Litter quality mediated the effect of nitrogen addition and precipitation reduction on the release and immobilization of plant litter nitrogen and phosphorus

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ARTICLE

Litter quality mediated the effect of nitrogen addition and precipitation reduction on the release and immobilization of plant litter nitrogen and phosphorus

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Abstract: A long-term field litterbag manipulation experiment was conducted to examine the effects of reduced precipitation (−30% of through-fall), nitrogen (N) addition (50 kg N·ha^{-1}·yr^{-1}), and their combination (−30% of through-fall and 50 kg N·ha^{-1}·yr^{-1}) on the release and immobilization of N and phosphorus (P) in four litter types (*Pinus koraiensis* (PK), *Tilia amurensis* (TA), *Quercus mongolica* (QM), and their mixture (MIX)). The results showed that N addition did not significantly stimulate litter decomposition, whereas precipitation reduction and the interaction significantly inhibited litter decomposition. N immobilization was significantly enhanced by N addition and reduced precipitation in the PK, QM, and MIX litters but was significantly inhibited in the TA litter. N addition, reduced precipitation, and their combination significantly increased the final P concentration of the litter in each sampling period. Furthermore, interestingly, there was a significant exponential correlation between the remaining N and final P concentration in the PK litter and a significant linear correlation for the QM and MIX litters, but no significant correlation for the TA litter, indicating that the dynamic relationship between the remaining N and final P concentration in the litter depended on the litter type. These results suggest that the forest litter layer may alleviate the effects of N deposition by increasing litter N immobilization and aggravating soil P limitation by inhibiting litter P release following N deposition. Reduced precipitation may further affect biogeochemical cycles by inhibiting the release of litter N and P.

Key words: nitrogen addition, reduced precipitation, litter decomposition, N immobilization, P release.

Résumé : Les auteurs ont réalisé une expérience sur le terrain de longue haleine avec des sacs à litière dans l'espoir de préciser les conséquences d'une réduction des précipitations (−30 % en automne), de l'addition d'azote (N) (50 kg par hectare annuellement) et de la combinaison de ces deux paramètres sur la libération et l'immobilisation du N et du phosphore (P) dans quatre sortes de litière [*Pinus koraiensis* (PK), *Tilia amurensis* (TA), *Quercus mongolica* (QM) et leur mélange (MIX)]. Selon les résultats, l'addition de N ne stimule pas de façon significative la décomposition de la litière, mais celle-ci est inhibée par la baisse des précipitations et la combinaison des deux facteurs. L'ajout de N et la diminution des précipitations accentuent sensiblement l'immobilisation du N dans les litières PK, QM et MIX, alors qu'elles l'inhibent nettement dans la litière TA. L'ajout de N, la réduction des précipitations et leur combinaison augmentent de façon sensible la concentration finale de P dans la litière, à chaque période d'échantillonnage. Par ailleurs, il faut mentionner l'existence d'une importante corrélation exponentielle entre le N résiduel et la concentration finale de P dans la litière PK, de même qu'une importante corrélation linéaire entre les litières QM et MIX et l'absence de corrélation significative avec la litière TA, signe que les liens dynamiques entre la concentration résiduelle de N et la concentration finale de P dans la litière dépend de la nature de cette dernière. Les résultats de l'expérience laissent croire que la litière forestière pourrait atténuer les conséquences d'une accumulation de N en raison d'une meilleure immobilisation de cet élément dans la litière et aggraver la carence en P dans le sol en empêchant la litière de libérer cet élément après dépôt du N. La baisse des précipitations pourrait également modifier le cycle biogéochimique en bloquant le N et le P dans la litière. [Traduit par la Rédaction]

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Mots-clés : addition d'azote, baisse des précipitations, décomposition de la litière, immobilisation du N, libération du P.

Introduction

Nitrogen (N) and phosphorus (P) limitation constrains ecosystem function in response to elevated N deposition and climate change (Du et al. 2020). Litter decomposition is a significant aspect of global terrestrial ecosystem N and P cycling, providing available N and P and other elements required for plant growth (Zheng et al. 2017; Chen et al. 2019). At regional scales, the rate of litter decomposition is primarily governed by local environmental conditions (e.g., precipitation pattern and soil properties), litter quality (e.g., element concentration of litter), and decomposer activity and community composition (e.g., soil fungal composition and biomass) (Garcia-Palacios et al. 2016; Zhou et al. 2018). However, any changes in factors associated with litter decomposition rates may influence ecosystem N- and P-cycling dynamics, with potential consequences for key ecosystem attributes (e.g., plant productivity) (Cornwell et al. 2008). Any factors affect the rates of litter decomposition and the pattern of litter N and P release via direct and indirect effects on factors associated with litter decomposition (Zheng et al. 2017; Zhang et al. 2018; Bernard et al. 2019).

As one of the critical components of global change, changes in both global- and regional-scale precipitation patterns may alter litter decomposition and N- and P-cycling dynamics directly through changes in soil moisture, which affect soil nutrient mobility and decomposer community composition, and indirectly through changes in plant growth and litter quality (Sanaullah et al. 2012; IPCC 2013; Zhou et al. 2018). Previous studies have shown that reduced precipitation might inhibit litter decomposition by limiting soil water availability, substrate diffusion, and decomposer communities (Sanaullah et al. 2012; Xu et al. 2012; Zhou et al. 2018). Moreover, reduced precipitation can decrease litter quality, thereby decreasing litter decomposition (Rennenberg et al. 2009). However, reduced precipitation might also increase litter quality by decreasing the ratios of carbon to N (C:N) which increases litter decomposition (Walter et al. 2013). These inconsistent results might be due to the difference in precipitation treatment intensity, background precipitation amount, and study species (Sanaullah et al. 2012; Walter et al. 2013; Zhou et al. 2018). Overall, the effects of reduced precipitation on litter quality could lead to alterations of the dynamics on litter N and P immobilization or release (Sanaullah et al. 2012). At this stage, although a few studies have studied the effects of reduced precipitation on litter decomposition (Sanaullah et al. 2012; Zheng et al. 2017; Zhou et al. 2018), the response of the pattern of litter N and P immobilization or release to reduced precipitation is still unclear.

