C and nutrients within these ecosystems [28]. Precipitation changes can influence litter decomposition directly by restricting the physical breakdown of litter and indirectly by affecting decomposer activities [29]. Paul et al. [30] found that SOM decomposition is governed by soil water conditions, with relatively weak decomposition in drier soils than in wetter soils, and Sanaullah et al. [25] found that drought inhibited grassland litter decomposition by more than 50%. Liu and Wang [23] noted that the N isotope values in Loess Plateau plant litter and soil respond to the amount of precipitation, declining with increasing precipitation. In addition, other studies have shown significant decomposition reductions with reduced water availability (e.g., in Chihuahuan Desert [31] and Mediterranean area [32]). 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, research that clarifies such dynamics and effects on the Loess Plateau also lays an important foundation for the study of C and N cycles during litter decomposition in general, which has significant value for predicting and comprehensively understanding the C and N cycles in terrestrial ecosystems. 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 correlation in soil; (2) higher precipitation increases the entry of leaf litter C and N into the soil and strengthens the C and N positive correlation relationship; and (3) more C and N from leguminous leaf litter sources enters the soil than from non-leguminous sources.

2. Materials and Methods

2.1. Study Site and Soil Properties

This study was carried out at the Northwest A&F University in Yangling, Shaanxi, China (34°16′18″ N, 108°04′59″ E; 525 masl), an area characterized by a warm temperate monsoon climate [33]. The area's mean annual precipitation and temperature are 580.5 mm and 15.6 °C, respectively, with most precipitation falling between July and September. The soil used in this study was collected from a farmland (pH = 7.8) that had been abandoned for at least 8 years. The soil is a Typ-Eum-Orthic anthrosol, which belongs to the Nitisols according to FAO taxonomy [34]. The soil's organic matter, total N, 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 a field capacity of 22%. The sand, silt, and clay contents were 27%, 41%, and 32%, respectively [35]. At the beginning of the experiment, the soil density was 1.35 g cm^{-3}. Given that all the soil used in this study was the same, the effects of the initial soil properties on litter decomposition were negligible.

2.2. Experimental Design

The greenhouse litter decomposition field experiments began by first sinking 16 cm diameter by 20 cm long PVC (polyvinyl chloride) tubes (N = 126) 15 cm into soil that had been screened through a 2 mm mesh. Then, 10 g of leaf litter (density about 0.02 g cm^{-3}) was placed on the surface of the soil inside each tube, and a 2 mm mesh nylon net (16 cm diameter) was carefully placed on the litter to minimize loss due to wind and other factors. Additionally, 18 control tubes were set up identically to the experimental tubes, except that they received no litter. Finally, the experimental site was roofed over, and the experiment was carried out in the shade to avoid photolysis of the litter (Figure A1). The decomposition experiment was conducted between April 2016 and August 2017.

The experimental design included the decomposition stages, precipitation treatments, and litter types. Decomposition was measured 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, which are amounts that reflect the actual ranges of average precipitation in the area. The scheduled rainfall and frequency are shown in Table 1. The artificial rainfall system was installed under the rooftop, and each sprinkler head had a short, stable, 360° full-circle spray range. Additionally, the sprinkler's water droplets were small enough to simulate raindrops (Figure A2). 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.

Table 1. Scheduled rainfall of the different precipitation treatments (800 mm, 600 mm, 400 mm) in different months.

Months	**800 mm**					**600 mm**					**400 mm**				
	Week 1	**Week 2**	**Week 3**	**Week 4**	**Sub-Sum**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Sub-Sum**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Sub-Sum**
November	15.9	0.0	15.9	0.0	31.8	12.7	0.0	12.7	0.0	25.4	8.0	0.0	8.0	0.0	16.0
December	6.4	0.0	6.4	0.0	12.8	4.8	0.0	4.8	0.0	9.6	3.2	0.0	3.2	0.0	6.4
January	6.4	0.0	6.4	0.0	12.8	4.8	0.0	4.8	0.0	9.6	3.2	0.0	3.2	0.0	6.4
February	9.6	0.0	9.6	0.0	19.2	8.0	0.0	8.0	0.0	16.0	4.8	0.0	4.8	0.0	9.6
March	19.1	0.0	19.1	0.0	38.2	14.3	0.0	14.3	0.0	28.6	9.6	0.0	9.6	0.0	19.2
April	38.2	0.0	38.2	0.0	76.4	28.7	0.0	28.7	0.0	57.4	19.1	0.0	19.1	0.0	38.2
May	25.5	25.5	25.5	25.5	102.0	19.1	19.1	19.1	19.1	76.4	12.7	12.7	12.7	12.7	50.8
June	19.1	19.1	19.1	19.1	76.4	14.3	14.3	14.3	14.3	57.2	9.6	9.6	9.6	9.6	38.4
July	38.2	38.2	38.2	38.2	152.8	28.7	28.7	28.7	28.7	114.8	19.1	19.1	19.1	19.1	76.4
August	5.1	0.0	5.1	0.0	10.2	3.8	0.0	3.8	0.0	7.6	2.5	0.0	2.5	0.0	5.0
September	41.4	41.4	41.4	41.4	165.6	30.3	30.3	30.3	30.3	121.2	20.7	20.7	20.7	20.7	82.8
October	25.5	25.5	25.5	25.5	102.0	19.1	19.1	19.1	19.1	76.4	12.7	12.7	12.7	12.7	50.8

2.3. *Isotope Labeling and Leaf Sample Collection*

We used a leguminous black locust (RP) and a non-leguminous poplar (PT) as study materials. There is significant variation in the ability of these two trees to use N from environments (leguminous vs. non-leguminous), which significantly affects the nitrogen and carbon contents as well as the abundance of labeled ^{13}C and ^{15}N in the litters. The two trees were all artificially planted in Yangling, among which RP was 4 m tall, with 3.2 m crown diameter and 20 cm trunk circumference, and PT was 5.5 m tall, with 2.4 m crown diameter and 30 cm trunk circumference. Since isotope labeling efficiency is greatly affected by water availability, we conducted the labeling during the rainy season (28 July 2015–3 August 2015), using the technique proposed by Glaser et al. [5]. During that process, we first drilled an approximately 2 cm hole into the tree trunks with a power drill tilted down about 30°. Then, the tree stems were injected with $^{15}NH_4{}^{15}NO_3$ to affect ^{15}N uptake, while the trees were simultaneously gassed with $^{13}CO_2$ to affect ^{13}C uptake via photosynthesis. Specifically, the stems were injected with a 2% $^{15}NH_4{}^{15}NO_3$ solution for 2 consecutive days. After ^{15}N labeling was completed, and ^{15}N was distributed in the leaves as evenly as possible, ^{13}C labeling was performed 1 day later. For that, we sealed each tree 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 stems and mixed in a high-purity $Na_2{}^{13}CO_3$ solution, which then released $^{13}CO_2$ gas. A fan fixed to the tree stems below the vessel evenly distributed the $^{13}CO_2$ around the tree body to aid ^{13}C uptake via photosynthesis. We performed labeling with the same concentrations of ^{13}C every 2 h during the day (09:00–15:00 h). 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 litters in this experiment. Ultimately, we obtained 2.18 kg (fresh weight) of RP leaves and 2.06 kg (fresh weight) of PT leaves. The $\delta^{15}N$ and $\delta^{13}C$ values for the labeled leaves were 3604.0‰ and 55.6‰ for RP and 8115.0‰ and 452.0‰ for PT, respectively. The quantities and isotopic abundances of the labeled materials met our study's requirements [5,10,36].

2.4. Litter Collection and Soil Sampling

Over the whole experimental 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 these particles. The litter was oven dried at 60 °C to a constant weight, the mass loss during decomposition was calculated, and then the litter was ground fine enough to pass through a 0.25 mm sieve for chemical analysis. The 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.

2.5. Chemical and Isotopic Analyses

The litter and soil samples were analyzed for total C and N contents and ^{13}C and ^{15}N 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).

