Litter decomposition and C and N dynamics as affected by N additions in a semi-arid temperate steppe, Inner Mongolia of China

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Abstract: Litter decomposition is the fundamental process in nutrient cycling and soil carbon (C) sequestration in terrestrial ecosystems. The global-wide increase in nitrogen (N) inputs is expected to alter litter decomposition and，ultimately, affect ecosystem C storage and nutrient status. Temperate grassland ecosystems in China are usually N-deficient and particularly sensitive to the changes in exogenous N additions. In this paper, we conducted a 1,200-day *in situ* experiment in a typical semi-arid temperate steppe in Inner Mongolia to investigate the litter decomposition as well as the dynamics of litter C and N concentrations under three N addition levels (low N with 50 kg N/(hm^2·a) (LN), medium N with 100 kg N/(hm^2·a) (MN), and high N with 200 kg N/(hm^2·a) (HN)) and three N addition forms (ammonium-N-based with 100 kg N/(hm^2·a) as ammonium sulfate (AS), nitrate-N-based with 100 kg N/(hm^2·a) as sodium nitrate (SN), and mixed-N-based with 100 kg N/(hm^2·a) as calcium ammonium nitrate (CAN)) compared to control with no N addition (CK). The results indicated that the litter mass remaining in all N treatments exhibited a similar decomposition pattern: fast decomposition within the initial 120 days, followed by a relatively slow decomposition in the remaining observation period (120–1,200 days). The decomposition pattern in each treatment was fitted well in two split-phase models, namely, a single exponential decay model in phase I (<398 days) and a linear decay function in phase II (≥398 days). The three N addition levels exerted insignificant effects on litter decomposition in the early stages (<398 days, phase I; *P*>0.05). However, MN and HN treatments inhibited litter mass loss after 398 and 746 days, respectively (*P*<0.05). AS and SN treatments exerted similar effects on litter mass remaining during the entire decomposition period (*P*>0.05). The effects of these two N addition forms differed greatly from those of CAN after 746 and 1,053 days, respectively (*P*<0.05). During the decomposition period, N concentrations in the decomposing litter increased whereas C concentrations decreased, which also led to an exponential decrease in litter C:N ratios in all treatments. No significant effects were induced by N addition levels and forms on litter C and N concentrations (*P*>0.05). Our results indicated that exogenous N additions could exhibit neutral or inhibitory effects on litter decomposition, and the inhibitory effects of N additions on litter decomposition in the final decay stages are not caused by the changes in the chemical qualities of the litter, such as endogenous N and C concentrations. These results will provide an important data basis for the simulation and prediction of C cycle processes in future N-deposition scenarios.

Keywords: litter decomposition; N deposition; N level; N form; C sequestration; semi-arid temperate grassland

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Litter decomposition is a fundamental ecological process in the control of nutrient cycling in most terrestrial ecosystems. This process provides soil nutrients for plant growth and influences terrestrial net primary production (Moore et al., 2004; Almagro and Martínez-Mena, 2012). Litter decomposition also regulates the buildup of soil organic matter (Jiang et al., 2013), which influences the fluxes of carbon (C) from soil (Wardle et al., 2003; Prescott, 2005). C storage in terrestrial ecosystems depends on the balance between the gain from net primary production and the loss through decomposition (Couteaux et al., 1995; Amundson, 2001; Mehta et al., 2013). This suggests that litter decomposition exhibits a critical function in C budget in terrestrial ecosystems (Berg and McClaugherty, 2008).

In recent years, exogenous nitrogen (N) additions into terrestrial ecosystems have increased sharply due to human activities, such as fertilizer use, fossil fuel combustion and intensive husbandry (Aerts et al., 2006; Gruber and Galloway, 2008; Liu et al., 2013). These activities not only elevate the amounts of N input into terrestrial ecosystems but also bring different forms of N (i.e. NO_y and NH_x) into the environments (Cleland and Harpole, 2010). China is one of the three regions with the highest N deposition rate in the world (Dentener et al., 2006; Zhang et al., 2006), and therein, the regional differences in the amounts and forms of N deposition can cause a suite of ecological changes including litter decomposition and, ultimately, affect C storage and N transfer in a specific ecosystem (Mo et al., 2006; Liu et al., 2010a, b; Peng et al., 2011a).

Grassland is one of the most widespread ecosystem types in China, occupying about 40% of the total land area of the country (Sun, 2005; Kang et al., 2007). Approximately 78% of grasslands in China are located in the northern arid and semi-arid temperate areas (Chen and Wang, 2000). However, due to severe climate conditions and overgrazing, most grasslands in these areas suffer from desertification or degradation, and are in N-deficient status (Hooper and Johnson, 1999; Cao et al., 2004; Zhang and Han, 2008). The N-deficient grassland ecosystem in China is particularly sensitive to increases in anthropogenic N inputs (Zhang and Han, 2008; Peng et al., 2011a, b).

Many previous N addition experiments have shown that increased N availability has consistent, positive effect on net primary production in a N-limited temperate grassland ecosystem (i.e. LeBauerand and Treseder, 2008), but studies regarding the effect of N additions (including different N addition levels or forms) on the process of litter decomposition were still poorly documented in grassland ecosystems. Some existing studies on grassland ecosystems have focused on short-term N addition levels in manipulative field experiments or laboratory simulation experiments (Liu et al., 2010b; Li et al., 2011); however, these studies disregarded the relatively long-term relationships between litter decomposition and *in situ* conditions of multiple N addition levels as well as N addition forms. The scarcity of studies on this subject limits the precise prediction of soil C sequestration in a specific grassland ecosystem with increased N deposition. It will also lead to poor understanding of the nutrient cycling in grassland ecosystems, given that N immobilization or release in litter is critical for understanding the patterns of N cycling in these ecosystems.