As another widespread global change factor, increasing atmospheric N deposition could directly affect litter decomposition and N- and P -cycling dynamics by raising soil N availability and indirectly altering soil decomposer communities and litter quality (Knorr et al. 2005; Zhang et al. 2018). The initial ratio of C:N is lower in microbes (7:1 on average) than that in litter (17:1 on average) (Xu et al. 2013). Because the stoichiometric balance must be maintained between litter and its decomposers (Keiblinger et al. 2010), N addition might contribute to N and P immobilization in decomposing litter by enhancing the N availability of litter and the soil layer (Parton et al. 2007; Zheng et al. 2017). Previous studies have generally shown that the decomposition rate of high-quality litter is stimulated by N addition, but that N addition limits the decomposition of low-quality litter (Knorr et al. 2005; Berg and McClaugherty 2008). The release or immobilization of litter N and P is controlled by litter decomposition (Zhu et al. 2016; Zheng et al. 2017). Thus, due to differences in litter quality, N addition might also have multiple effects on litter N and P dynamics (Knorr et al. 2005; Hobbie et al. 2012; Zhang et al. 2018; Chen et al. 2019). It is unclear how the pattern of litter N and P immobilization or release is regulated by litter quality and exogenous N supplies (Chen et al. 2019).

Furthermore, changes in soil water are always coupled with changes in soil N availability (Zhou et al. 2016). Changes in precipitation patterns might modulate the litter decomposition response to increased N deposition (Schuster 2016; Zheng et al. 2017). Under reduced precipitation conditions, soil N mobility is reduced, and plant access to N is inhibited (Everard et al. 2010). Thus, reduced precipitation could alleviate the effect of increased N deposition on litter decomposition. Although reduced precipitation is potentially crucial in regulating the impact of elevated N deposition on litter decomposition, the combined influence of reduced precipitation and N addition on the patterns of litter N and P immobilization or release remains mostly unknown.

This study aims to investigate the effects of reduced precipitation, simulated increased N deposition, and a combination of these factors on litter mass loss and the pattern of litter N and P immobilization or release. A field manipulation experiment was conducted with N addition (50 kg N·ha^{-1}·yr^{-1}), precipitation exclusion treatments (−30% of through-fall), and a combination of these factors (−30% of through-fall and 50 kg N·ha^{-1}·yr^{-1}). Litter quality might modulate the effects of reduced precipitation, N addition, and their combination. Thus, litters of three dominant species *Quercus mongolica* (QM), *Tilia amurensis* (TA), and *Pinus koraiensis* (PK) and a mixture of three litter types (MIX) were collected. According to the previous findings reported above (Sanaullah et al. 2012; Zheng et al. 2017; Zhang et al. 2018; Bernard et al. 2019), we hypothesized that (*i*) litter quality could modulate the responses of litter N and P dynamics to N addition and reduced precipitation; (*ii*) reduced precipitation would alleviate the effect of N addition on litter mass loss and the pattern of litter N and P immobilization due to limited available N diffusion.

Materials and Methods

Study site

The study sites are located in the Changbai Mountains Natural Research Station Forest Ecosystem (42°24′N, 128°06′E) in Jilin Province, northeastern China, and belong to a temperate-continental monsoon climate. The average height above sea level is 758 m in the experimental fields. The average annual temperature is 3.6 °C with an average nongrowing season (from November to April) temperature of −0.6 °C and an average growing season temperature of 15.0 °C (from May to October, Yan et al. 2019*a*). The average annual precipitation is approximately 750 mm, primarily occurring from June to August (Yan et al. 2019*b*). According to the FAO classification and USDA Soil Taxonomy, the study area's soil is classified as Eutric Cambisol and Inceptisols. The vegetation type of the experimental site is an old broad-leaved Korean pine mixed forest with an age exceeding 200 years. *Pinus koraiensis*, TA, and QM are the three dominant tree species, with shrub species dominated by *Euonymus alatus*, *Corylus mandshurica*, *Philadelphus schrenkii*, and *Lonicera japonica*, herbaceous species dominated by *Anemone cathayensis*, *Filipendula palmate*, *Fumaria officinalis*, *Brachybotrys paridiformis*, *Anemone raddeana*, and *Cyperus microiria*. The tree density was 432 trees·hm^{-2}. Detailed information on the study site is shown in Supplementary Table S1[1].

Experimental design

In this study, six 50 m × 50 m plots were established randomly with a >20 m wide buffer strip between every two plots in May 2009. Three plots were treated with a 30% reduction in natural through-fall, and the other three plots received ambient treatments. A previous study found that mean annual precipitation has decreased in northern China over the last 50 yr (Wang et al. 2006). The reduced precipitation treatment was based on the precipitation recorded in drought years (e.g., 1985, 1997, 1999, 2001, and 2003), which was approximately 30% less than the long-term mean annual precipitation of 750 mm at the study site (Chinese Ecosystem Research Net). The details of this experimental design have been described in our previous publication (Supplementary Fig. S1[1]; Zheng et al. 2017; Yan et al. 2019*a*, 2019*b*; Yan et al. 2020*a*, 2020*b*). The through-fall interception facility was installed using V-shaped polycarbonate panels with high light transmittance (transparency = 95%). The V-shaped panels were fixed on an aluminum frame and covered 30% of the plot area to remove approximately 30% of the natural through-fall in a growing season. They were removed in winter to avoid the effects of uneven snow distribution on the soil system. To understand the impact of N addition, each plot was separated into two 25 m × 50 m subplots. One of the subgroups of each plot received exogenous N supply, and the other did not. Ammonium nitrate (NH_4NO_3) was applied as a form of exogenous N addition in this study. The N addition level was 50 kg N·ha^{-1}·yr^{-1}, which was approximately two times the historic N deposition rate in the study area (Lü and Tian 2007). In each N treatment subplot, NH_4NO_3 was weighed (2.38 g of NH_4NO_3·m^{-2} each time), mixed with 40 L of deionized water, and then sprayed monthly onto the forest floor of each plot using a backpack sprayer during the growing season (from May to October, six times total in each year), beginning in May 2009. In the subplots that did not receive additional N, 40 L of deionized water was applied to prevent a difference in moisture application difference. All the treatments started in May 2009 and are ongoing.

