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Precipitation and Nitrogen Deposition Alter Litter Decomposition Dynamics in Semiarid Temperate Steppe in Inner Mongolia, China☆

Zhongqing Yan [a,b], Yuchun Qi [a], Yunshe Dong [a,*], Qin Peng [a], Shufang Guo [a,b], Yunlong He [a,b], Zhaolin Li [a,b]

[a] Key Laboratory of Land Surface Pattern and Simulation, Institute of Geographic Sciences and Natural Resources Research, CAS, Beijing 100101, China
[b] University of the Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

Plant litter decomposition is one of the most important links connecting plants to the soil through the carbon (C) and nitrogen (N) cycles. Climate change scenarios predict changes in precipitation and N deposition, and previous studies have demonstrated that increases in the availability of water and N affect the litter decomposition rate and nutrient release. We studied the effects of increased N deposition and precipitation on changes in the remaining mass and the C and N contents of shoot litter after decomposition in a typical steppe in Inner Mongolia, China. The treatments included the addition of NH_4NO_3 at rates equivalent to 0, 25, 50, and 100 $kg \cdot N \cdot ha^{-2} yr^{-1}$ with and without added water. The addition of water proved to be a more effective practice than amendment with NH_4NO_3 for improving the litter decomposition rate; the addition of water significantly increased the rate of litter decomposition ($P < 0.001$), whereas the addition of N alone had no apparent effect on litter mass loss. However, a repeated measures analysis of variance (ANOVA) showed that the interaction of water and N significantly affected both mass loss and litter N content ($P < 0.05$), and a linear relationship was identified between litter mass loss and litter decomposition time ($P < 0.001$). No correlation was found between litter mass loss and organic C content, but a significant positive correlation was found between residual litter mass and N content ($P < 0.01$). Although the study was conducted over a relatively short period, our results indicate that increased precipitation could potentially promote litter decomposition, whereas increased N input has little effect. The effects of time on litter mass loss and residual C and N concentrations indicate the need for long-term trials that measure the complete process of litter decomposition and the peaks of C and N release.

Introduction

Litter decomposition is a necessary component of nutrient cycling as it releases stored nutrients and energy for consumption by microbes (Kang et al., 2007). In terrestrial ecosystems, > 50% of net primary production is transferred to the soil by the litter decomposition pathway (Wardle et al., 2004). The decomposition of plant residues involves numerous physical, chemical, and biological factors; mainly climatic variables; initial litter quality; the physicochemical environment, and the decomposer community of microorganisms and soil animals, all of which operate at different scales (Bontti et al., 2009). Previous studies have found strong links between decomposition and site-specific characteristics, such as soil carbon (C), nitrogen (N), and phosphorus (P) availability, and climatic variables, such as the mean annual precipitation, temperature, and water content (Finn et al., 2015).

Changes in precipitation patterns in response to global climate change have already manifested broadly, and they have resulted in more frequent extreme precipitation and drought events (Easterling et al., 2000). Furthermore, an approximate 0.5%–1% increase in global precipitation per decade is predicted to occur over the course of this century (Houghton et al., 2001), and in northern China, annual precipitation is predicted to increase by 14–155 mm by the end of the century (Jiang et al., 2008). Previous studies have shown that water availability could become the predominant factor affecting litter decomposition at local scales, particularly in desert or semiarid regions where water is the primary limiting factor (Zhang et al., 2008). Water availability affects decomposition directly and indirectly. Direct abiotic effects include litter fragmentation and leaching of labile compounds from the litter surface. In addition, soil water status directly affects the biotic activity of the soil microorganisms responsible for litter decomposition (Swift et al., 1979). Soil water availability affects decomposition indirectly through changes in the species composition and the abundance of plants and microorganisms (Gonzalez and Seastedt, 2001).

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* Correspondence: Dr. Yunshe Dong, Professor of Institute of Geographical Science & Natural Resource Research, Chinese Academy of Sciences, All Datun Road, Anwai, Beijing 100101, China. Tel.: +86 10 64889320.
E-mail address: Dongys3736@163.com (Y. Dong).