To elucidate the C cycles and nutrient transfers in N-deficient, temperate grasslands in China, more studies (especially long-term experimental researches) should be conducted on litter decomposition under different exogenous N addition treatments in site-specific grassland ecosystems. The present study selected *Leymus chinensis* in Inner Mongolia of China and investigated its litter decomposition under field conditions with different N availabilities and N-form inputs in 1,200 days by the litter bag method, and aimed to examine the changes in natural litter mass loss and the dynamics of litter C and N concentrations under different N addition levels and forms in a typical semi-arid temperate steppe ecosystem.

1   Materials and methods

1.1   Site description

The field study was conducted in a typical *L. chinensis* steppe (43°33′10″N, 116°40′29″E; 1,265 m asl) located in the Xilin River Basin near the Inner Mongolia Grassland Ecosystem Research Station, Chinese Academy of Sciences. The study area is characterized by a semi-arid temperate climate with an annual mean temperature of about 0°C. The maximum

monthly mean air temperature is 19°C in July, whereas the minimum is –21°C in January. The mean annual precipitation ranges from 350 to 450 mm, with 70% occurring between July and September. A soil property survey was conducted in June 2007 prior to the experiment (Table 1; Peng et al., 2011b). The soil is classified as chestnut soil according to the Chinese soil classification system or Calcic-orthic Aridisol according to the US soil taxonomy classification system. The soil depth varies from 100 to 150 cm, and the horizon ranges from 20 to 30 cm in thickness. Soil particle is composed of 60% sand and 21% clay. The plant community is mainly composed of *L. chinensis*, *Stipa grandis*, *Agropyron michnoi* and *Cleistogenes squarrosa*. Among these species, *L. chinensis* and *S. grandis* are the constructive species in the study site, and they are also identified as the dominant species throughout the entire Xilin River Basin (Zhou et al., 2006).

1.2 Experimental design and treatments

We performed N addition treatments beginning from 1 July 2007. In this study, seven N addition treatments were designed: (1) control with no N addition (CK); (2) three N addition levels with calcium ammonium nitrate as N source, namely, low N with 50 kg N/(hm²·a) (LN), medium N with 100 kg N/(hm²·a) (MN), and high N with 200 kg N/(hm²·a) (HN); and (3) three N addition forms, namely, ammonium-N-based with 100 kg N/(hm²·a) as ammonium sulfate (AS), nitrate-N-based with 100 kg N/(hm²·a) as sodium nitrate (SN), and mixed-N-based with 100 kg N/(hm²·a) as calcium ammonium nitrate (CAN). Three replicate experimental plots for each treatment were set, and every plot has an area of 11 m×6 m. Notably, MN and CAN were similar treatments and applied to the same plots, so there were 18 plots in total. The N fertilizer was added in the aqueous solution and divided into two applications (20 L solution per plot per application; control plots received water only) on the starting days of the two wettest months (July and August) at the study site in 2007, respectively, considering that relatively high N deposition rate often occurs accompanying with the rainfall during the growing seasons. Two passes were made across each plot to ensure an even distribution of fertilizers.

Before the initial N application, we prepared litter bags (15 cm×20 cm) for litter decomposition experiments and assigned them evenly on different N treatment plots. Fallen litters produced in the non-growth season between 2006 to 2007 were collected by handpicking in mid-June 2007. Litters from the two constructive species (*L. chinensis* and *S. grandis*) together with the other two main component species (*A. michnoi* and *C. squarrosa*) in the *L. chinensis* steppe were selected carefully and mixed evenly according to their natural composition ratios at the study site. Mixed litters were air-dried in the laboratory and then selected randomly for grinding and sieving through a 0.15-mm mesh to analyze the initial chemical properties (total organic C and total N). The litter mass remaining from a 2-mm mesh polyester (7.5 g per bag) was placed in litter bags. Before placement, the litters in some randomly selected litter bags were heated at 65°C for 48 h to determine the ratio of air-dried to oven-dried mass. This mass ratio was used to accurately calculate the initial oven-dried weight of litter in each litter bag from the air-dried mass. On 26 June 2007 (four days before the first N fertilizer application), the remaining litter bags were evenly assigned into each plot and fixed to the ground surface by 10-cm metal pins in length. The 1,200-day field experiment was conducted from 26 June 2007 to 28 September 2010. The litter bags were retrieved at time intervals of about 1 to 2 months during the growth seasons of the first two years. The sampling frequency was reduced once every three months during the growth seasons in the remaining experimental years.

Table 1 Soil physical-chemical properties in the sampling site

Soil depth (cm)	Organic C (%)	Total N (%)	C/N	NH₄⁺ (mg/kg)	NO₃⁻ (mg/kg)	pH	Soil bulk density (g/cm³)
0−10	1.995	0.188	10.63	1.43	1.91	7.88	1.22
10−20	1.496	0.160	9.33	1.38	1.47	–	1.28
20−30	1.301	0.131	9.91	0.85	0.75	–	1.28

Note: Data are from Peng et al. (2011b).

Litter bags were not collected in the non-growth seasons during the experimental years. A total of 14 subsequent litter collections were performed during the 1,200-day experiment. At each sampling time, 18 litter bags were collected from 18 plots, including three replicates for each treatment.

After collection, litter bags were immediately transported to the laboratory, and then cleaned and oven-dried at 65°C for 48 h to determine the remaining dry mass. The concentrations of litter C (total organic C) and N (total N) of the samples were determined by wet oxidation using the $K_2Cr_2O_7$ and the semi-micro Kjeldahl methods, respectively.