Litter decomposition experiment

In this study, senescent litter leaves from the three dominant species (PK, TA, and QM) were collected using nylon mesh litter traps in each subplot during the growing season of 2016 (from May to October). Six litter traps (1 m × 1 m) were installed in each subplot. All the leaves were oven-dried at 65 °C. The litter leaves of an individual species from the same treatment were mixed to obtain a uniform mixture before being placed in 20 cm × 20 cm (1 mm mesh) nylon litterbags. The mass proportion of PK, TA, and QM litter in each litter trap was approximately 4:3:3. Thus, four litter types (PK, TA, QM, and MIX (40% PK, 30% TA, 30% QM)) were used in this study. Five grams of dried litter from each litter type was placed in a nylon litterbag. A total of 720 litterbags (180 litterbags for each litter type, 3 replications × 5 sampling time × 12 subplots) were prepared at the beginning of the field litter decomposition study. In October 2017, 15 litterbags of each litter type were randomly placed on each the surface of subplot's forest floor and marked with red nylon ropes. The random placement point was determined by random values between 0 and 20 and between 0 and 50 generated by Excel (Microsoft Excel, 2016; Softonic International Ed. Media TIC). The litterbags were placed near the randomly generated points, with 30% of the surface of each litterbag under the precipitation interceptor panels.

[1]Supplementary data are available with the article at https://doi.org/10.1139/cjss-2021-0068.

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Three litterbags of each litter type were harvested from each subplot (12 litterbags for each subplot) after 112, 161, 364, 487, and 536 d. During the experimental periods, each litterbag received 0.2 g of N after six N addition treatments. After collection, all the litterbags were transported to the laboratory for analysis. In the laboratory, each litterbag's remaining litter was cleaned to remove soil particles, ingrown roots, and other material and then oven-dried at 65 °C to a constant mass and weighed. The harvested litter samples and each species' initial litter from each subplot were ground using a ball mill (Retsch MM 400; Retsch, Haan, Germany) for chemical analysis. The total C and N concentrations of litter were measured using an N/C analyzer (MultiN/C 2100; Analytik Jena AG, Jena, Germany). The P concentration of the litter was determined using an AA3 Continuous Flow Analyzer (Seal Analytical, Norderstedt, Germany).

Soil samples were collected in July 2018. Six soil cores (diameter = 2 cm) were taken from the top layer (0–10 cm of mineral layer) of each subplot by using a handheld auger, and then the all soil cores from the same subplot were pooled into one composite sample. All the soil samples were immediately brought to the laboratory and sieved to 2 mm. Soil NH_4^+ and NO_3^- were extracted from 10 g of fresh soil using 0.05 $mol \cdot L^{-1}$ K_2SO_4 extraction procedures and determined by using an autoanalyzer (AA3; Bran-Luebbe, Hamburg, Germany). The soil total N (TN) was determined by using a MultiN/C 3100 analyzer (Analytik Jena AG). The soil pH was determined with a pH meter (Sartorius PB-10, Gottingen, Germany).

Statistical analyses

The percentages of mass remaining ($(M_t/M_0) \times 100\%$, where M_t is the mass remaining at time t and M_0 is the original mass) or N or P remaining ($[(C_t \times M_t)/(C_0 \times M_0)] \times 100\%$, where C_t is the N:P concentration at time t, M_t is the mass remaining at time t, C_0 is the original N concentration, and M_0 is the original mass) was calculated as the amount of litter mass or N or P lost as a percentage of the total initial litter mass or N:P concentration. The Olson model calculated the decomposition rate coefficient (k):

$$X/X_0 = e^{-kt}$$

where X/X_0 is the fraction of mass remaining at time t, X is the final mass remaining at time t, X_0 is the initial mass, and t is the harvested time.

A general linear mixed model (GLMM) for a split-plot with Tukey's honestly significant difference test was used to investigate the effects of litter type, sampling time, N addition, reduced precipitation, and the combined effect of N addition and reduced precipitation on litter mass loss, remaining N, and remaining P. The litter types, sampling time, N addition, reduced precipitation, and combination of N addition and reduced precipitation were fixed factors, and the plots were random effects. Regression analysis was performed to explore the relationship between the remaining N and remaining P and the expected remaining mass and the observed remaining mass. All statistical analyses were performed using R software version 3.2.2 (R Development Core Team), and the graphs were plotted with SigmaPlot 12.5 software (Systat Software Inc., Chicago, IL, USA).

Results

Initial litter quality

The climate conditions during the experimental periods are shown in Table 1. The initial total C concentrations and the C:N ratio were the highest in the PK litter, followed by the QM and MIX litters, and lowest in the TA litter (Table 2). The initial total N and P concentrations were the highest in the TA litter, which also had the highest N:P ratio, followed by the QM and MIX litters, and lowest in the PK litter (Table 2). N addition, reduced precipitation, and their combined effect significantly decreased the initial C concentration of the TA litter compared with that of the control. However, there was no significant difference in the N concentrations, initial P concentrations, the C:N or N:P ratio in the initial litter among the different treatments ($p > 0.05$). The soil property data are shown in Supplementary Table S2[1], suggesting that reduced precipitation increased the inorganic NH_4^+ concentration and the pH value, while N addition decreased the pH value.

Effects of N addition, reduced precipitation, and their combination on litter mass loss

The litter mass decreased by 35–65% during the study period (Fig. 1). The mass loss patterns of all the litter types showed rapid mass loss during the growing season (from May to October) and slowed mass loss during the nongrowing season (from November to April) (Fig. 1). Moreover, the mass loss also varied significantly by litter type (Table 3), and the mass loss was considerably faster for the TA and QM litter than that for the PK litter during the study period ($p < 0.05$; Fig. 1). At the end of the study period, the mass loss of all the litter types decreased significantly with reduced precipitation and the combined effect of N addition and reduced precipitation ($p < 0.05$) but was not significantly affected by N addition alone ($p > 0.05$) (Fig. 1, Table 3). The interactions between N addition and reduced precipitation, litter types and sampling time, reduced precipitation and litter types, and reduced precipitation and sampling time also had significant effects on litter mass loss ($p < 0.05$, Table 3).

According to the single exponential model, the decomposition coefficients (k) for all the litter types were significantly lower in the treatments with reduced precipitation and the combination effect than that in

Table 1. Climate factors during the experimental year of 2016/2017, 2017/2018, and 2018/2019.