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Due to human activities, such as fertilizer application, fossil fuel combustion, and legume cultivation (Pinder et al., 2012), the deposition of reactive N has increased from 32 Mt N yr^{-1} to approximately 116 Mt N yr^{-1} since 1860 on a global scale, and further increases are expected in the future (Hewins and Throop, 2016). Increased N deposition can dramatically alter soil N availability, N cycling, litter quantity and quality, and the soil physicochemical environment, affecting litter decomposition processes in terrestrial ecosystems (Li et al., 2015). The addition of N can increase the availability of inorganic N and decrease litter C:N ratios (Henry et al., 2005) due to an increased rate of litter decomposition (Liu et al., 2006). However, other studies have found that N addition had no effect on litter decomposition (Zhang et al., 2013) and did not reduce the rate at which decomposition occurs (Fang et al., 2007)). These inconsistent responses can be attributed to substrate chemistry, differences in N levels, fertilizer types used, and the distribution of species (Chen et al., 2013). The N content, C content, C/N ratios, and lignin/N ratios of litter strongly affect litter decomposition (Liu et al., 2009; Peh et al., 2012). The decomposition rate of litter is mediated by many interacting environmental (chemical, physical, and biotic) factors (Sariyildiz and Anderson, 2003). Recent models have shown that climate and litter quality explain approximately 60%–70% of global litter decomposition rates (Parton et al., 2007). The effects of increased precipitation and atmospheric N deposition on litter decomposition are of considerable interest to researchers given that both factors can influence decomposition rates (Gong et al., 2014; Liu et al., 2011; Wang et al., 2015; Zhu et al., 2016). Therefore, identifying the effects of increased precipitation and N deposition on litter decomposition is essential for the quantitative analysis of C content, N content, and nutrient cycling in grassland ecosystems.

Grassland ecosystems are important components of terrestrial nutrient cycles and are strongly influenced by climate change and anthropogenic activities (Bontti et al., 2009). Arid and semiarid grasslands cover 40% of the terrestrial surface of the earth and have characteristically slow decomposition rates (Gill and Burke, 2002). Grasslands also account for 40% of the land area within the borders of China (Kang et al., 2007), whose main grassland region is the Inner Mongolian steppe in the northwest (Ma et al., 2016). However, the response of litter decomposition to the coupled effects of increased precipitation and N deposition has seldom been discussed for grassland ecosystems. We hypothesized that decomposition rates would increase with increasing precipitation and N deposition due to improved soil conditions, including a higher water content and greater N availability, on a local scale. We also hypothesized that the C content in litter would decrease due to microbial activity enhancement, while the N content in litter would increase due to additional N. Therefore, to better understand the effects of increasing precipitation and N deposition and to define the main factors that regulate litter decomposition processes in grassland ecosystems, we conducted a 2-yr decomposition experiment in a semiarid grassland in northern China, with treatments including both water and N addition. Our specific objectives were 1) to determine how increases in precipitation and N deposition affect decomposition rates and the C and N contents of grassland litter and 2) to characterize the relationship between litter mass loss and the release of C and N from litter.

Materials and Methods

Site Description and Treatment

Our study was carried out in a typical *Leymus chinensis* steppe (43°33′51.3″N, 116°40′44.1″E; 1 225 m asl) near the Inner Mongolia Grassland Ecosystem Research Station of the Chinese Academy of Sciences, which is located within the Xilin River basin. Within the study site, the vegetation coverage is approximately 44.7%, and the plant community mainly consists of *Agropyron michnoi, Stipa grandis, L. chinensis,* and *Cleistogenes squarrosa*. Of these species, *S. grandis* and *L. chinensis* are the constructive species, and they have also been identified as the dominant species throughout the entire Xilin River basin (Zhou et al., 2006). The soil type is classified as chestnut soil according to the Chinese classification or as Calcic Orthic Aridisol according to the Soil Taxonomy classification. It consists of 60% sand, 21% clay, and 19% silt; its depth is < 100 − 150 cm; and its organic layer is 20 − 30 cm thick. The study site has a semiarid temperate climate with a mean annual precipitation of 350 − 450 mm, and 70% of this precipitation falls between July and September (Wang et al., 2015). The mean annual temperature is − 0.3°C − 1°C, and the annual accumulated temperature ≥10° is 1800°C − 2000°C. The area has a typical semiarid temperate climate with moist summers and dry spring seasons. The growing season typically begins in early May and ends in late September (Liang et al., 2001). The experimental plots were used for grazing for 11 yr, and the grazing intensity was approximately 2.25 sheep/ha before enclosure. Other key soil properties are shown in Table 1.