1.3 Data analyses

All statistical analyses were performed and graphs were plotted using SPSS 17.0 (SPSS Inc., Chicago, USA) and Origin 8.5 (Origin Lab Corporation, USA), respectively. The litter mass remaining (X_r) was determined using the following equation:

$$X_r = \frac{X_t}{X_0} \times 100 \times 100\%. \tag{1}$$

Where X_0 is the initial dry mass of litter, and X_t is the dry mass of the remaining litter for each sample in each sampling time. The litter C and N remaining were also calculated by Eq. 1. The litter mass remaining and C:N ratios in all treatments during the whole experimental period were simulated by the programs in Origin 8.5. Repeated-measure ANOVAs were conducted to test the significance of the overall differences of the litter mass remaining as well as the litter N and C concentrations among different N addition levels or forms during the decomposition period, where time was regarded as the within-subject factor, and N addition level or form was evaluated as the between-subject factor. Significant differences were tested at a significance level of 0.05, unless otherwise stated.

2 Results

2.1 Dynamics of litter mass remaining under different N addition treatments

Within the initial 120 days (July to October in 2007), the average litter mass remaining in all treatments decreased by about 30%. Further, about another 30% mass loss was also observed during the following relatively longer observation period (120 to 1,200 days; Fig. 1).

The fast decay pattern, followed by a relatively slow decrease in the litter mass remaining for each treatment during the whole experimental period, can be fitted roughly into the classic decomposition dynamic models, such as the double exponential model ($y=y_0+e^{-ax}+e^{-bx}$) and the allometric equation ($y=ax^{-b}$) (Fig. 2).

The process of litter decomposition can be divided into two distinct phases when considering the effects of N additions on litter mass loss (Fig. 3). According to the results from the repeated ANOVA measures (Table 2), the litter mass remaining in all treatments significantly varied with time during the whole
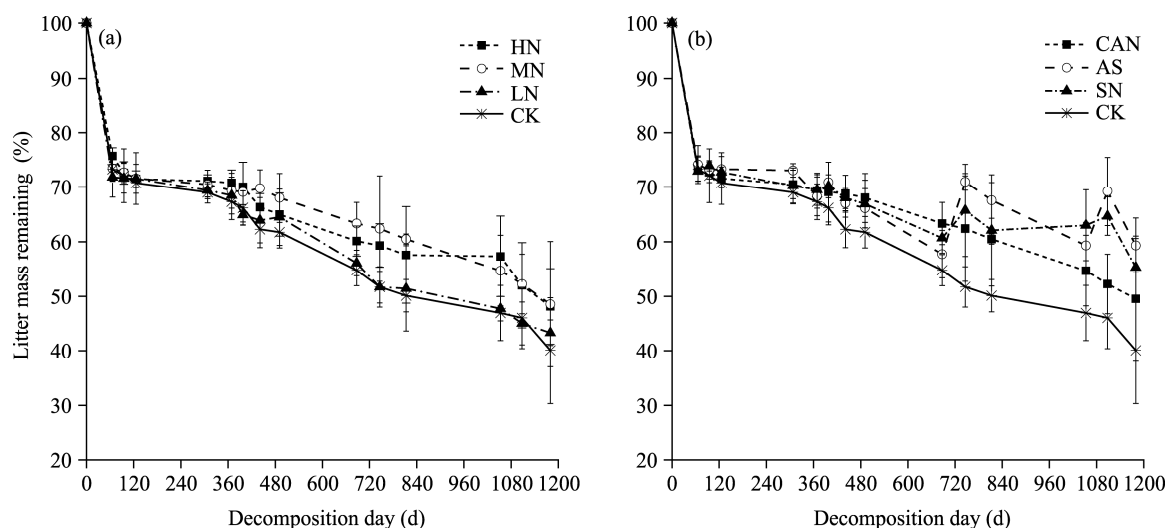

Fig. 1   Litter mass remaining in different N addition levels (a) and forms (b) during the whole experimental period. Dots are measured in mean±SE (*n*=3). HN: high N with 200 kg N/(hm²·a); MN: medium N with 100 kg N/(hm²·a); LN: low N with 50 kg N/(hm²·a); CK: control with no N addition; CAN: mixed-N-based with 100 kg N/(hm²·a) as calcium ammonium nitrate; AS: ammonium-N-based with 100 kg N/(hm²·a) as ammonium sulfate; SN: nitrate-N-based with 100 kg N/(hm²·a) as sodium nitrate.

observation period (P<0.001). Whether N addition levels or forms have no significant effects on litter mass remaining on the collection dates in phase I (<398 days; P>0.05). However, during the observation period in phase II ($\geq$398 days), the differences in the litter mass remaining in different N addition levels and forms reached significant (P=0.017) and marginally significant levels (P=0.056), respectively.

The litter mass remaining during the experimental period across each treatment can also be efficiently simulated using two separate models in two split observation phases: a single exponential decay model ($y=A_1e^{-at}+A_2$, P<0.001) in phase I and a linear decay function ($y=kx+b$, P<0.001) in phase II (Fig. 2 and Table 3). Compared with the double exponential model and the allometric equation across the whole experimental period, the simulations from the two split-phase models were more accurate (Fig. 2).