Environmental parameters	2017/2018 Nongrowing season	2018 Growing season	2018/2019 Nongrowing season	2019 Growing season
Lowest air temperature (°C)	−34.2	NA	−30.6	NA
Days with the highest temperature below 0 °C	115	NA	93	NA
Precipitation (mm)	NA	740.9	NA	769.3
Max-snow thickness (cm)	41.3	NA	7.1	NA
Snow cover days	137	NA	78	NA
Mean soil temperature (°C)	−2.63	12.49	−4.12	13.53
Mean air temperature (°C)	−7.11	15.62	−4.65	15.49

Note: NA means no data. In this study, "Nongrowing season" was defined as from November to April, and "Growing season" was defined as from May to October.

Table 2. Initial chemical characteristics (mean values ± SE) of litter ($n = 3$).

Treatments	Litter types	Total C (%)	Total N (%)	Total P (%)	C:N	N:P
Control	PK	58.41 ± 1.84aA	0.14 ± 0.01cA	0.14 ± 0.01cA	455.38 ± 79.70aA	1.01 ± 0.25cA
	TA	54.84 ± 0.76bA	1.01 ± 0.08aA	0.26 ± 0.03aA	54.81 ± 5.14cA	4.07 ± 0.23aA
	QM	55.97 ± 1.53bA	0.51 ± 0.06bA	0.20 ± 0.01bA	114.14 ± 14.99bA	2.69 ± 0.50bA
	MIX	56.04 ± 1.28bA	0.51 ± 0.03bA	0.20 ± 0.03bA	109.21 ± 8.76bA	2.75 ± 0.34bA
+N	PK	56.07 ± 1.11aA	0.14 ± 0.03cA	0.14 ± 0.01cA	430.09 ± 73.46aA	1.02 ± 0.15cA
	TA	52.52 ± 0.26cB	1.02 ± 0.08aA	0.27 ± 0.03aA	52.35 ± 4.02cA	4.06 ± 0.33aA
	QM	54.97 ± 0.20bA	0.52 ± 0.02bA	0.21 ± 0.06bA	111.80 ± 13.54bA	2.68 ± 0.45bA
	MIX	54.38 ± 0.26bB	0.53 ± 0.04bA	0.20 ± 0.03bA	105.75 ± 6.80bA	2.77 ± 0.32bA
−W	PK	56.74 ± 0.61aA	0.14 ± 0.02cA	0.14 ± 0.01cA	433.48 ± 70.10aA	1.01 ± 0.25cA
	TA	52.51 ± 0.27cB	1.01 ± 0.08aA	0.25 ± 0.03aA	52.35 ± 4.03cA	4.10 ± 0.33aA
	QM	54.63 ± 0.78bA	0.51 ± 0.06bA	0.20 ± 0.02bA	111.53 ± 15.16bA	2.70 ± 0.48bA
	MIX	53.38 ± 0.26cC	0.52 ± 0.04bA	0.20 ± 0.03bA	103.81 ± 6.67bA	2.76 ± 0.24bA
+N−W	PK	57.74 ± 1.16aA	0.14 ± 0.03cA	0.14 ± 0.01cA	439.96 ± 68.73aA	1.02 ± 0.15cA
	TA	52.84 ± 0.40cB	1.01 ± 0.08aA	0.25 ± 0.03aA	52.65 ± 3.84cA	4.08 ± 0.20aA
	QM	54.63 ± 0.78bA	0.51 ± 0.06bA	0.20 ± 0.02bA	111.53 ± 15.16bA	2.71 ± 0.40bA
	MIX	53.71 ± 0.34bC	0.52 ± 0.04bA	0.20 ± 0.03bA	104.37 ± 6.11bA	2.77 ± 0.24bA

Note: +N, N addition; −W, reduced precipitation; PK, *Pinus koraiensis*; QM, *Quercus mongolica*; TA, *Tilia amurensis*; MIX, 40% PK + 30% TA + 30% QM. Different lowercase letters under same treatment indicate significant difference among litter types, and different capital letters under same litter type indicate significant difference among different treatments ($p < 0.05$).

the control treatment (Table 4, Fig. 1). However, N addition did not have a significant impact on the k value under ambient conditions (i.e., no reduced precipitation) (Table 4).

Effects of N addition, reduced precipitation, and their combination on the litter remaining N

The effect intensity and directions of N addition, reduced precipitation, and their combination on the remaining N in the litter depended significantly on the litter type and sampling time during the study period (Fig. 2). N addition and reduced precipitation significantly increased the remaining N (N immobilization) in the PK, QM, and MIX litters during the growing season. Nevertheless, there is an obvious opposite effect (N loss) on the TA litter ($p < 0.05$; Fig. 2). Moreover, the combined effect of N addition and reduced precipitation significantly increased the remaining N in the PK, QM, and MIX litters during the whole study period. Nevertheless, there was a significant positive effect on the TA litter during only the early stages of the decomposition process (the first six months) ($p < 0.05$; Fig. 2). Furthermore, N addition, reduced precipitation, litter type, sampling time, and their two-way interactions significantly affected the remaining litter N (Table 4).

Effects of N addition, reduced precipitation, and their combination on the litter remaining P

The effect patterns of all the treatments on the remaining litter P also strongly depended on litter types

Fig. 1. Changes in the mass loss in decomposing litter for *Pinus koraiensis* (PK), *Quercus mongolica* (QM), *Tilia amurensis* (TA), and a mixture (MIX = 40% PK + 30% TA + 30% QM) with different treatments (+N, N addition; −W, precipitation reduction; −W+N, precipitation reduction with N addition). Fitted curves are derived from the single exponential model (Olson model). Vertical bars represent standard errors ($n = 3$). [Colour online.]