2.6. Calculations and Statistical Analysis

The proportion of remaining mass (R_M, %) after the litter decomposition for each treatment was calculated as follows:

$$R_M(\%) = \left(1 - \frac{M_0 - M_t}{M_0}\right) \times 100 \tag{1}$$

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, year^{-1}) of the litter types were calculated using the Olson [37] negative exponential decay model:

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

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

The time required for 50% and 95% decomposition of leaf litter in each treatment was calculated as follows:

$$t_{0.5} = \frac{\ln(0.5)}{(-k)} \tag{3}$$

$$t_{0.95} = \frac{\ln(0.05)}{(-k)} \tag{4}$$

where $t_{0.5}$ and $t_{0.95}$ are the time for 50% (half-life period) and 95% decomposition of leaf litters, respectively.

The fraction of litter-derived C and N in the soil was obtained from the variation in δ^{13}C or δ^{15}N in the soil and litter and by applying a two-source mixing model [7]:

$$f_{litter} = \frac{\delta_s(t) - \delta_c(t)}{\delta_l(t) - \delta_c(t)} \tag{5}$$

where f_{litter} (%) is the fraction of litter-derived C or N in the soil over the total soil C or N; δ_s (t) is the δ ^{13}C or δ ^{15}N of the soil at the end of incubation; δ_c (t) is the δ ^{13}C or δ^{15}N of the control soil at the end of incubation (c = control); and δ_l (t) is the δ ^{13}C or δ^{15}N of the litter sample.

All correlation analyses were calculated using Pearson correlation coefficients. The direct effects of precipitation (400, 600, and 800 mm) on litter mass-loss rates, C and N releases, and ^{13}C and ^{15}N isotope abundances were examined by using one-way analyses of variance (ANOVAs). Linear regression and further statistical analyses were performed using SPSS version 19.0 (IBM, Armonk, NY, USA).

3. Results

3.1. Remaining Mass of Litters

When averaged across the two leaf types and three precipitation treatments, the remaining mass (R_M) of the leaf litters decreased linearly with time during the 16-month decomposition period, with rapid decomposition in the first 2 months (73.52 ± 1.61% for RP and 66.44 ± 1.23% for PT, about 60% of the total mass loss) and slow changes after 8 months (60.80 ± 1.71% for RP and 45.68 ± 4.05% for PT) (Figure 1). Additionally, the R_M varied with leaf type, with the decay rate constants (*k*) of 0.41 year^{-1} and 0.48 year^{-1} for RP and PT leaves, respectively. After 16 months of decomposition, the PT leaf R_M (53.17 ± 2.48%) was significantly lower than that of RP leaves (60.08 ± 1.47%) ($p = 0.027$).

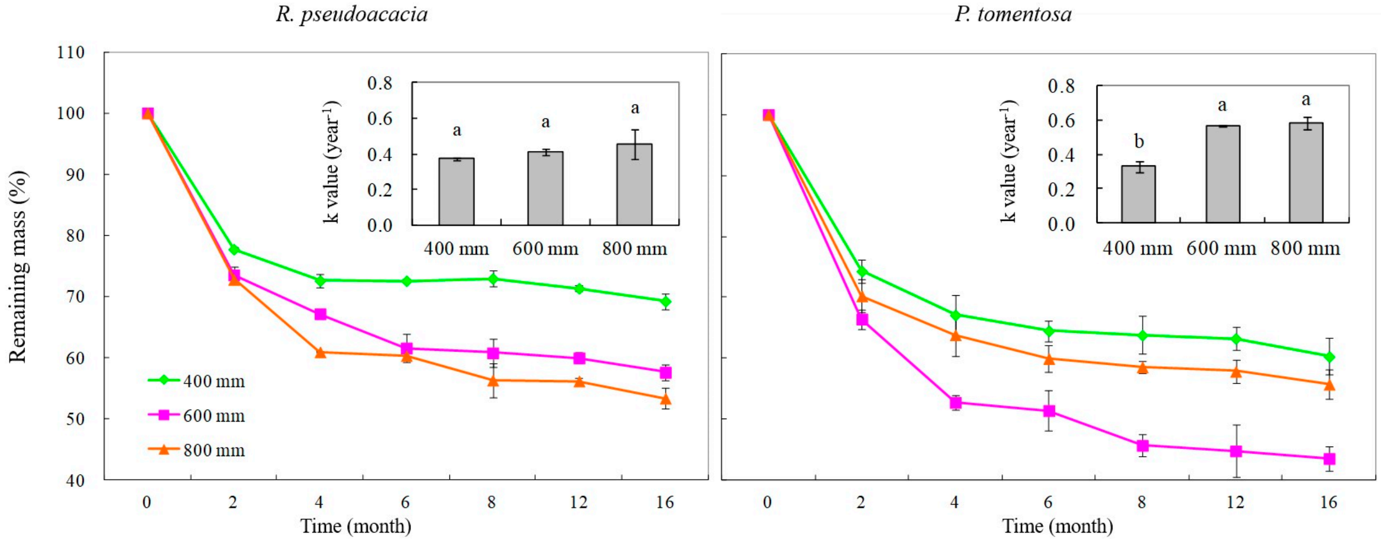

Figure 1. Leaf litter mass loss and annual decay rate constants (*k* value mean, SE) for the 400, 600, and 800 mm precipitation treatments during decomposition (time) of *Robinia pseudoacacia* (legume) and *Populus tomentosa* (non-legume). The data are represented as means (SD), and the means with different letters are significantly different at $p < 0.05$.

The R_M of both leaf types was significantly affected by precipitation regime during litter decomposition ($p < 0.01$, Table 2), and the effects varied with leaf type ($p = 0.028$ for the interaction). After 16 months of decomposition, The PT leaf R_M under 600 mm (43.53%) precipitation treatment was significantly lower than that under 400 mm (60.27%) and 800 mm (55.73%) treatments ($p < 0.05$); Same as the $R_{M,}$ the *k* values were 0.33 ± 0.03 year^{-1}, 0.56 ± 0.01 year^{-1}, and 0.58 ± 0.04 year^{-1}, respectively. The R_M for RP leaves under 400 mm (69.24%) precipitation treatment was significantly higher than that under 600 mm (57.52%) and 800 mm (53.39%) ($p < 0.05$), while the *k* values ($p = 0.552$) were 0.37 ± 0.01, 0.41 ± 0.02, and 0.45 ± 0.08 year^{-1}, respectively. The half-life period of the RP leaf litters was not affected by precipitation treatments, while the half-life period of the PT leaf litter under 400 mm precipitation was significantly higher than that under 600 mm and 800 mm precipitation treatments.

Table 2. The remaining mass (R_M, %) and annual decay rate constants (k, year^{-1}) for the decomposition of *Robinia pseudoacacia* (leguminous) and *Populus tomentosa* (non-leguminous) leaf litters, and the carbon (C) and nitrogen (N) contents (g kg^{-1}) as well as C:N ratio of both leaf litters at the beginning (Initial) and after 16 months of decomposition (Final), respectively.

	R. pseudoacacia			***P. tomentosa***		
Precipitation	**400 mm**	**600 mm**	**800 mm**	**400 mm**	**600 mm**	**800 mm**
R_M	72.73 a	63.44 b	60.00 b	65.53 a	50.76 b	61.03 a
k	0.37 a	0.41 a	0.45 a	0.33 b	0.56 a	0.58 a
Initial C	492.0 ± 3.0	492.0 ± 3.0	492.0 ± 3.0	517.0 ± 2.0	517.0 ± 2.0	517.0 ± 2.0
Final C	385.2 ± 8.0 a	399.0 ± 10.0 a	384.4 ± 8.0 a	385.1 ± 5.0 a	364.7 ± 3.0 a	375.6 ± 6.0 a
Initial N	44.2 ± 0.6	44.2 ± 0.6	44.2 ± 0.6	36.6 ± 1.4	36.6 ± 1.4	36.6 ± 1.4
Final N	37.6 ± 0.6 a	37.6 ± 0.8 a	35.9 ± 1.0 a	23.7 ± 2.2 a	25.4 ± 1.8 a	29.6 ± 1.9 a
Initial C/N	11.1 ± 0.6	11.1 ± 0.6	11.1 ± 0.6	14.14 ± 1.28	14.14 ± 1.28	14.14 ± 1.28
Final C/N	10.2 ± 0.6 a	10.6 ± 0.8 a	10.7 ± 1.0 a	16.2 ± 1.2 a	14.4 ± 1.8 ab	12.7 ± 1.2 b

Note: Means with different lowercase letters are significantly different ($p < 0.05$) among the 3 precipitation treatments for each variable.