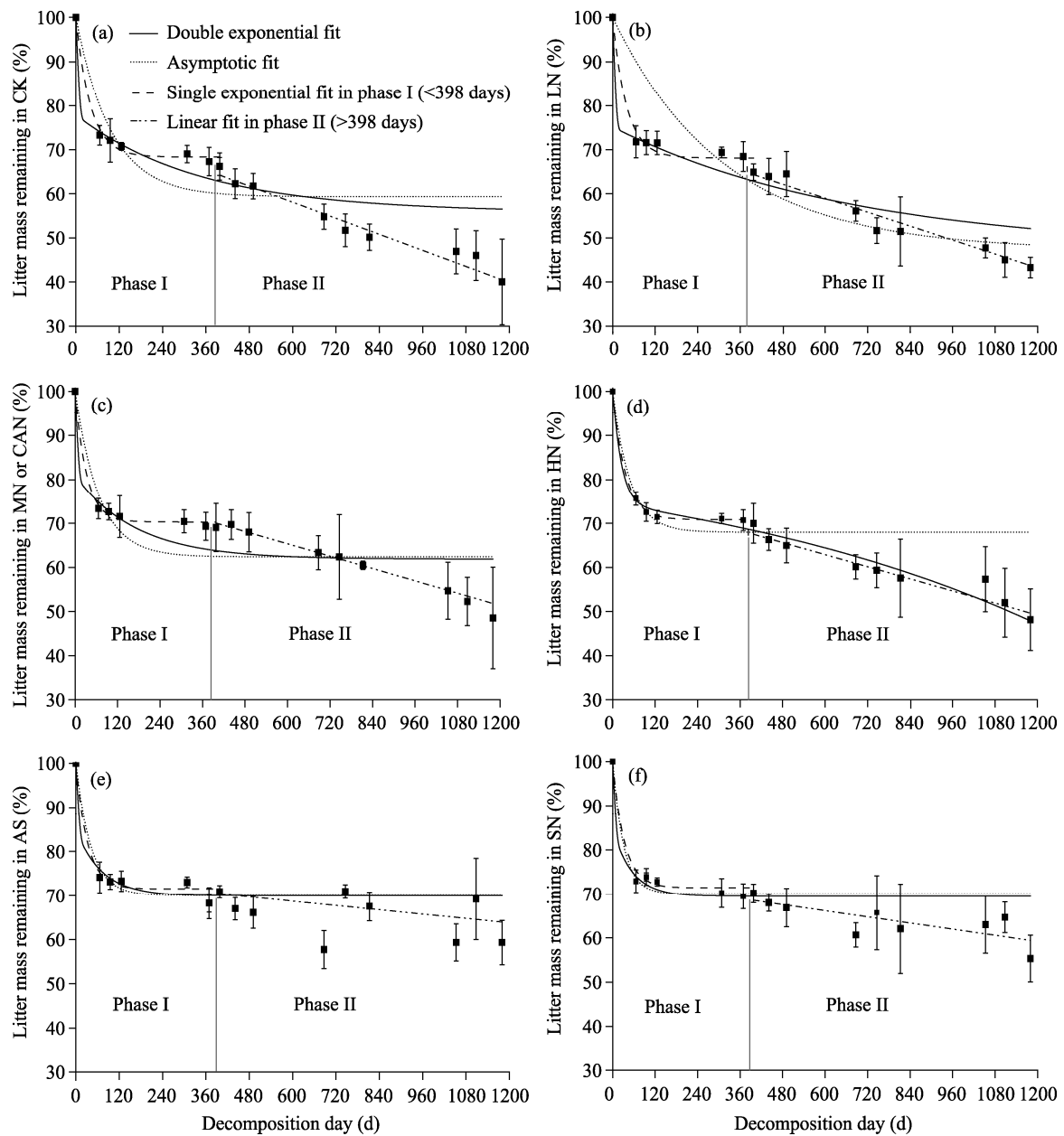Calculating the turnover periods of litter deposition in different treatments by using the simulated models

Fig. 2 Three simulations of litter mass remaining in different N addition levels (a, b, c, d) and forms (c, e, f) during the whole experimental period. Dots are measured in mean±SE (n=3).

in two split phases showed that, the time required for 95% litter decomposition (approximated complete decomposition) was 8.5, 9.3, 7.3 and 6.5 years in HN, MN, LN and CK treatments, respectively. However, for AS, SN and CAN treatments, the corresponding time was 14.8, 15.5 and 9.3 years, respectively (Table 3). Paired comparison analysis (data were not shown) further showed that the MN and HN treatments inhibited litter mass loss after 398 (P=0.029) and 746 days (P=0.047), respectively. LN treatment had no significant effect on litter decomposition in the whole obser-

vation period (P>0.05). Among the three N addition levels, litter mass remaining in MN treatment was significantly higher than that in LN treatment after 1,053 days (P=0.045) during the whole observation period; however, no significant differences were observed in the other two pairs (P>0.05). Among the three N addition forms, litter mass remaining in AS and SN treatments differed significantly from that in CAN treatment after 746 days (P=0.028) and 1,053 days (P=0.049), respectively. However, litter mass remaining between AS and SN treatments have no
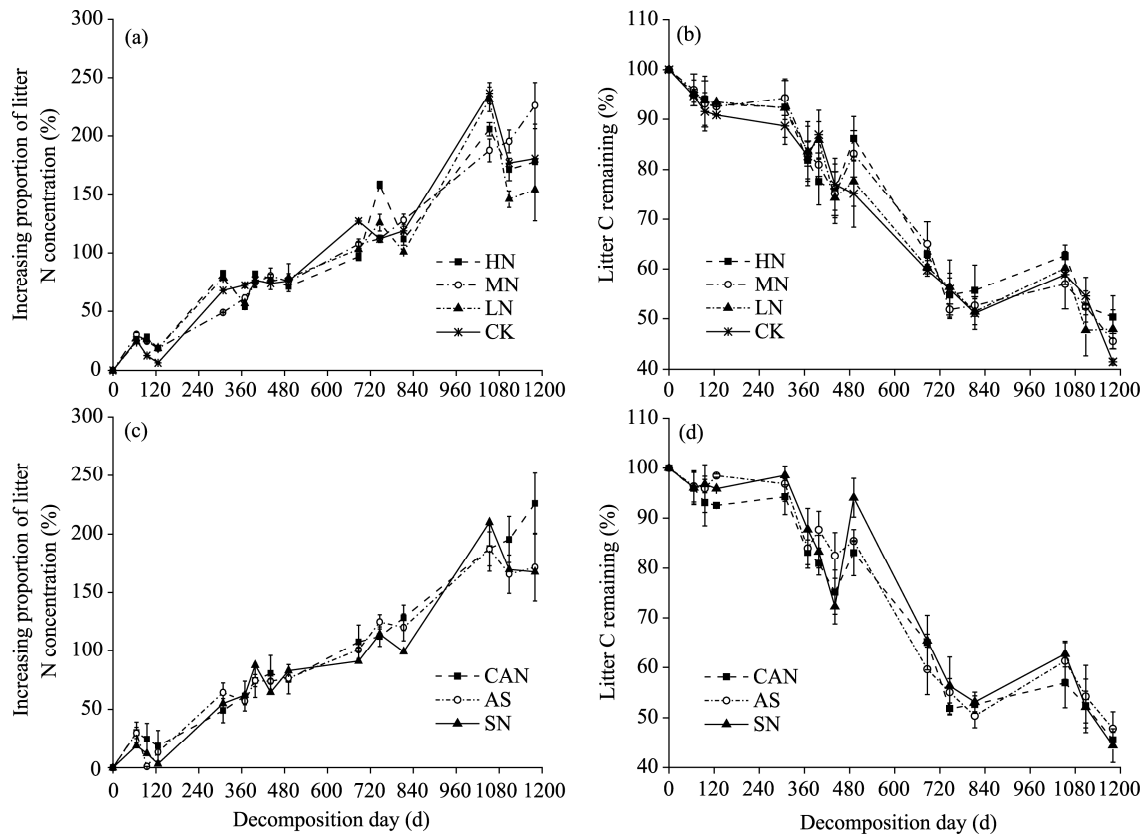