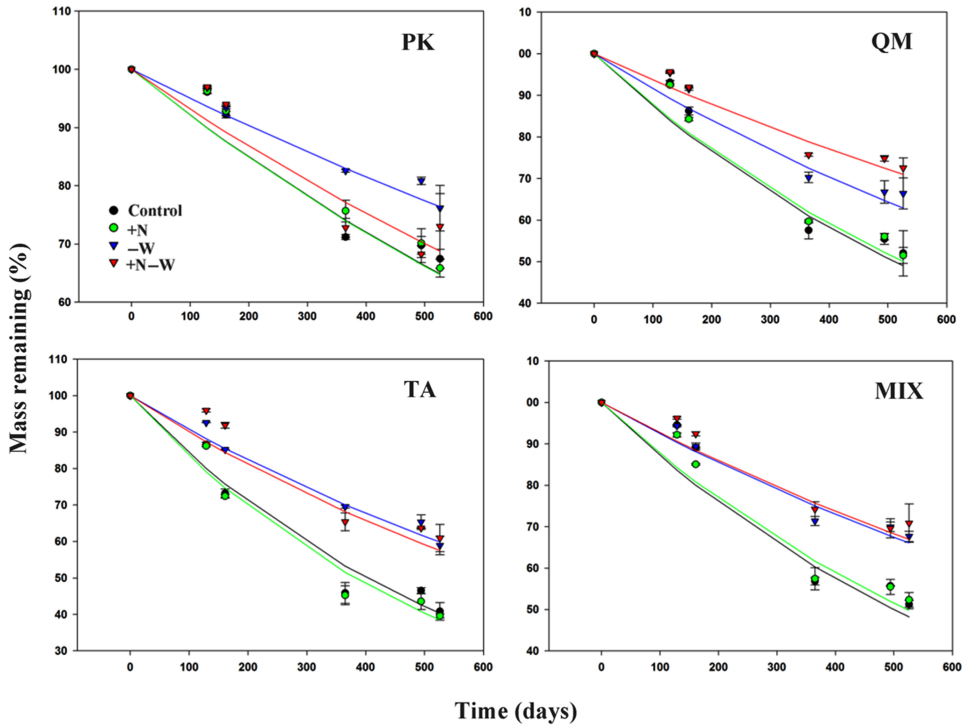


Table 3. Results of the mixed model analysis of the responses of mass remaining, N remaining, and P remaining to N addition, reduced precipitation, litter type, and time of sampling.

	Mass remaining		N remaining		P remaining	
Effects	df	Pr > F	df	Pr > F	df	Pr > F
Litter types	3	<0.001	3	<0.001	3	<0.001
Time	4	<0.001	4	<0.001	4	<0.001
+N	1	0.051	1	0.001	1	0.001
−W	1	<0.001	1	0.005	1	0.003
Litter types × Time	12	<0.001	12	<0.001	12	<0.001
Litter types × +N	3	0.523	3	<0.001	3	0.006
Litter types × −W	3	<0.001	3	0.017	3	0.105
Time × +N	4	0.935	4	0.025	4	<0.001
Time × −W	4	<0.001	4	0.020	4	0.018
+N × −W	1	0.014	1	0.013	1	0.003
Litter types × Time × +N	12	0.554	12	0.189	12	<0.001
Litter types × Time × −W	12	0.028	12	0.172	12	0.120
Litter types × +N × −W	3	0.254	3	0.338	3	0.934
Time × +N × −W	4	0.310	4	0.625	4	0.690
Litter types × Time × N × W	12	0.496	12	0.088	12	0.007

Note: +N, N addition; −W, reduced precipitation; Time, time of sampling; Litter, litter type; df, numerator degrees of freedom.

Table 4. Decomposition rates (day^{-1}) and the coefficient of determination of the regression (K, ($X/X_0 = e^{-kt}$) where X/X_0 is the fraction mass remaining at time *t*, *X* is the mass remaining at time *t*, X_0 is the initial mass, and *k* is the decomposition coefficient.

			Single exponential model	
Species	Precipitation	N	K	*p* value
PK	Ambient	Ambient	0.0008230a	<0.001
		Addition	0.0008264a	<0.001
	Reduction	Ambient	0.0005117c	<0.001
		Addition	0.0007713b	<0.001
TA	Ambient	Ambient	0.0017262b	<0.001
		Addition	0.0018161a	<0.001
	Reduction	Ambient	0.0009757c	<0.001
		Addition	0.001051c	<0.001
QM	Ambient	Ambient	0.0013546a	<0.001
		Addition	0.0013142a	<0.001
	Reduction	Ambient	0.0008813b	<0.001
		Addition	0.0006514c	<0.001
Mix	Ambient	Ambient	0.001386a	<0.001
		Addition	0.001325a	<0.001
	Reduction	Ambient	0.0007876b	<0.001
		Addition	0.0007627b	<0.001

Note: PK, *Pinus koraiensis*; TA, *Tilia amurensis*; QM, *Quercus mongolica*; MIX, 40% PK + 30% TA + 30% QM. Treatments with the same letter are not significantly different ($p > 0.05$).

Fig. 2. Effects of N addition and reduced precipitation on litter N remaining for *Pinus koraiensis* (PK), *Quercus mongolica* (QM), *Tilia amurensis* (TA), and a mixture (MIX = 40% PK + 30% TA + 30% QM) after decomposing for 526 d with different treatments (+N, N addition; −W, precipitation reduction; −W+N, precipitation reduction with N addition). Vertical bars represent standard errors ($n = 3$). Values greater than 100 indicate nutrient immobilization, and values less than 100 indicate net mineralization. [Colour online.]