The experiments were performed from 15 May 2014 to 15 September 2015, and Fig. 1 presents the monthly air temperature and precipitation during the study period. The water was manually added to the designated experimental plots using backpack sprayers at two levels: ambient precipitation (W0) and an approximate 15% increase (W15) over the mean annual precipitation. This amount was based on the 12–18% increase in precipitation predicted by the end of the 21st century according to the models for northern China of the Fourth Assessment Report of the 13th Intergovernmental Panel on Climate Change (IPCC-AR4) (Jiang et al., 2008). This increase corresponds to an additional 51.68 mm to the long-term mean annual precipitation of 344.5 mm. Water was supplemented each year during the growing season, from June to September. The water input for each month was determined on the basis of the proportion of rainfall during each month of the average total amount over 4 mo. The amount determined for each month was split into halves for two applications, except for September, in which only one application was carried out (Table 2). N additions were applied using NH_4NO_3 in late June and July, and the amount of each N addition was half of the total N treatment level. The N was surface broadcast at rates of 0 (N0), 25 (N25), 50 (N50), and 100 (N100) kg·N·ha^{-2} yr^{-1} based on the current N deposition rate of 22.6 kg·N·ha^{-1} yr^{-1} (Liu et al., 2013) and its predicted change over the next 30 yr (Galloway and Cowling, 2002). A randomized block design was used to prepare three replicates of the eight treatments (W0N0, W0N25, W0N50, W0N100, W15N0, W15N25, W15N50, and W15N100) to study the effects of water and N addition on the litter decomposition process. Each experimental plot measured 8 × 8 m.

Litter Bag Experiment

Polyester litter bags were 15 × 20 cm in size with a mesh size of 2 mm. They contained 15 g of litter (dry weight) from two constructive species mixed at a ratio of 2:1 based on their natural abundance at the study site. In early May 2014, litter from *L. chinensis* and *S. grandis* was collected from standing dead material in the study area. The litter was then dried in an oven at 65°C for 48 h and clipped into fragments of 5 cm in length before being placed in the bags. A total of 144 litter bags were placed on the soil surface on 15 May 2014 and fixed with 10-cm metal pins; 6 litter bags were randomly placed each plot. The litter bags were retrieved at 0, 60, 156, 361, 424, and 489 d after initial deployment. Each time, three litter bags that had undergone treatment were collected.

Chemical Analysis

After collection, the litter bags were immediately transported to the laboratory. The undecomposed litter in each litter bag was dried in an oven at 65°C for 48 h and then weighed. The concentrations of litter C (total organic C) and N (total N) in the samples were determined

Table 1
Soil physiochemical properties of the experimental plots (mean ± standard deviation).

Soil depth (cm)	Organic C ($g \cdot kg^{-1}$)	Total N ($g \cdot kg^{-1}$)	C/N	NH_4^+ ($mg \cdot kg^{-1}$)	NO_3^- ($mg \cdot kg^{-1}$)	pH	Soil bulk density ($g \cdot cm^{-3}$)
0-10	18.97 ± 0.41	1.80 ± 0.09	10.54 ± 0.58	4.15 ± 0.28	2.54 ± 0.50	7.95 ± 0.07	1.30 ± 0.05
10-20	18.19 ± 0.52	1.47 ± 0.07	10.28 ± 0.68	3.81 ± 0.69	1.38 ± 0.31	8.12 ± 0.07	1.28 ± 0.05
20-30	16.26 ± 0.47	1.14 ± 0.05	13.17 ± 1.20	3.60 ± 0.24	0.84 ± 0.30	8.29 ± 0.03	1.29 ± 0.05

using a Total Organic Carbon Analyser (Elementar Vario TOC Cube, Hanau, Germany).