3.2. Leaf Litter Carbon and Nitrogen Dynamics

The C concentration of both leaf litters was similar, while that of N was significantly higher in the RP litter than in the PT litter (Table 2). Additionally, the C and N concentrations decreased as decomposition progressed, and the decreases were not significantly different for PT and RP ($p = 0.567$), while C decreased more overall than N did. For instance, the C and N concentrations in the RP litter decreased by 102.49 g kg^{-1} and 7.20 g kg^{-1}, respectively, while those in the PT 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 2). In the 400, 600, and 800 mm precipitation treatments, the mean C contents in the RP leaf litter were 450 g kg^{-1}, 436 g kg^{-1}, and 431g kg^{-1}, respectively, and the mean N concentrations were largely unaffected by precipitation (40.15 g kg^{-1}, 39.84 g kg^{-1}, and 35.87 g kg^{-1}, respectively). For the PT leaf litter, the mean C contents were 445 g kg^{-1}, 421 g kg^{-1}, and 428g 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 ($p = 0.003$) in RP (11.10–10.74) than in PT (14.75–14.43) leaves primarily because the RP leaves began with higher N concentrations. The average C:N ratio of the RP leaf litter during decomposition was not significantly different with increasing precipitation, but for the PT leaf litter, the ratio was significantly higher in the 400 mm treatment (16.2) ($p = 0.052$) but not in the 800 mm treatment (12.7).

3.3. Leaf Litter $\delta^{13}C$ and $\delta^{15}N$ Dynamics

The initial $\delta^{13}C$ of the PT leaf litter was significantly higher (452.00‰) than that of the RP leaf litter (55.60‰) regardless of precipitation regime and decomposition time (Figure 2). As decomposition progressed, $\delta^{13}C$ decreased and the decrease was greater for PT than for RP. The drop occurred mainly in the first two months (reduced about 70%); in the RP litter, $\delta^{13}C$ decreased to 16.28‰, and it decreased to 133.84‰ in the PT litter. While $\delta^{13}C$ in the RP leaf litter was relatively stable (16.28‰ to 23.44‰) in 2–16 months, it was significantly lower than the initial value; in the PT leaf litter, it decreased over the decomposition time from 133.84‰ to 38.50‰ in 2–16 months. For the RP litter, $\delta^{13}C$ was significantly higher in the 400 mm treatment (30.73‰) than in the 600 mm (21.31‰) and 800 mm (23.47‰) treatments ($p < 0.05$) from 4 to 12 months of decomposition, but the PT litter had no such significant differences.

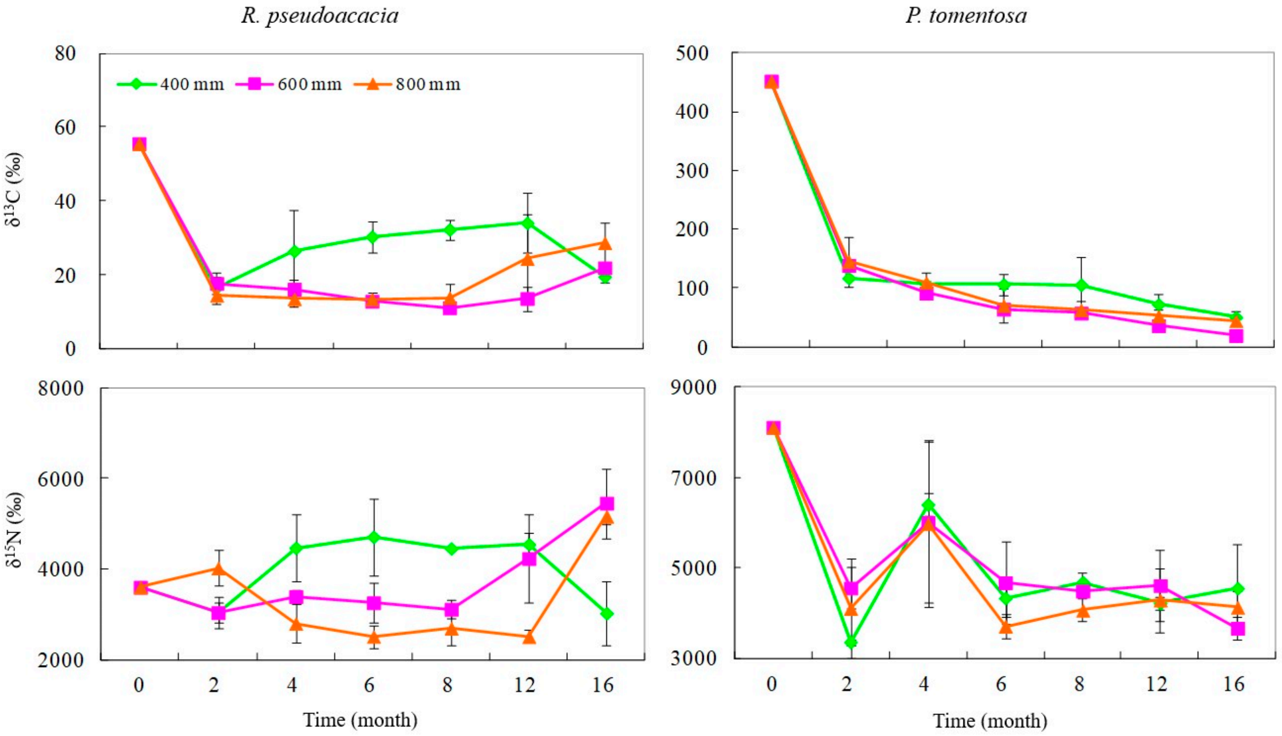

Figure 2. Leaf litter δ^{13}C and δ^{15}N values for the 400, 600, and 800 mm precipitation treatments during decomposition (time) of *Robinia pseudoacacia* (legume) and *Populus tomentosa* (non-legume).

The δ^{15}N value was also significantly higher in the PT leaf litter than in the RP leaf litter across the three precipitation regimes ($p < 0.05$). The RP leaf litter's δ^{15}N increased from 3604‰ to 4552‰ as decomposition progressed, while that of the PT leaf litter decreased from 8115‰ to 4118‰. The δ^{15}N value of the PT leaf litter decreased sharply in the first two months, and it decreased by 58.57%, 43.84%, and 49.38% in the 400 mm, 600 mm, and 800 mm precipitation treatments, respectively. Additionally, the average δ^{15}N was not significantly different among different precipitation regimes for both litters ($p > 0.3$, Figure 2).

3.4. Soil Carbon and Nitrogen Dynamics

As precipitation increased from 400 to 800 mm, soil moisture increased from 14.97% to 32.00%, and conductivity increased from 7.57 to 19.14 dS m^{-1}, but soil temperature decreased from 19.27 to 17.74 °C (Figure 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) ($p < 0.001$, Figure 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; Figure 4). Because previous research showed that litter decomposition mainly changed surface soil C and N contents, we only analyzed the 0–2 cm soil layer's large variations [38,39]. Researchers believe that microorganisms are active in litter layers, and SOC and TN accumulate significantly in the surface layer. In addition, about half of the underground plant biomass is concentrated in the soil surface layer, and organic matter in a lower layer is less returned. Both the RP and PT leaf litter-treated soils began with similar C and N concentrations, but over time, the C concentration peaked at 8 months for the RP soil and at 12 months for the PT soil. For N concentrations, the peak values appeared at six months for both RP and PT soils. Although soil C and N contents first increased and then decreased, these variations were not significantly different. Compared with the blank soil, the leaf litter treatment slightly increased the C and N contents in the 0–2 cm soil layer, but the effect of this increase was not significant ($p > 0.05$).