Fig. 3 Increasing proportion of litter N concentration and litter C remaining in different N addition levels (a, b) and forms (c, d) during the whole experimental period. Dots are measured in mean±SE (*n*=3).

Table 2 Repeated-measure ANOVA of the litter mass remaining with different N addition levels and forms in two observation phases

Treatment	Phase I (<398 days)			Phase II (≥398 days)		
	Mean Square	F	P	Mean Square	F	P
Within-subject effect						
Date	100.787	8.524	<0.001	95.612	10.431	<0.001
Time×N level	6.095	0.515	0.937	37.022	0.612	0.818
Time×N form	2.844	0.215	0.978	163.948	2.633	0.012
Between-subject effect						
N level	23.498	3.296	0.179	473.350	6.249	0.017
N form	5.354	2.083	0.206	92.447	4.131	0.056

Table 3 Simulated models of the litter mass remaining in two split-phase under different N addition levels and forms during the whole experimental period and the total turnover time required for 95% litter decomposition

Treatment	Phase I (<398 days)			Phase II (≥398 days)			Mean time for 95% decomposition (a)
	Simulation function	P	R^2	Simulation function	P	R^2	
HN	$y = 70.9 + 29.1e^{-\frac{x}{36}}$	0.001	0.999	$y = -0.023x + 76.6$	0.001	0.926	8.5
MN or CAN	$y = 70.3 + 29.7e^{-\frac{x}{32}}$	0.001	0.998	$y = -0.022x + 79.3$	0.001	0.985	9.3
LN	$y = 68.3 + 31.7e^{-\frac{x}{39}}$	0.001	0.994	$y = -0.026x + 74.8$	0.001	0.968	7.3
AS	$y = 71.5 + 28.5e^{-\frac{x}{32}}$	0.001	0.996	$y = -0.013x + 75.0$	0.030	0.500	14.8
SN	$y = 71.4 + 28.6e^{-\frac{x}{33}}$	0.001	0.997	$y = -0.012x + 73.2$	0.029	0.508	15.5
CK	$y = 68.4 + 31.5e^{-\frac{x}{38}}$	0.001	0.997	$y = -0.030x + 76.3$	0.001	0.940	6.5

significant differences in the whole observation period (P>0.05).

2.2 Dynamics of litter N and C under different N addition treatments

Litter N in all N addition treatment plots (including CK) generally showed a clear accumulation trend during the entire observation period (Fig. 3), except for a small transient downward trend exhibited by litter N concentration after 1,053 day for HN, LN, AS, SN and CK treatments (Figs. 3a and c). By contrast, litter C generally exhibited a decreasing trend during the whole decomposition period in all N addition treatments, although occasional small fluctuations were also observed (Figs. 3b and d).

The temporal dynamics of litter C:N ratios in all treatments followed an exponential decay pattern ($y=e^{-ax+c}+b$, P<0.001; Fig. 4), which was attributed to the general retention of litter N and the loss of litter C. The litter C:N ratios in all treated plots were about 50 on the first day of the experiment. These ratios decreased below 25 and 15 after 441 and 746 days during the whole decomposition period, respectively. At the end of the observation period, the litter C:N ratios further decreased to values lower than 10. Repeated-measure ANOVA indicated that both N addition levels and forms exerted no significant effects on litter N and C concentrations (P>0.05 and data not shown). In addition, the litter mass remaining in each treatment was negatively correlated with litter N while positively correlated with litter C. The relationships between litter N and C or between litter mass and litter N as well as litter mass and litter C can be described well using the linear functions (P<0.001; Fig. 5).

3 Discussion

3.1 Dynamics of litter mass remaining under different N addition treatments

In the present study, a higher rate of litter mass loss was observed during the initial 120 days of the whole decomposition period, followed by a relatively low rate of mass loss. This decay pattern was typically reported for numerous litter decomposition studies regardless of the N additions (Aber et al., 1990; Mo et al., 2006; Oladoye et al., 2008). This pattern was often attributed to the changes of litter components in decomposition process. Some litter components, such as carbohydrates, hemicelluloses and water-soluble compounds, were easily decomposed in the early stages, whereas other compounds such as waxes, lignin and suberins, were difficult to decay (McClaugherty et al., 1985; Rovira and Vallejo, 2000). Given that the former disappears and the latter decomposes with lower rate during the late stages, the litter decomposition rate decreased (Berg and McClaugherty, 2008; Rovira and Rovira, 2010). The relatively warm and rainy weather conditions at the study sites during the first two months (July and August) of the growing season could also accelerate the litter decomposition rate and the leaching of the easily decomposed litter compounds (e.g. water-soluble compounds; Mo et al., 2006).