Statistical Analysis

Data analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL). The remaining litter mass *(Mr)* was determined using the following equation:

$$M_r = M_t/M_0 \times 100 \times 100\% \qquad (1)$$

where M_0 = initial dry mass, and M_t = mass at time *t*. The remaining litter C and N were also calculated using Eq. (1). All data were checked for deviations from normality and homogeneity of variance before analysis. The annual decomposition rate, soil organic C, and total N content were analyzed under the added water and N treatments, respectively, using ANOVA for repeated measurements. Correlation analysis was performed to determine the correlations among these variables. Linear regression was used to identify significant relationships between the remaining mass of litter and time. Values of $P < 0.05$ were considered statistically significant. All graphs were created using the 2016 version of the Origin Pro software package (http://www.originlab.com/).

Results

Litter Mass Remaining Under Different Water and N Treatments

The cumulative mass losses associated with the eight treatments over the 489-d period of litter decomposition showed similar trends and seemed to have occurred in a stepwise process (Fig. 2A): Mass declined 13 − 18% in the first 156 d, the average daily decomposition rate with N treatments under ambient rainfall was 0.07 − 1.10 $mg \cdot g^{-1}d^{-1}$, and the average decomposition rate of litter with N treatments under increased rainfall was 1.13 − 1.16 $mg \cdot g^{-1}d^{-1}$. During d 156 − 361 (i.e., from the end of October of the first year to the end of April in the following year), the cumulative mass losses decreased by 0.27 − 2.67%

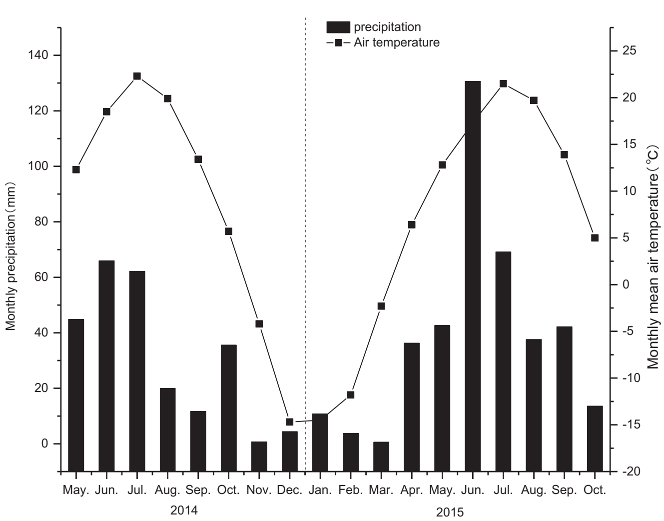

Figure 1. Monthly mean air temperature (lines) and precipitation (bars) at the study site from May 2014 to October 2015.

during the nongrowing season. During this period, the decomposition rate of litter in the plots that received N additions was 0.04 − 0.11 $mg \cdot g^{-1}d^{-1}$, and the decomposition rate under water plus N treatments was 0.01-0.13 $mg \cdot g^{-1}d^{-1}$. Litter decomposed quickly during the summer, when the temperature and moisture level were most suitable for microbial activity. After the start of the second growing season, the litter decomposition rates accelerated; in the 128-d period of d 361 − 489, the mass loss was 12.56 − 15.33%, the decomposition rate with N treatments was approximately 0.98 − 1.20 $mg \cdot g^{-1}d^{-1}$, and the rate with water plus N treatments was 1.00 − 1.12 $mg \cdot g^{-1}d^{-1}$. At the end of the experimental period, the residual mass percentage of the different treatments ranged between 65.96% and 73.09% (i.e., ~7 percentage points). During the entire experimental period, the decomposition rates of litter under different N additions were 0.55 − 0.68 $mg \cdot g^{-1}d^{-1}$, while the decomposition rates under water plus N treatments were 0.66 − 0.70 $mg \cdot g^{-1}d^{-1}$.