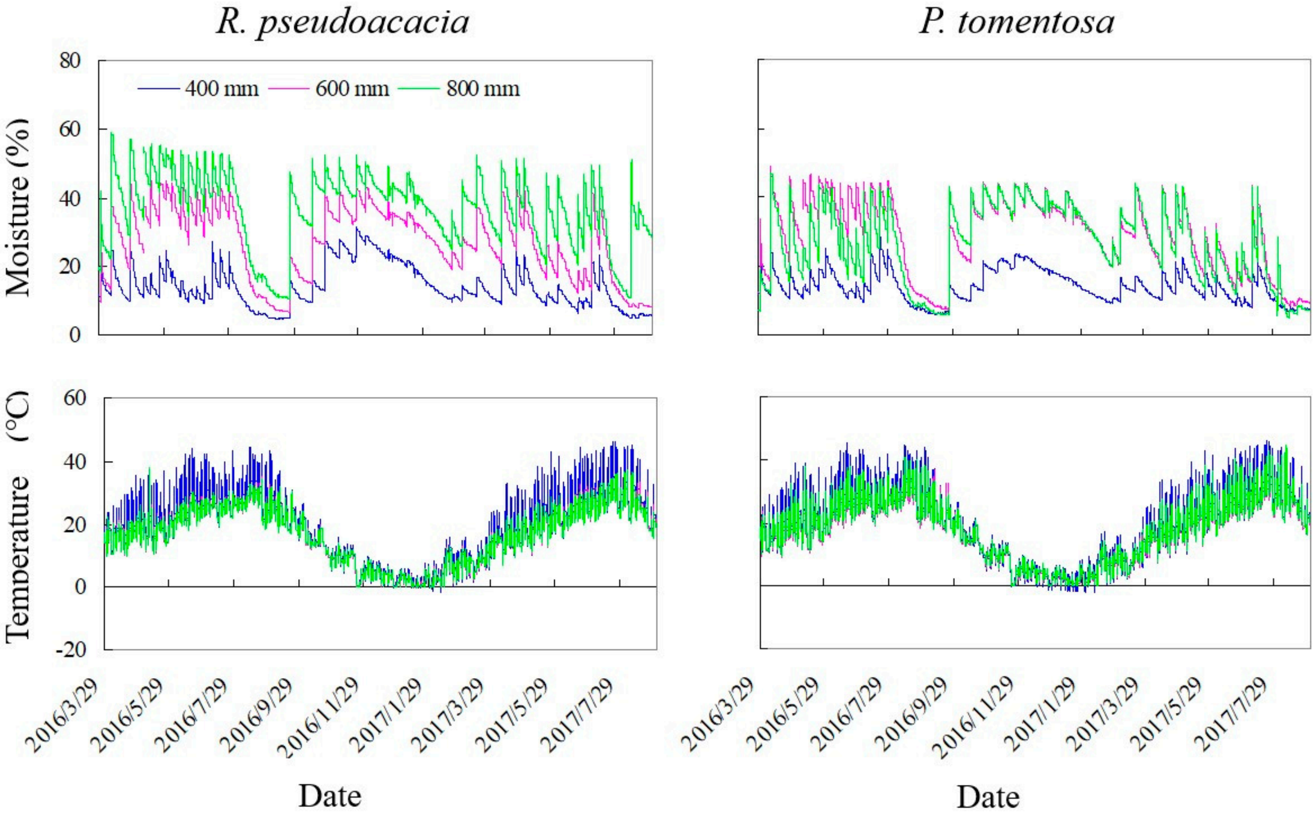

Figure 3. Soil temperature and moisture in the 400, 600, and 800 mm precipitation treatments during decomposition (Date).

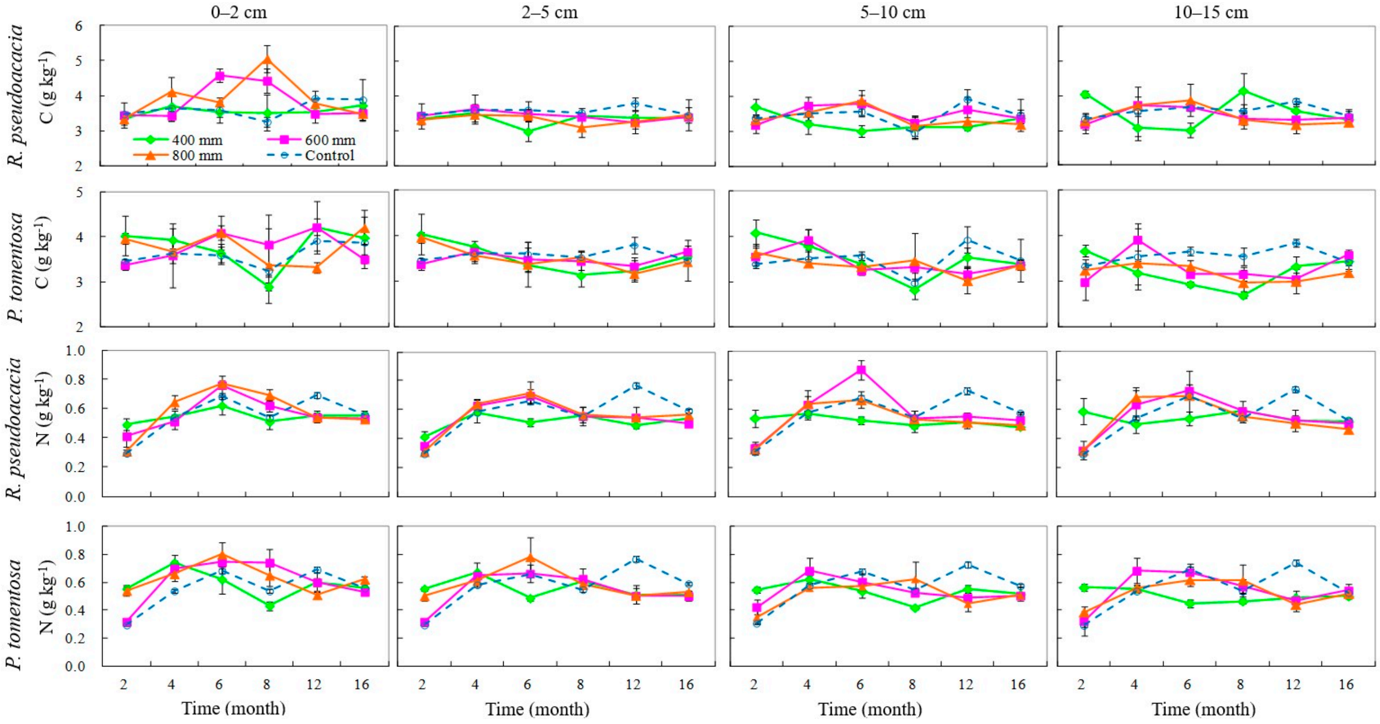

Figure 4. Soil C and N contents for the 400, 600, and 800 mm precipitation treatments during decomposition (time) of *Robinia pseudoacacia* (legume) and *Populus tomentosa* (non-legume) leaf litters. The control has no litter. The data are represented as means (SD).

Precipitation regimes did not significantly affect soil C and N dynamics during decomposition ($p > 0.05$, Figure 4). In the 0–2 cm soil layer, the mean values of C and N concentrations for the RP soil increased slightly as precipitation increased. In the 400, 600, and 800 mm precipitation treatments, the RP soil's C concentration was 3.55, 3.81, and 3.93 g kg^{-1}, respectively, and the N concentration was 0.54, 0.56, and 0.58 g kg^{-1}, respectively. For the PT soil, the C concentration was almost unaffected by precipitation, and the N concentration was 0.58, 0.61, and 0.63 g kg^{-1}, respectively.

3.5. Soil δ^{13}C and δ^{15}N Dynamics

As soil depth increased, the δ^{13}C and δ^{15}N values gradually decreased (Figure 5), and the values in the 0–2 cm soil layer were markedly higher than those in the 2–15 cm soil layers. Additionally, the δ^{15}N decrease was significantly greater than that of δ^{13}C. However, the control soil's δ^{13}C value was evenly distributed in the soil profile (focused on −21‰), and the δ^{13}C value was significantly lower than the leaf litter-treated soil. In the 0–2 cm soil layer, the mean δ^{13}C was not significantly different in both PT (−10.49‰) and RP soils (−13.34‰) ($p = 0.237$), whereas the mean δ^{15}N was significantly higher in the RP soil (445.75‰) than in the PT soil (126.75‰) ($p < 0.05$). The δ^{13}C peak values of the RP and PT soils appeared at six months (−10.82‰) and two months (−8.14‰) of decomposition, respectively, and δ^{15}N peaked at eight months for both RP and PT soils (557.83‰ and 414.89‰, respectively) (Figure 5). In the 0–2 cm soil layer, the δ^{13}C and δ^{15}N values were not significantly affected by the different precipitation treatments, but the PT soil's δ^{13}C value in the 600 mm treatment was higher than those in the 400 and 800 mm treatments ($p < 0.05$).