Despite the predictability of the dynamics of litter mass remaining by a single exponential model in the initial 398 days, this relationship may not be predictable over a relatively long term (1,200 days; Fig. 2). This result agrees with the study by Berg and McClaugherty (2008), which suggests that the most

commonly used mathematical model, i.e. Olson's exponential model, is often used for the early stages (often shorter than 2 years) of litter decomposition, but it frequently fails to fit observations from the late phases of decomposition. In addition to the single exponential model, the slightly complex models such as the double exponential model and the asymptotic function, were often the desirable choices for simulating the dynamics of litter mass remaining (Berg andMcClaugherty, 2008). However, these two models

are not suitable for describing the litter mass remaining dynamics in this study. Although the allometric equation can simulate the dynamics of the litter mass remaining during the entire observation period in this study, the equation can also overestimate the loss of litter mass in the early stages and underestimate the litter mass remaining in the late stages to a certain extent (Fig. 2). To improve the simulation accuracy, we used two different models separately in the two stages of the whole decomposition period: the single
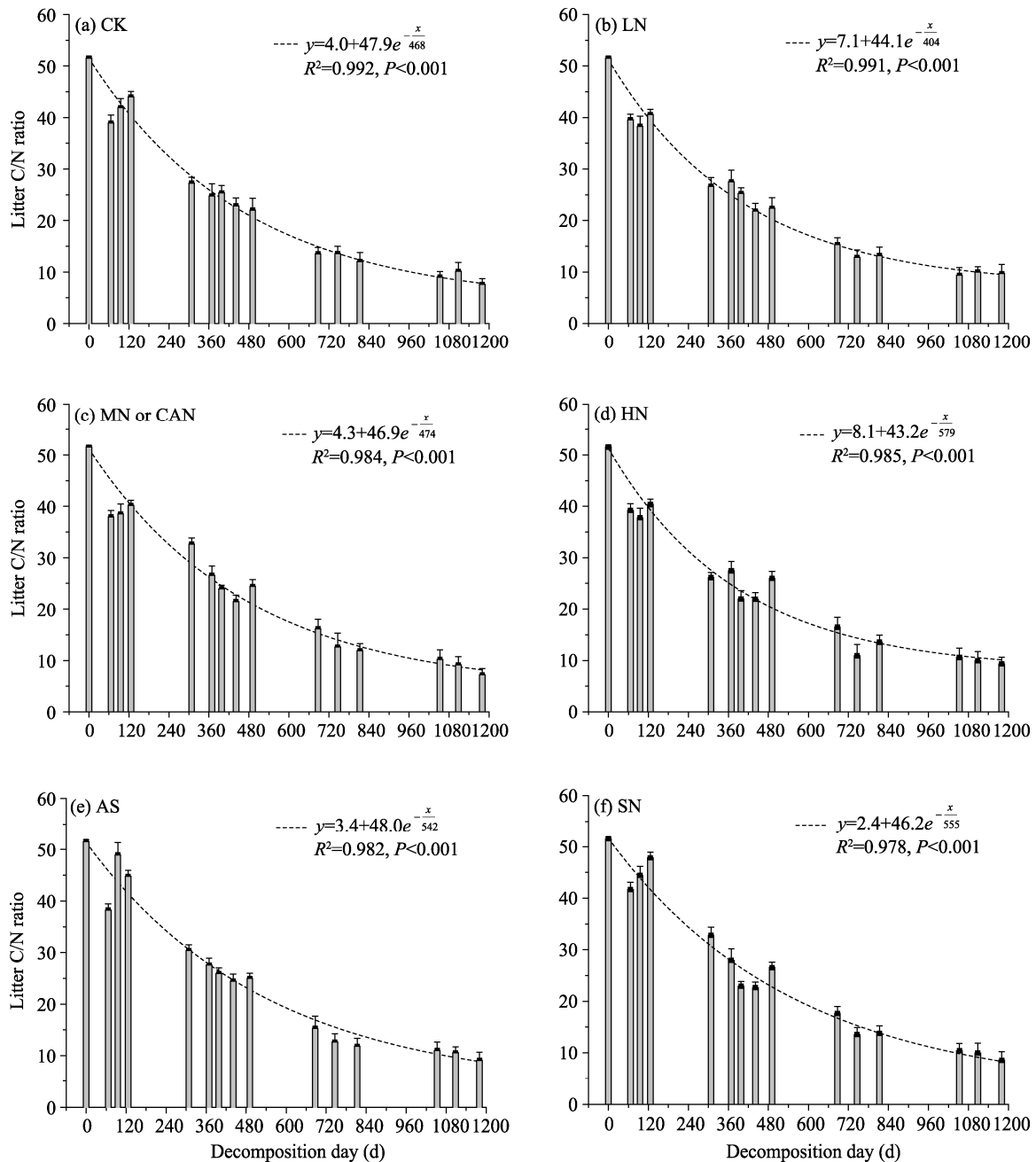

(a) CK $y=4.0+47.9e^{-\frac{x}{468}}$
$R^2=0.992, P<0.001$

(b) LN $y=7.1+44.1e^{-\frac{x}{404}}$
$R^2=0.991, P<0.001$

(c) MN or CAN $y=4.3+46.9e^{-\frac{x}{474}}$
$R^2=0.984, P<0.001$

(d) HN $y=8.1+43.2e^{-\frac{x}{579}}$
$R^2=0.985, P<0.001$

(e) AS $y=3.4+48.0e^{-\frac{x}{542}}$
$R^2=0.982, P<0.001$

(f) SN $y=2.4+46.2e^{-\frac{x}{555}}$
$R^2=0.978, P<0.001$

Decomposition day (d)