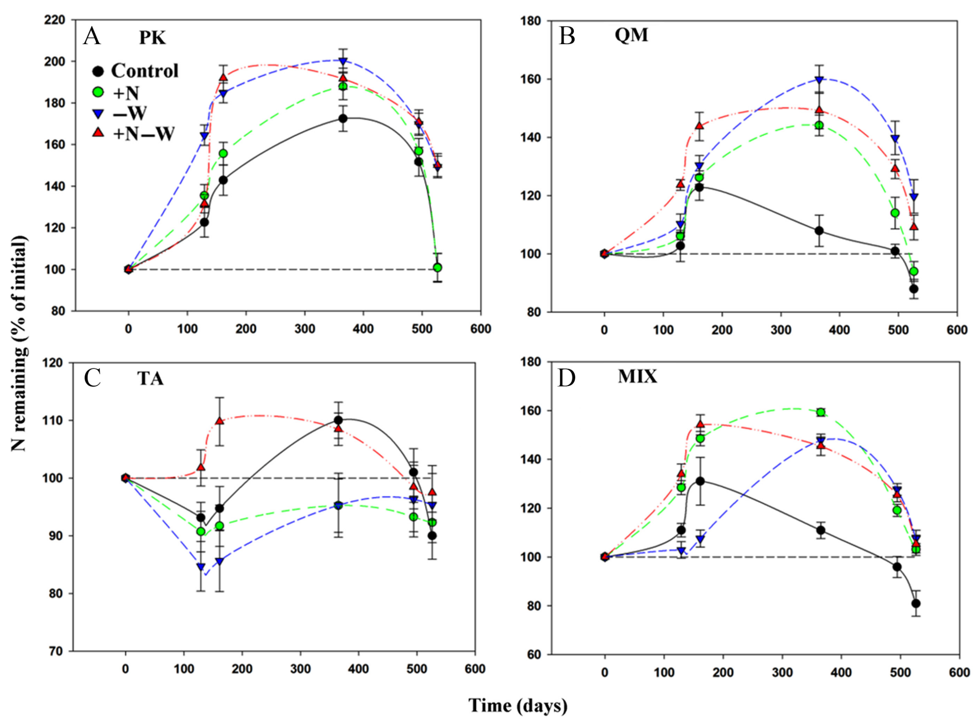
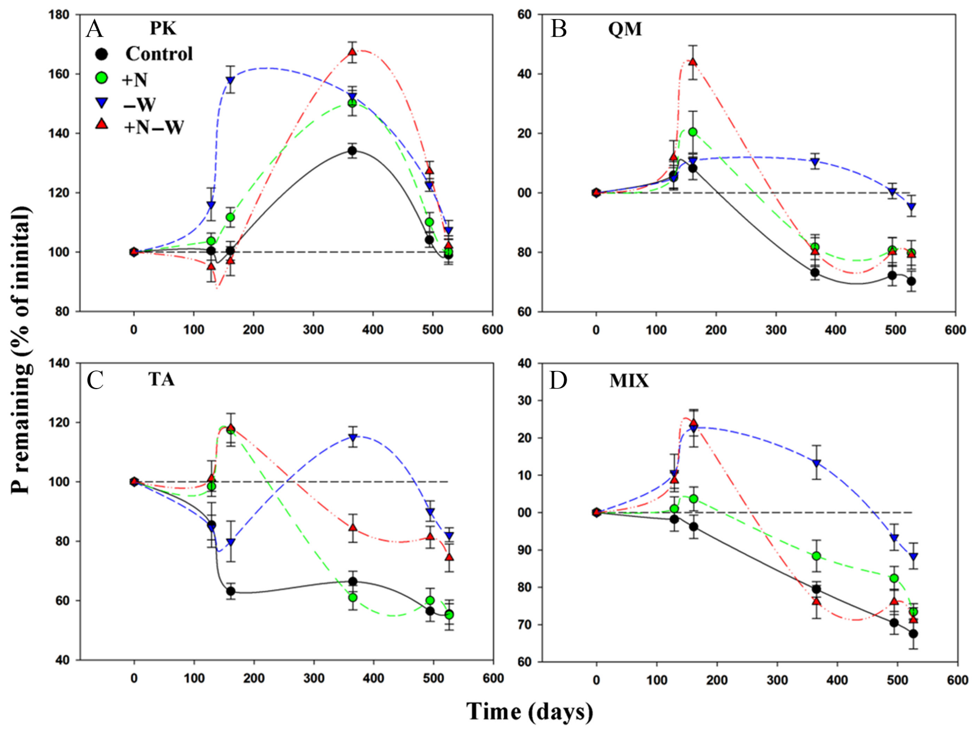


Fig. 3. Effects of N addition and reduced precipitation on litter P remaining for *Pinus koraiensis* (PK), *Quercus mongolica* (QM), *Tilia amurensis* (TA), and a mixture (MIX = 40% PK + 30% TA +30% QM) after decomposing for 526 d with different treatments (+N, N addition; −W, precipitation reduction; −W+N, precipitation reduction with N addition). Vertical bars represent standard errors ($n = 3$). Values greater than 100 indicate nutrient immobilization, and values less than 100 indicate net mineralization. [Colour online.]



and sampling time (Table 4, Fig. 3). For the PK litter, the N addition and reduced precipitation treatments increased litter P immobilization during the whole study period, while the positive impact occurred only after the first two sampling times under their combined influence (Fig. 3). For the QM and MIX litters, reduced precipitation promoted litter P immobilization. N addition and its effect combined with other factors increased litter P immobilization only during the first winter, while it decreased litter P loss during the late stages of the experiment (Fig. 3). Similarly, for the TA litter, N addition and its effect combined with other factors increased litter P immobilization during the early stages. However, reduced precipitation did not significantly affect litter P immobilization during the early stages but did positively affect litter P immobilization during the growing season.

The results of the regression analysis between remaining litter N and remaining P varied significantly across different litter types (Fig. 4). There was a significant exponential correlation between the remaining litter N and remaining P for the PK litter, and a significant linear relationship for the QM and MIX litter, but no significant relationship for the TA litter (Fig. 4).