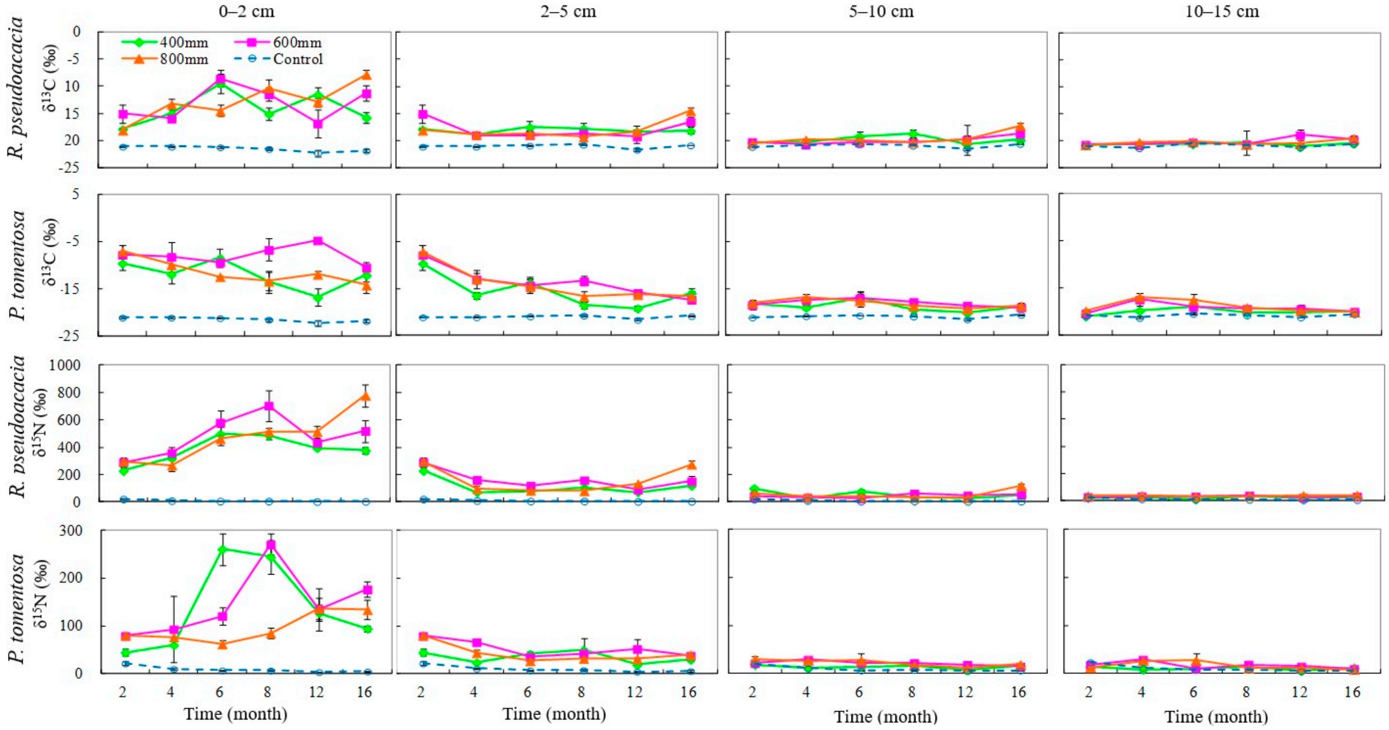

Figure 5. Soil δ^{13}C and δ^{15}N values for the 400, 600, and 800 mm precipitation treatments during decomposition (time) of *Robinia pseudoacacia* (legume) and *Populus tomentosa* (non-legume) leaf litters. The control has no litter. The data are represented as means (SD).

3.6. The Fractions of Litter-Derived Carbon and Nitrogen in 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 (Figure 6). Moreover, in the 0–2 cm soil layer, there was no significant difference between the RP and PT fractions of litter-derived C (20.79% and 14.60%, respectively), while the fraction of litter-derived N from the RP soil (14.13%) was significantly higher than that from the PT soil (3.19%, $p = 0.009$). Additionally, the fraction of litter-derived C (17.34%) was significantly higher than that of litter-derived N (8.7%, $p = 0.036$).

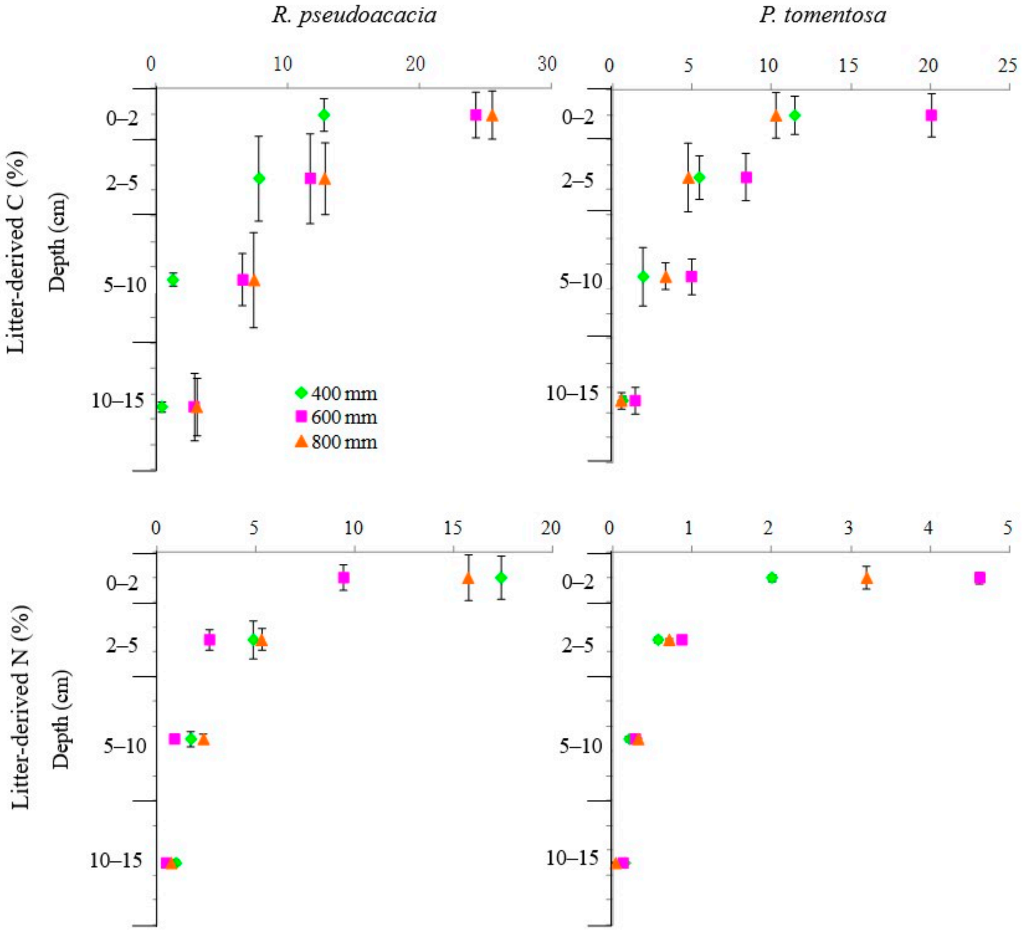

Figure 6. The fractions (%) of litter-derived C and N in various soil layers (0–2, 2–5, 5–10, and 10–15 cm depths) under the 3 precipitation regimes (mm) after 16 months of *Robinia pseudoacacia* (legume) and *Populus tomentosa* (non-legume) leaf litter decomposition. The data are represented as means (SD).

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 layer, the fraction of litter-derived C from the RP soil was significantly higher in the 600 (24.22%) and 800 mm (25.44%) precipitation treatments than in the 400 mm (12.72%) precipitation treatment ($p < 0.05$), and the fraction of litter-derived N from the RP soil was significantly higher in the 400 (17.34%) and 800 mm (15.67%) precipitation treatments than in the 600 mm (9.38%) precipitation treatment ($p < 0.05$). However, the fractions of litter-derived C (20.01%) and N (4.61%) from the PT soil were highest in the 600 mm precipitation treatment (Figure 6, $p < 0.05$). In addition, with the deepening of soil layers, the differences among the three precipitation regimes were smaller.