Fig. 4 Litter C:N ratios in different N addition levels (a, b, c, d) and forms (c, e, f) during the whole experimental period. Dashed curves correspond to regression fitting for the C:N ratios to decomposition time series.
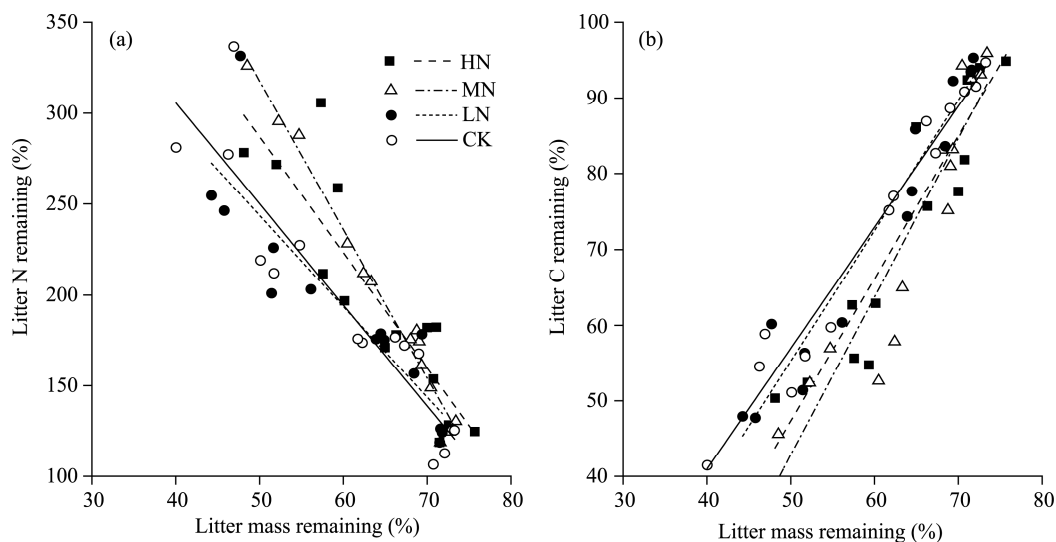
Fig. 5   Relationships between litter mass remaining and litter C (a) and N (b) remaining under different N addition levels

exponential model in phase I and the linear model in phase II. The dynamics of litter mass loss in this study differs from those reported previously (e.g. Berg and McClaugherty, 2008), especially in short-term (<2 years) decomposition researches (Liu et al., 2010b; Li et al., 2011). Therefore, experiments should be conducted over an extended period to clarify the actual litter decomposition pattern.

3.2   Influences of different N addition levels and forms on litter mass remaining

In this study, no significant effects on litter mass remaining were induced by all N addition treatments within a relatively short decomposition period (<398 days). This result contradicts many previous short-term (less than 1 or 2 years) studies, which reported that N addition to a N-limited ecosystem could commonly accelerate the litter decomposition (Knorr et al., 2005; Mo et al., 2006; Vivanco and Austin, 2011). N stimulation of litter decomposition in such studies often relates to the positive effect of N on labile C fractions, which are the dominant components of litter during the early decomposition stages (Knorr et al., 2005). In the present study, the litters collected were mixed evenly with different plant species. Thus, in addition to labile C fractions, other fractions that are easily suppressed by N additions (e.g. lignin) more likely produce and coexist during the early stages in different plant species than those in single plant species. In such circumstances, any stimulatory effect of N on the decay of labile C fractions (Fog, 1988;

Waldrop et al., 2004) may be offset by the direct N suppression of the degradation of difficult fraction decomposition (Berg, 1986; Conn and Day, 1996). Therefore, the neutral effects of N additions on litter decomposition can be detected.

The treatments of HN and MN inhibited litter decomposition in the late stages of the experimental period in this study. This result agrees with many previous studies on litter decomposition with N additions (Sinsabaugh et al., 2002; Hobbie, 2008; Allison et al., 2009). Meanwhile, LN treatment did not significantly inhibit the loss of litter mass ($P>0.05$), suggesting that a threshold of N addition level for the inhibition of litter decomposition could exist between the LN and MN levels at the study sites. N additions inhibiting litter decomposition have been reported in many ecosystems (Sinsabaugh et al., 2002; Berg and Laskowski, 2006). Potential underlying mechanisms have been proposed explaining the negative effects of N additions on litter decomposition (Dijkstra et al., 2004; Allison et al., 2008; Berg, 2008). Relatively high N addition levels have aggravated C limitation for microbial degradation (Berg et al., 1998), reduced microbial diversity and activity (Mo et al., 2006; Allison et al., 2008), and altered the microbial community. As a result, the overall degradation of litter was impeded. N additions can also suppress the synthesis of lignolytic enzymes, which can completely degrade lignin (Magill and Aber, 1998; Sinsabaugh et al., 2002), thereby inhibiting litter decomposition. Furthermore, the additions of relatively high N-level

fertilizers to ecosystems can produce more additional recalcitrant compounds from added N reaction with litter components (Ågren et al., 2001; Dijkstra et al., 2004). The formation of recalcitrant compounds is also considered as the main contributor for the suppression of litter decomposition rates in the late stages with decreased water-soluble compounds (Hobbie, 2000; Berg and Laskowski, 2006).

In summary, regardless of the mechanisms involved, the neutral or inhibitory influences of N additions on litter decomposition suggest that C sequestration through litter could not decrease in this semi-arid temperate grassland ecosystem in future exogenous N input conditions. Even if a threshold level (near the LN level) of N addition inhibiting litter decomposition has been found in the current study, the exact threshold value of N addition should be determined by further experimentation.