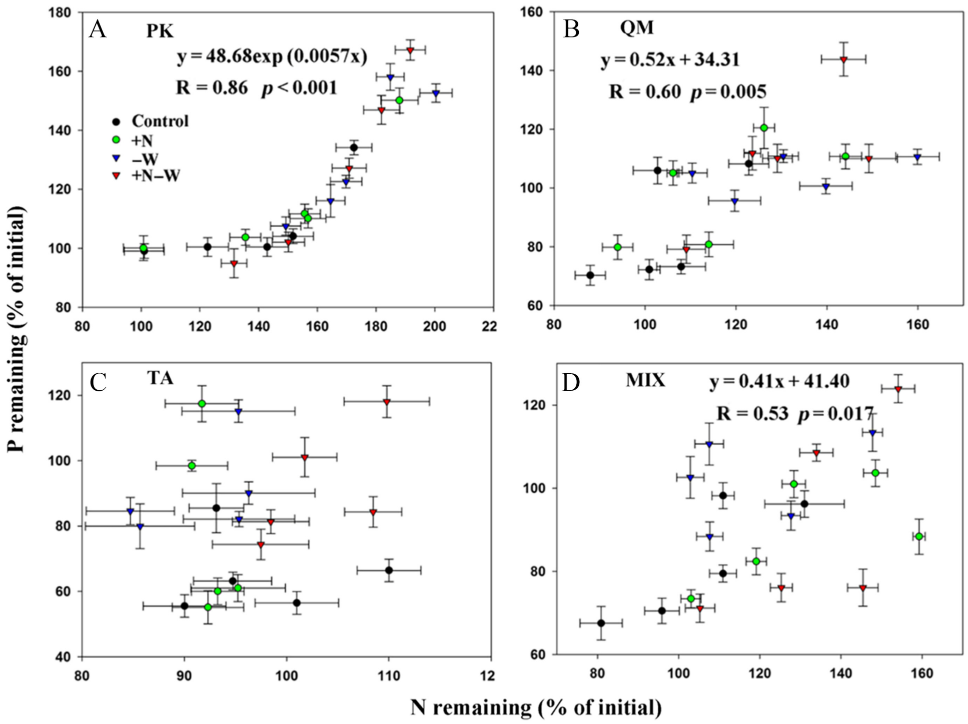
Discussion

Many previous studies have shown that the rate of litter decomposition is often correlated with the initial chemistry of litters such as litter N concentration and the C:N ratio (Hoorens et al. 2003; Zhang et al. 2016; Liu et al. 2017; Zhang et al. 2018). However, due to plant stoichiometric homeostasis and nutrient resorption, N addition or reduced precipitation did not alter total plant N uptake or litter N concentrations in our study site (Zhou et al. 2019; Yan et al. 2019*c*). Furthermore, trees in the studied forest were mature that have strong stability and resistance to external interference, which also might partly explain why there is no change in litter N concentrations. The initial litter N concentration and the ratios of C:N did not change significantly under N addition or reduced precipitation in this study, suggesting that the revised litter decomposition rates were not caused by the historical effects of N addition or reduced precipitation on initial litter chemical characteristics. Furthermore, for PK, QM, and MIX litters, N concentration increased until third sampling, indicating that N from exogenous sources can be immobilized in litter by microbial uptake or chemical immobilization (Hobbie 2008; Zheng et al. 2017; Chen et al. 2019).

The response of litter mass loss and N and P dynamics to N addition

Traditionally, the plant and microbe growth are generally N limited in temperate forests (LeBauer and Treseder 2008). Thus, N addition can improve litter

Fig. 4. Correlation analysis between N remaining and P remaining. The equation indicates the significant correlation between N remaining and P remaining. PK, *Pinus koraiensis*; QM, *Quercus mongolica*; TA, *Tilia amurensis*; MIX, a mixture (40% PK + 30% TA + 30% QM); +N, N addition; −W, precipitation reduction; −W+N, precipitation reduction with N addition. Vertical bars represent standard errors ($n = 3$). [Colour online.]



decomposition by releasing microbes from N limitation in a temperate forest (Li et al. 2017). However, much experimental evidence shows that this is often not the case; in contrast, N addition can hamper litter decomposition rates (Janssens et al. 2010; Buchkowski et al. 2015). Inorganic N addition-associated declines in pH and other nutrients (such as P or calcium) were shown to suppress litter decomposition rates by inhibiting microbial activity (Averill and Waring 2017). Our previous study and this study also confirmed that N addition decreased the soil pH value and phosphorus concentration (Chen et al. 2019). Across all the litter types, N addition did not significantly affect the litter mass remaining in the study period, which could be because of the decreased soil pH value and break nutrient balance, which was consistent with previous studies (Sinsabaugh and Follstad Shah 2012; Averill and Waring 2017).

The effects of N addition on remaining litter N and P were significant and varied with litter quality and the study period. Consistent with some previous studies (Zhang et al. 2018; Chen et al. 2019), in this study, N addition increased the immobilization of litter N and P in low-quality litter with a relatively high ratio of C to nutrients (such as the PK litter). It stimulated N release in the high-quality litter with a relatively low C:N or C:P ratio (such as the TA litter), indicating that the quality of the decomposing litter regulates the effects of N addition on the capacities of litter N immobilization. Moreover, N addition increased the N and P immobilization in early sampling in the study (such as the first three sampling) in relatively low-quality litter. The pattern of the N addition effect on litter N and P dynamics might be explained by the stoichiometric balance between the decomposers and litter. Because the ratio of C:N or C:P in microbes is significantly lower than that in low-quality litter, soil microbes have to take up extra nutrients (such as N or P) in the early litter decomposition from decomposing litter or soil to meet their nutrient demands in the process of litter decomposition (Aponte et al. 2012). Less N and P can be released during the decomposition process from low-quality litter (i.e., litter that has higher C:N and C:P ratio, such as the PK litter). Thus, N from exogenous sources can be immobilized in low-quality litter by microbial uptake or chemical immobilization (ammonia-N can react with byproducts of microbial breakdown and hummus) to achieve a balance of C:N and C:P ratios between microbes and litter (Hobbie and Vitousek 2000; Hobbie 2008; Zheng et al. 2017; Chen et al. 2019). This could explain the increase in N and P immobilization in low-quality litter under N addition in the early litter decomposition. However, high-quality litter (such as the TA litter) releases N faster than low-quality litter (Zheng et al. 2017). The amount of N or P released from the high-quality litter could meet or exceed microbial demands, which may partly explain why N addition does not increase N and P immobilization in high-quality litter. However, with the consumption of litter C, the C:N and (or) C:P ratios of litter would gradually decrease. In the late stage of litter decomposition, when the C:N and (or) C:P ratios of litter were similar to that of microorganisms, microorganisms in litter altered from fixed N:P to consumed N:P, resulting in the reduction of N:P fixation or the transformation from N fixation to release. In the later stage of the experiment, N addition resulted in relatively accelerated N and P release, which might be due to the early microbial colonization accelerating the decomposition of litter.

The response of litter mass loss and N and P dynamics to reduced precipitation

Reduced precipitation significantly decreased litter mass loss, which was consistent with some previous studies (Vogel et al. 2013; Santonja et al. 2015; Zheng et al. 2017). However, reduced precipitation did not alter the C:N ratios (as the most critical predictors of decomposition) of the initial litter, so this inhibition of reduced precipitation was not related to the initial litter quality. Our previous studies at this study site found that reduced precipitation significantly reduced soil moisture (Yan et al. 2019*a*, 2019*b*). First, reduced precipitation could decrease the leaching of soluble compounds, reducing the loss of litter mass (Santonja et al. 2015; Zhou et al. 2018). Moreover, because water shortages limit the diffusion of nutrients in soil pore space, reduced precipitation could inhibit litter mass loss by decreasing the activity and community composition of soil biota (including soil microbes and fauna) (Vogel et al. 2013; Bernard et al. 2019). These reasons might support our results.