3.7. Linear Carbon and Nitrogen Relationship

The C and N residue contents of the RP leaf litter were significantly positively correlated under different precipitation treatments ($p < 0.001$), but the positive correlation between the C and N residue contents of the PT leaf litter was not significant ($p > 0.1$) (Table 3). Additionally, the slope value of the linear relationship was higher for the RP leaf litter (0.0671) than for the PT leaf litter (0.0257). The slope values of the linear relationships between C and N residue contents in the RP and PT leaf litters under the 400 mm precipitation treatment were significantly lower than those under the 600 and 800 mm treatments ($p < 0.05$). For example, the linear slope values of the C and N residue contents of the RP leaf litter under the 400 mm, 600 mm, and 800 mm precipitation treatments were 0.0504, 0.0821, and 0.0834, respectively, and those of the PT leaf litter were 0.0019, 0.0295, and 0.0500, respectively. These results indicate that the N response to the changes in C was enhanced as precipitation increased. As decomposition proceeded, the C and N residue contents in the RP leaf litter were always positively correlated. However, the C and N residue contents in the PT leaf litter were negatively correlated at 2 and 16 months of decomposition, but positively correlated between 4 and 12 months of decomposition, although none of these correlations were significant ($p > 0.05$). In addition, the slope value of the relationship between the C and N residue contents was significantly higher at two months of decomposition (RP = 0.137 and PT = -0.129) than at other decomposition periods.

Table 3. The correlation relationship between C and N (N = a × C + b) of the leaf litters under different precipitation regimes (400, 600, and 800 mm) and at different decomposition times (2, 4, 6, 8, 12, and 16 months).

	r		**R^2**		***p***		**RMSE**		**a**		**b**	
Whole	**0.396** **		**0.149**		**<0.001**		**5.775**		**0.070**		**4.557**	
	RP	**PT**	**RP**	**PT**	**RP**	**PT**	**RP**	**PT**	**RP**	**PT**	**RP**	**PT**
Precipitation regimes												
400 mm	0.740 **	0.018	0.515	–0.071	**0.001**	0.948	1.746	4.498	0.050	0.002	17.177	28.872
600 mm	0.853 **	0.391	0.710	0.096	**<0.001**	0.121	1.607	2.585	0.082	0.030	4.121	15.318
800 mm	0.843 **	0.314	0.711	0.046	**<0.001**	0.190	1.766	4.539	0.083	0.050	3.993	9.341
Decomposition times												
2 months	0.661	–0.390	0.356	0.031	0.053	0.299	1.803	5.494	0.137	–0.129	–22.231	90.290
4 months	0.331	0.416	0.039	0.035	0.423	0.306	1.485	3.688	0.039	0.049	24.010	6.288
6 months	0.285	0.186	0.072	0.126	0.494	0.659	1.332	3.938	0.018	0.037	32.438	14.839
8 months	0.467	0.704	0.106	0.411	0.205	0.051	1.543	3.561	0.026	0.119	28.009	–20.271
12 months	0.962 **	0.131	0.909	0.147	**0.001**	0.757	0.663	3.101	0.073	0.023	5.696	18.576
16 months	0.573	–0.095	0.245	–0.101	0.083	0.782	1.420	3.994	0.048	–0.030	18.149	39.741

Abbreviations: r: correlation coefficient; RP: *R. pseudoacacia*; PT: *p.* tomentosa; **: significantly correlated at 0.01 level; RMSE: root mean square error.

While the C and N contents in the 0–2 cm, 5–10 cm, and 10–15 cm soil layers were significantly positively correlated ($p < 0.01$) (Table 4), they were not significantly correlated in the 2–5 cm soil layer ($p = 0.272$). During the whole decomposition period (16 months), the C and N contents in the leaf litter-treated soil were always positively correlated ($p < 0.001$). As decomposition time progressed, the linear relationship slope value gradually decreased from 0.299 (initial) to 0.095 (eight months), and then it remained basically unchanged. This indicated that while the litter cover weakened the response of N to C changes in the soil, they did not uncouple. In addition, the C and N contents in the leaf litter soil were positively correlated under different precipitation treatments ($p < 0.001$). We also found that the slope values of the linear relationship of C and N contents in all soil layers of the legume RP were significantly higher than those of the non-legume PT, indicating that the responses of N to C changes in the leguminous leaf litter-treated soil were stronger than those of the non-legume treatment.

Table 4. The correlation relationship between C and N (N = a × C + b) in the litter-treated soil in different layers (0–2, 2–5, 5–10, and 10–15 cm), precipitation regimes (400, 600, and 800 mm), and decomposition times (2, 4, 6, 8, 12, and 16 months) in this study.

	r	R^2	*p*	RMSE	a	b
Layers						
0–2 cm	0.604 **	0.347	<0.001	0.096	0.171	–0.065
2–5 cm	0.188	0.007	0.272	0.101	0.090	0.239
5–10 cm	0.502 **	0.230	0.002	0.089	0.170	–0.047
10–15 cm	0.557 **	0.289	<0.001	0.084	0.169	–0.035
Precipitation regimes						
400 mm	0.614 **	0.363	<0.001	0.050	0.102	0.182
600 mm	0.586 **	0.329	<0.001	0.104	0.229	–0.249
800 mm	0.457 **	0.192	0.001	0.107	0.145	0.048
Decomposition times						
Control soil	0.533 **	0.251	0.007	0.134	0.299	–0.509
2 months	0.882 **	0.767	<0.001	0.048	0.276	–0.560
4 months	0.731 **	0.514	<0.001	0.042	0.183	–0.041
6 months	0.723 **	0.501	<0.001	0.077	0.198	–0.047
8 months	0.675 **	0.431	<0.001	0.057	0.095	0.243
12 months	0.857 **	0.723	<0.001	0.020	0.104	0.165
16 months	0.753 **	0.547	<0.001	0.022	0.107	0.147

Abbreviations: r: the correlation coefficient; **: significantly correlated at 0.01 level; RMSE: root mean square error.

4. Discussion

4.1. Remaining Mass of Litters

The leaf litters rapidly lost mass in the first two months (simulated rainy season) of irrigation (Figure 1), indicating that many unstable water-soluble compounds were lost. Similarly, Rubino et al. [7] demonstrated that mass loss stabilized after 100 days of incubation, and Gautam et al. [6] 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 [40]. Afterward, decomposition rates reduce during later decomposition phases because of a predominance of recalcitrant metabolites [6].

The greatest R_M in our study was in the 400 mm precipitation treatment ($p < 0.05$), possibly because drought-limited conditions did not favor microbial decomposition in the litter. Santonja et al. [32] also showed that drier climate led to slower mass-loss rates. For the RP leaf litter, the mass loss increased with an increase in precipitation. Typically, populations of major decomposers, including bacteria, fungi, and arthropods, may be affected by precipitation, and low humidity can inhibit fungal growth and/or activity [41]. Increasing precipitation not only promotes the leaching of soluble substances in litter, but also increases soil surface water content and improves the living environment of microorganisms, thus accelerating the decomposition of litter. Zhou et al. [42] studied decomposition in Sichuan (with an average annual rainfall of 1700 mm) and found that both 20% and 50% reduction in rainfall significantly reduced litter mass loss. Studies in both Hawai'ian (500–5500 mm) and the Mediterranean (annual mean of 830 mm) found that decomposition reduced significantly as water availability reduced [32,43]. However, for the PT leaf litter, the mass loss was the highest under the 600 mm precipitation treatment. We believe that the shape and size of leaves should also be considered in the study of litter decomposition in experimental tubes. The PT leaves we selected were thin and large, resulting in the loss of excess water flow from the short-term, high-intensity pulse precipitation along the soil column wall. Moreover, greater precipitation (i.e., 800 mm) in humid soil hinders oxygen supply to microorganisms and inhibits the biological activity of decomposers [44]. Thus, not surprisingly, precipitation regimes significantly affected the R_M values during litter decomposition in our study.