The application of different mineral N forms exerted different effects on litter decomposition in this study. AS and SN treatments exhibited similar inhibitory effects on litter mass remaining; however, the treatments of AS and CAN or SN and CAN exerted significantly different inhibitory effects on litter mass remaining during the whole decomposition period. Previous studies indicated that different effects of N additions on litter mass remaining depended on the forms of mineral N (Liao et al., 2000; Zhang and Wang, 2012). However, these studies did not completely agree with the present study. For example, Zhang and Wang (2012) observed that the increase in ammonium N inputs more considerably decreased the litter decomposition rates in the late decay stages than the increase in nitrate N inputs. Liao et al. (2000) found that nitrate N significantly stimulated litter decomposition, whereas ammonium N showed no significant effect on litter decomposition. Wang et al. (2011) found that ammonium N and nitrate N induced similar effects on litter mass remaining in broadleaf and coniferous forests in Zijin Mountain in China, which is consistent with the present study. However, the direction of the two N-form-induced effects was completely contrary to our observation (stimulatory effects vs. inhibitory effects). This inconsistency may be related to the different combinations of N forms with different litter components or the inconsistent preferences of soil microorganisms for different N components. As indicated in previous studies (Bardgett et al., 2003; Schadt et al., 2003), the microbial preferences for N forms varied over the decomposition period because of marked temporal shifts in the composition and functional attributes of the microbial community. This finding could explain the different effects of AS and CAN treatments or SN and CAN treatments on litter mass remaining in the late decomposition stages. The lack of a significant difference in the effects of ammonium-N and nitrate-N treatments on litter decomposition in the present study may be attributed to the mechanisms by which the two forms of inorganic N were used efficiently by soil microorganisms or the rapid nitrification of soil NH_4-N after NH_4-N addition (Hu et al., 2010; Wang et al., 2011).

3.3 Dynamics of litter N and C under different N addition treatments

Litter C commonly functions as the primary energy source for litter decomposers (Bosatta and Staff, 1982; Ågren and Bosatta, 1996; Manzoni et al., 2008, 2010) and the main constituent of total litter mass. Thus, it is not surprising that the amount of litter C decreased continuously during the whole decomposition period in the present study (Figs. 3b and d), which is similar to some researches in other ecosystems (Kochsiek, 2010; Zhang and Wang, 2012).

In contrast to litter C, litter N generally showed an accumulation pattern in all treatments throughout the observation period, expect for a transient downward trend after 1,053 days (Figs. 3a and c). This observation agrees with other reports (Hobbie et al., 2000, 2008; Parton et al., 2007), in which the retention of N in litter occurred in the early stages of decomposition, followed by N release in the late stages. The accumulation of litter N before 1,053 days in the present study may be a function of litter decomposer organisms that import N from the soil solution to the decomposing litter (Hobbie et al., 2000, 2008; Moore et al., 2006; Parton et al., 2007; Oladoye, 2008; Manzoni et al., 2010; Berglund and Ågren, 2012). This assumption indicates a pre-existing N limitation for the growth of microorganism as litter decomposer. The accumulation of litter N could also be attributed to the chemical N immobilization (Axelsson and Berg, 1988; Moore et al., 2006). Generally, N tends to be retained more in litter on rich N addition plots (Berg

and Tamm, 1994; Chadwick et al., 1998; Liu et al., 2011; Apolinário et al., 2013). However, this phenomenon was not observed in the present study. The reasons need to be further explored.

Litter N demonstrated a downward trend after 1,053 days in almost all N addition treatments except for MN in this study. Previous studies showed that N was usually released when litter C:N ratios dropped to a critical value (Hobbie et al., 2000, 2008; Oladoye, 2008; Manzoni et al., 2010; Berglund and Ågren, 2012). At this critical value, N in litters is expected to begin satisfying the requirements of the main decomposer communities which consume the associated C for energy (Moore et al., 2006; Parton et al., 2007). The critical C:N ratios for N release generally ranged from 25 to 30 for agricultural residues (Haynes, 1986) and 23 to 80 for forest foliage litter (Edmonds, 1980; McClaugherty et al., 1985; Hart et al., 1992; Hart, 1999). In the present study, a downward trend in litter N was observed at the average C:N ratios which are between 10 and 15 for three N-level treatments (HN, LN and CK) and three N-form treatments (AS, SN and CAN). These points are markedly lower than those in agricultural and forest ecosystems. Meanwhile, the period for N accumulation in the present study are relatively long (lasted for about 1,053 days). This finding suggests that litter decomposers could be more strongly N-limited in a semi-arid temperate grassland in Xilingol, Inner Mongolia.

4 Conclusions

The 1,200-day field experiment in this study showed that exogenous N additions can inhibit litter decomposition. The inhibitory effects were determined by N addition treatments and litter decomposition time because the relatively high inputs of N (MN and HN) additions exerted negative effects on litter mass loss in the late stages of decomposition (≥398 days). Meanwhile, N addition treatments exerted no significant differences on litter decomposition in the early stages of the decomposition period (<398 days). The roles of the N addition forms cannot be neglected in the litter decomposition at the study sites because the litter mass remaining in the AS- and SN-added plots were significantly different from that in the CAN-added plots in the late stages of the litter decomposition period; however, the litter mass remaining in the AS- and SN-added plots were not different from each other.

During the decomposition period, litter C decreased whereas litter N increased. Litter N was strongly associated with litter C, and the C:N ratios fitted a good exponential regression that provides a good prediction tool for the changes in chemical elements in litter. N retention and C loss in the decomposing litter were not affected by exogenous N additions. This finding suggests that N retention or C loss in this typical temperate steppe ecosystem in China depends more likely on the chemical quality of litter than on the N status of the surrounding soils. In addition, the inhibitory effects of N additions on litter decomposition in the final decay stages were not caused by the changes in the chemical qualities of the litter such as endogenous N and C concentrations.

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