Litter mass loss was highly dependent on time. Therefore, we calculated the litter decomposition rate for each treatment by fitting a negative linear function (Fig. 2B), and the litter mass loss was estimated using the linear equation y = b − kx (Table 3), where *x* is the cumulative number of decomposition days and *y* is the remaining mass. The slopes of the fitting lines *(k)* were 0.053, 0.048, 0.053, and 0.055 for W0N0, W0N25, W0N50, and W0N100, respectively, and 0.058, 0.051, 0.058, and 0.058 for W15N0, W15N25, W15N50, and W15N100, respectively. Under the same N application condition, the slope k of the fitting line of water addition was lower than that of ambient precipitation.

The times to 50% and 95% decomposition are also shown in Table 3. Experiments have shown that the remaining mass over time follows a curvilinear relationship (Perez-Suarez et al., 2012), with the greatest mass loss occurring during the first days, known as the *leaching phase,* and driven by the loss of water-extractable compounds that physically leak from the sample. In the presence of more digestible fractions of the litter substrate, such as soluble carbohydrates including sucrose or glucose, after the initial leaching phase when the substrate is less decomposable, the litter mass loss rate slows (Berg and Laskowski, 2005; Cotrufo et al., 2015). Therefore, due to our relatively short experimental period, the time required to reach 95% mass loss provides only a rough estimate in this manuscript.

Dynamics of Litter C and N Concentrations Under Different Water and N Treatments

In Figs. 3 and 4, the C and N contents of the litterbags are expressed as the percentages of the initial contents along the decay continuum of mixed *S. grandis* and *L. chinensis* litter for different experimental water and N treatments. Percentages > 100% reflect net C or N immobilization, and lower percentages indicate net C or N mineralization. The initial total C and N concentrations of mixed litter were 44.58% and 0.50%,

Table 2
Time and amount of water input.

Amount of 474water added (mm)	Date of water addition	
	2014	2015
5.6	2014/06/15, 2014/06/29	2015/06/18, 2015/06/28
9.7	2014/07/16, 2014/07/28	2015/07/12, 2015/07/27
7.2	2014/08/13, 2014/08/28	2015/08/16, 2015/08/27
6.6	2014/09/11	2015/09/07