In our study, the non-legume leaf litter's R_M values were less than those of the legume (higher N content and lower C:N ratio) leaf litter. In contrast, other studies have shown that decomposition is rapid when the N contents are high and the C:N ratios are low [45]. This might be because the PT litter's surface area was larger, more microorganisms could be carried on the litter per unit mass, thus contributing to decomposition [46]. Alternatively, its low tannin content might have aided PT leaf decomposition, as Drechsel et al. [47] suggested when they found that the low decomposition rate of *Acacia* was caused by its high tannin content. However, we believe that in the Loess Plateau, leaf litter mass loss of deciduous vegetation is mainly determined by the N/p ratio, and the N/p ratio is negatively correlated with mass loss [35]. Therefore, the PT leaf litter (N/p ratio was 15.91) had a higher mass loss than the RP leaf litter (N/p ratio was 34.53).

4.2. Leaf Litter Carbon and Nitrogen 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. [35] found that the C content in plant litter gradually reduced after 1 year, and Connin et al. [48] reported that plant litter N contents decreased 60% during the first 120 days of decomposition. Hart et al. [49] found that the C mineralization rate of fresh litter in soil was accelerated because of the soil's high N content at the initial decomposition stage, and the C mineralization rate gradually decreased as the high N litter was decomposed. However, Parton et al. [50] reported a simultaneous net increase in N content in decomposing litter, perhaps because N may be immobilized by bacteria and/or nutrients may be transferred to the decomposing litter from humus or mineral soil via fungal hyphae [7]. In our study, the leaf litter N content was >24 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 mg g^{-1} and C:N < 25) favor organic matter decomposition and, thus, larger N loss [51]. Therefore, the dynamic characteristics of C and N are related to litter quality.

In our study, the litter C and N losses were not significantly affected by precipitation (Table 2), thus indicating that increases in precipitation did not increase the loss of litter C and N. Santonja et al. [24] also showed that a 10% reduction in rainfall did not alter the C content in a Mediterranean shrub forest with an average annual precipitation of 552 mm. However, Austin and Vitousek [43] 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). Additionally, the extent of the effects of reduced rainfall may depend on the type of ecosystem and on the amount of rainfall that is removed [24].

4.3. Leaf Litter $\delta^{13}C$ and $\delta^{15}N$ Dynamics

All $\delta^{13}C$ values decreased gradually, while the $\delta^{15}N$ value for the RP leaf litter showed an enrichment and that for the PT leaf litter decreased. These findings indicate that different types of leaf litters may have greatly different isotope distributions. The loss of ^{13}C in leaf litter is usually accompanied by a loss of dry mass and total C [52]. The variation in $\delta^{13}C$ values in litters during decomposition may also result from isotope fractionation during microbial respiration, which leads to preferential release of ^{13}C [53]. In the Loess Plateau, Bai et al. [10] found that ^{13}C recovery in the leaves of *Populus* decreased from nearly 90% at 6 h ($\delta^{13}C = 568 \pm 71‰$) after labeling to 40% at 21 days. Other studies found that $\delta^{13}C$ decreased by 75.83% after 11 months [7], enriched over time [6], or shifted negligibly in residual litters [54]. Connin et al. [48] also reported two different isotopic shifts within the same geographic region for two different species after two years of decomposition—one shift led to enrichment (*S.gerardi*) and the other led to depletion (*p.elliotii*). Other studies found that $\delta^{15}N$ was depleted [1] and ^{15}N was enriched [55] in litters. The rapid $\delta^{13}C$ depletion was caused mainly by decreased tannin content and the destruction of aromatic C structures in the initial stage of decomposition [56]. The $\delta^{15}N$ enrichment might be explained by a preferential retention of ^{15}N-enriched substrates [48] and/or by fungaldominant decay processes [57]. However, decreases in $\delta^{15}N$ might reflect decomposition processes dominated by bacteria [58]. Perakis et al. [59] observed a correlation between $\delta^{15}N$ in litters and N availability. Thus, when N is limited, ^{15}N will be lost. Similarly, when C availability is relatively high, the fractionation in the N assimilation process leads to a low ^{15}N enrichment level [60].

Our results also showed that the $\delta^{13}C$ and $\delta^{15}N$ values of the leaf litters were positively correlated at 0.01 level ($r = 0.414$, $p < 0.01$). We also found that the N content was negatively correlated with $\delta^{13}C$ ($r = -0.305$, $p < 0.01$) and $\delta^{15}N$ ($r = -0.284$, $p < 0.01$). Therefore, in our study, the $\delta^{13}C$ and $\delta^{15}N$ values of the RP leaf litter (with high N content) were lower than those of the PT leaf litter. In addition, Gautam et al. [6] found that when the C/N value is high, ^{15}N depletion may be related to microbial demand for extra N during litter degradation. Thus, the ^{15}N loss of the PT (with high C/N value) leaf litter was greater than that of the RP (with low C/N value) leaf litter in our study.

The $\delta^{13}C$ and $\delta^{15}N$ values of our leaf litters were not significantly different among the precipitation gradients, except for the RP leaf litter's $\delta^{13}C$ (2–12 months) (Figure 2). This indicates that the increase in precipitation in this study did not change the isotope distributions in the litters. Water availability is arguably the most influential factor affecting leaf $\delta^{13}C$ and $\delta^{15}N$ in semi-arid and arid environments [28]. Additionally, a large body of literature has reported negative correlations between plants' $\delta^{13}C$ and $\delta^{15}N$ values and the availability of precipitation [61]. Handley et al. [62] found that *Acacias*'s $\delta^{13}C$ ($-24‰$ to $-26.6‰$) in arid sites without access to groundwater had smaller negative values than other sites with groundwater access, and this was possibly a response to water stress. Welker et al. [63] reported that $\delta^{15}N$ in the leaves of 15 Eurasian Arctic coastal *Carex* plants differed significantly among the plots, and these $\delta^{15}N$ values were negatively correlated with the mean annual precipitation. This correlation suggests that the $\delta^{15}N$ values decreased when water availability was sufficient. Liu et al. [23] showed that $\delta^{15}N$ of the Loess Plateau ecosystem was more sensitive to precipitation than previous data, and the $\delta^{15}N$ value of the litter was $-1.39‰/100$ mm. The nitrogen isotope values of the litter decreased with an increase in annual rainfall or summer precipitation. In our study, precipitation had no significant effect on the $\delta^{15}N$ value, possibly because the abundance of leaf litter markers was too high, which did not respond significantly to the change caused by the difference of 200 mm precipitation. Future research should employ larger differences.

4.4. Soil Carbon and Nitrogen Dynamics

Compared to the control (no litter), the C and N contents in the 0–2 cm layer of litter-treated soil were significantly greater than those in the 2–15 cm layers ($p < 0.001$), and the profile difference was larger. Because litter can alter the microenvironment, thereby affecting both biotic and abiotic processes in the topsoil [64], it has no significant effect on soil C in the deep layers because the faster degradation of old C in the soil offsets the increased input of soil-conditioning matter [65]. In their 6-year study, Cao et al. [66] found that forest leaf litter increased the SOC content in the 0–10 cm soil layer but did not cause a change in the 10–20 cm soil layer. Garten et al. [67] also found that the mean soil C contents declined with soil depth during litter decomposition. Hatton et al. [68] observed that after 10 years, a relatively large proportion of N produced by litter was retained in the topsoil layer, and they demonstrated time delays in the transfer of litter-derived N from the soil organic layer to the mineral layer (vertical delay) and from plant debris to the mineral–organic assemblage (horizontal delay). 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 topsoil layers [64].

Although the C and N contents of the litter-treated soil was significantly higher than the control soil (no litter) during 4–8 months of decomposition (August to December, during which there was heavy rainfall), the leaf litter treatment did not significantly increase soil C and N contents in terms of the mean values after the 16-month decomposition period.

In Pennsylvania, Bowden et al. [69] found that 20 years of double litter treatment did not increase the content and storage of SOC. Similarly, Lajtha et al. [70] found in their 20-year study in the Harvard Forest that double litter treatment increased the C content of the organic layer, but it had no effect on the C content of mineral soil. This is because new C input from litter sources can compensate for the decomposition of soil old OC caused by priming effect [65]. Thus, an increase in litter input during the initial years of forest restoration is likely to be offset by C loss due to its higher C degradation rate [71].