Reduced precipitation increased litter N immobilization in the low-quality litter and litter N release in the high-quality litter, which was consistent with the effects of N addition. Previous studies have shown that fungi have high resistance to drought, which regulates organic matter decomposition under reduced precipitation conditions (Yuste et al. 2011; Haugwitz et al. 2016). Drought-adapted fungi might have maintained litter mass loss (Vogel et al. 2013). At the same time, fungal hyphae can transfer N from soil to litter due to the slow-release rate of N in low-quality litter (such as the PK litter), which would increase the N immobilization of low-quality litter. Moreover, the rate of N release from litter is controlled by the C:N ratio, which provides information on whether N will be immobilized or released (Zhou et al. 2018). In this study, the C:N ratio in the TA litter was approximately 50, suggesting that net N release would occur in the TA litter. The above might partly explain why reduced precipitation promoted N release from high-quality litter and N immobilization from low-quality litter. Reduced precipitation also increased litter P immobilization for relatively low-quality litter (except the TA litter) in the first two samples, which was in line with the studies of Zhou et al. (2018) and Zhong et al. (2017) showing significantly reduced P release in response to reduced precipitation. Under reduced precipitation, in the early sampling, litter P immobilization in relatively low-quality litter increased suggesting that fungal litter decomposers were limited by P availability and then translocated substantial amounts of P into the litter (Xu and Hirata 2005). Similarly, with the consumption of litter C, litter P changed from fixed to release in relatively low-quality litter. In any case, the release rate of P was lower than that of the control under reduced precipitation, which might also be due to the inhibition of nutrient flow and litter decomposition rate by reduced precipitation. Reduced precipitation facilitates a delay in P release, meaning that reduced precipitation might have aggravated soil P limitation for forests with relatively low-quality litter (Zhou et al. 2018). However, P dynamic of the high-quality litter (TA litter) was opposite to that of low-quality litter in the study, which was released first and then fixed under reduction precipitation. This might be because the P content of high-quality litter meets the microbial demand for early decomposition of litter. With the progress of litter decomposition, when phosphorus could not meet the microbial demand, phosphorus would be fixed again under reduction precipitation. In addition, reduced precipitation might mitigate the P element leaching from litter, leading to increase of P accumulation and P content in litter. However, because reduced precipitation inhibited litter decomposition, P immobilization and the P release delay could also be attributed to fungal assimilation and chemical immobilization.

Reduced precipitation regulates the effects of N addition on litter mass loss and N and P dynamics

Consistent with some previous studies (Zheng et al. 2017; Zhong et al. 2017; Chen et al. 2019), reduced precipitation altered the N addition effect on litter mass loss and N and P dynamics, but the response pattern depended on litter quality and sampling time. In this study, reduced precipitation strengthened the inhibition of N addition on the mass loss and N and P release of the PK and QM litters during the early stage of litter decomposition while changing the direction of the N addition effect on the TA litter. One potential reason for these results is that reduced precipitation inhibited the leaching of soluble compounds from the litter and microbial activity but increased N accumulation on the litter surface during the early decomposition stage, thereby inhibiting litter mass loss and increasing litter N immobilization under N addition. Additionally, reduced precipitation might mitigate the mismatch in stoichiometry between decomposing litters and microbial decomposers by decreasing element leaching from litter. More P would likely be immobilized in litters with a higher N:P ratio, leading to more P immobilization in litter under N addition. A previous study at this study site also found that the duration of this drought effect on N immobilization was extended by N addition, suggesting that reduced precipitation with N addition is likely to increase the sequestration of atmospheric N deposition (Zheng et al. 2017). However, the effects of reduced precipitation on litter N and P immobilization seemed to be weakened by N addition during the later decomposition stage. This could be because N addition harmed lignin degradation and increased the formation of recalcitrant substances (Berg and McClaugherty 2008), which might weaken the effect of reduced precipitation on litter N and P immobilization.

Furthermore, we found a significant exponential correlation between the remaining N and P for the PK litter, a meaningful linear relationship for the QM and MIX litter, but no significant TA litter association. The low N and P content in the low-quality litter and the imbalance between the litter and microbial N and P tend to cause litter N and P to be immobilized in the litter to a certain extent. Thus, the correlation between the remaining N and P increases with decreasing litter quality, while in high-quality litter, the litter N and P tend to be released, so there is no correlation between the remaining N and P. Overall, the potential stoichiometric mismatch between decomposing litter and litter decomposer demand could explain the dynamic relationship between the remaining N and P in different litter types during litter decomposition. Although our study had found some interesting results, we did not have a good understanding of the mechanism behind the phenomenon. Therefore, we still need to continue to study the mechanism of litter decomposition response to N deposition and precipitation pattern change in the future.

Conclusions

Our results indicate that N addition and reduced precipitation could enhance litter N and P immobilization or delay litter N and P release under natural conditions (for the MIX litter). For each litter type, litter quality affected the litter N and P dynamic response to N addition and reduced precipitation in decomposing litter across the whole study period. N addition and reduced precipitation generally increased N immobilization and delayed P release during the decomposition of low-quality litter (e.g., the PK litter); however, the opposite results were found for the decomposition of high-quality litter (e.g., the TA litter). These results suggested that lower-quality litter with higher C:N and C:P ratios tended to immobilize more exogenous N and P under N addition and reduced precipitation. Overall, the present study provides evidence that the direction and intensity of the effects of N addition and reduced precipitation on litter N and P dynamics can be primarily explained by litter quality and the stoichiometric balance between decomposers and litter.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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Authors' Contributions

QW and YX designed the study, awarded funding, supervised data collection, and edited manuscripts. QW, GY, YX, GL, and SH contributed the whole manuscript preparation and designed and wrote the main manuscript text. QW, GY, YX, GL, and SH prepared all the figures, QW, GY, GL, YX, and SH prepared field experiments, prepared tables, and collected literatures. All authors reviewed the manuscript.

Availability of Data and Material

Data are available from the corresponding author on reasonable request.

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