In the litter-treated soil, the surface soil C and N contents did not change significantly as precipitation increased. A previous study showed that rainfall positively correlated with litter decomposition rate, which in turn affected surface soil C and N dynamics [72]. This difference might be because our precipitation gradient was too small to reflect the impacts of precipitation differences on soil surface C and N. 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 δ^{13}C and δ^{15}N Dynamics

Compared with the δ^{13}C and δ^{15}N values in the control soil, these values were significantly enriched in the 0–5 cm layer of the litter-treated soils ($p < 0.01$) (Figure 5). Similarly, litter-treated soils in other studies were significantly enriched in δ^{13}C [7] and δ^{15}N [1] in the 0–5 cm soil layers. As the δ^{15}N content became gradually enriched, the δ^{13}C content remained unchanged in the 0–2 cm soil layer during decomposition (Figure 5). Overall, these values were higher than the values in the control soil, most likely because of the decomposition of organic matter [73]. Consistent with our study, Bimüller et al. [1] found that δ^{15}N increased after 140 days of decomposition, and Díaz-Ravinã et al. [73] observed a 4.4‰ enrichment in the 0–5 cm layer after 12 years. Our results show that ^{15}N peaked in winter, with most of it as dissolved organic N that was strongly retained in the upper 5 cm of soil [52]. In addition, the δ^{13}C value of the 0–2 cm soil layer was positively correlated with the δ^{13}C value of the leaf litter ($r = 0.431$, $p < 0.01$) in this study. It has been verified that soil organic matter mainly comes from the vegetation layer, and the δ^{13}C value of the surface layer is closely related to the δ^{13}C value of the litter [23]. Altogether, these results show that litter decomposition significantly changes the δ^{13}C and δ^{15}N profile distribution characteristics in soil.

4.6. The Fractions of Litter-Derived Carbon and Nitrogen in Soil

The fractions of litter-derived C and N gradually decreased as the soil layers deepened (Figure 6). This indicates that litter-derived C and N accumulate mainly in topsoil (C: 17.35%, N: 7.89%), and part of the C and N remain in the soil as microbial by-products [7]. Changes in aboveground litter inputs can cause significant changes in the SOC pool in the upper soil layer [66], so litter-derived C and N accumulate mainly in the top mineral soil, progressively increasing the topsoil's ^{13}C and ^{15}N signatures [1]. Studies have shown that the fraction of litter-derived C accounts for 5% (after 200 days [7]) and 8% (after one year [74]) of all soil C; however, the fraction of litter-derived N is 16–23% after 2.25 years of decomposition [4], and it can even reach about 90% at the soil surface [68]. Rubino et al. [75] found that litter-derived C in soil accounted for 13.3% of the total C loss, so C input was directly proportional to the total C loss from the litter ($r = 0.780$, $p < 0.01$). Therefore, they posited that a fixed proportion of C in the total C lost by litter decomposition entered the SOM, but there was no such correlation in our study. In addition, we found that soil particles wrapped in the humified leaf litter formed on the soil surface (Figure A3). This might also account for the high proportions of litter-derived C and N in the surface soil. Zeller and Dambrine [76] found that ^{15}N released from decaying litter was recovered mainly in the coarse particulate organic matter fraction (>200 μm). 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 [7].

Although the mass loss of the PT leaf litter in this study was significantly higher than that of the RP leaf litter, the fractions of litter-derived N from the RP leaf litter were higher than those from PT leaf litter, indicating that leguminous leaf litter contributed more to soil N than non-leguminous litter did. This might be because the difference in mass loss between the two was only 7%, which was reduced when RP, with its high N content, entered the soil. This reflects RP's advantage of having a higher proportion of N in the leaf litter. After investigating 130 cases, Berg [77] 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 [78]. Therefore, due to their higher N content, leguminous leaf litters can boost soil N content faster than other plants [79].

We found that different precipitation gradients significantly affected the percentages of soil C and N from the 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 SOC. In addition, drought conditions promoted the transformation of legume litter N to soil total nitrogen (TN), but inhibited the transformation of non-legume litter N.

4.7. Linear Carbon and Nitrogen Relationship

The concentrations of C and N in the litter and soil, and the fractions of litter-derived C and N, had a positive correlation (Table 3). This does not support our first hypothesis that C and N would eventually decouple as decomposition progressed. Marichal et al. [80] also reported that soil and litter C and N were highly coupled in tropical forest plantations, and Fan et al. [81] reported that N and C released from *Maize* litter were coupled in the first year. These findings suggest that microbes preferentially decompose litter C and N simultaneously when the labile organic compounds are composed of both C and N during the early decomposition stage [82]. Compared to the control soil, the litter-treated soils experienced significantly reduced soil N intensity in response to C change, and that might, in turn, alter SOC dynamics by changing plant litter inputs and decomposition rates [66]. Our results also showed that the slopes of the linear relationships between C and N contents were highest under the highest precipitation treatment (Table 3). This shows that the response of N to C change becomes stronger as precipitation increases. The study of linear C and N relationship during litter decomposition provides an important basis for the modeling and prediction of the material cycles of ecosystems [83].

5. Conclusions

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 show that the dual-isotope tracing method can be used to study both C and N fluxes and how C and N pathways diverge during litter decomposition. While the δ^{15}N value of the leguminous leaf litter increased gradually, that of the non-leguminous leaf litter decreased. In the surface soil, the overall fraction of litter-derived N from the leguminous leaf litter was higher than that of the non-leguminous leaf litter, 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 SOC. Additionally, drought conditions promoted the transformation of legume litter N to soil TN, but inhibited the transformation of non-legume litter N. The C and N concentrations of both litters and soils always has a positive correlation during decomposition, and their entering the soil did not change that relationship. Additionally, the N responses to C changes in the soil were reduced by the litter cover. However, increased precipitation enhanced the positive relationship between C and N in the litters. These results have implications for how C and N fluxes respond to precipitation changes, thus providing new insights for further research on litter decomposition and its ecological significance under various global climate change scenarios.

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Appendix A

Figure A1. Experimental setups and PVC decomposition tubes used in this study. (**a**) A technician embeds probes that use time-domain reflectometry to measure soil moisture and temperature. (**c**) Screened, native soil beds under shade and with sunken experimental tubes. (**b**,**d**) Sunken tubes containing (**b**) uncovered leaf litter and (**d**) leaf litter covered by nylon netting.

Figure A2. Sample sprinkler head used in the artificially simulated rainfall system in this study. Each sprinkler’s diameter is 1.4 mm and each rotates 360° with an injection diameter of 1.1 m. This setup allows uniform watering over all experimental tubes.

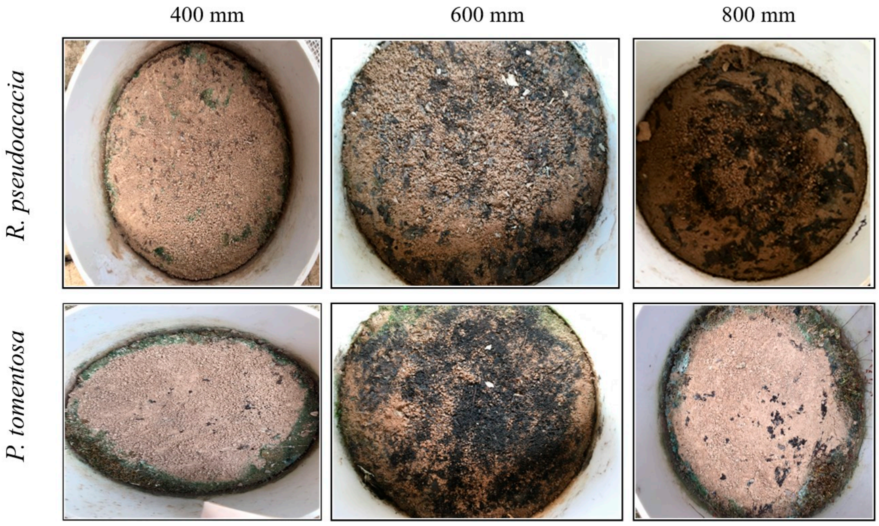

Figure A3. Experimental settlements and decomposition container used in this study.

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