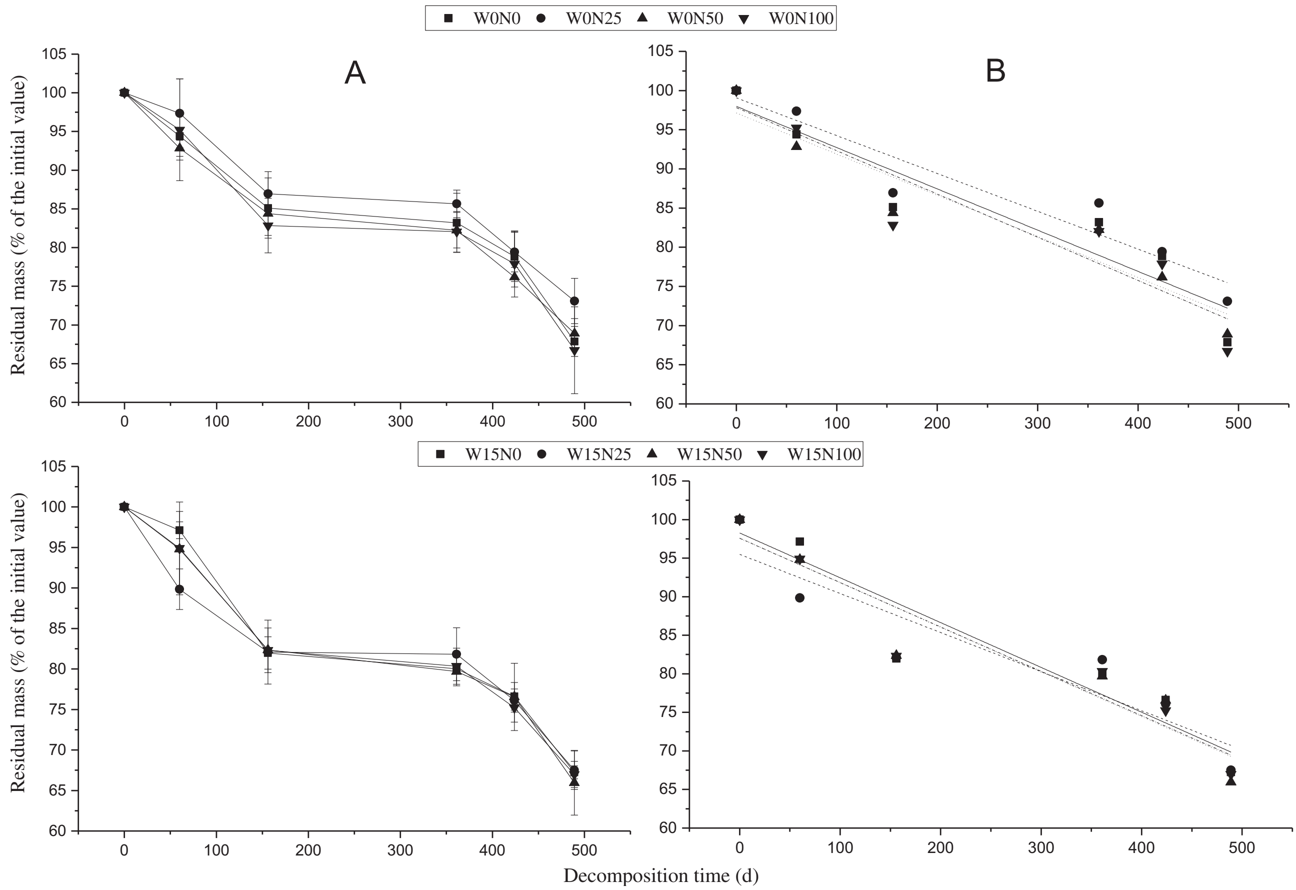

Figure 2. Residual mass (% of the initial mass) of litter under different water and N treatments over the course of the experimental period. The values are the means ± standard deviation ($n = 3$) in part A, and part B is the fitted straight line.

respectively. In general, our results show that the C loss rates with different treatments followed the same pattern. During the first growing season, the organic C concentration initially increased. During the non-growing season, the organic C concentration in the litter tended to increase gradually. In the second year's growing season, the organic C concentration decreased initially due to water and heat factors at the end of July, enhancing microbial activity and increasing the utilization of carbon compounds, which reduce the organic C concentration. At the end of the growing season, litter decomposition decelerated and the organic C concentration increased with decreased microbial decomposition activity. Throughout the 489 d of the trial, the average organic C concentrations in the plots of W0N25 and W0N100 were lower than that of the W0N0 plot by approximately 0.45% and 1.04%, respectively. The C concentration in the W0N50 plot was 0.51% higher than that of the W0N0 plot, the C concentrations in the W15N25 and W15N100 plots were lower than those of W15N0 plot by approximately 0.53% and 0.93%, and the C concentration of the W15N50 plot was 0.07% higher than that of the W15N0 plot, although the differences were not statistically significant.

The N concentration in decomposing litter changed over time, revealing the same trends for different treatments. N loss from the litter showed high temporal variation (see Fig. 4); the N concentration initially increased for the first 60 d, released during d 60 − 156, and then accumulated until d 489 in the field. The N concentration remaining in the litter was 147.83% on average across all treatments, indicating net N accumulation. Throughout the trial, the mean values of the N concentration under W0N50 and W0N100 treatments were 2.55% and 5.37% higher than those under W0N0 treatment, while the N content under W0N25 treatment was 1.01% lower than that under W0N0 treatment. When water and N were added together, the N contents of the W15N25 and W15N100 plots were higher than those of the W15N0 plot by 4.55% and 7.47%, respectively, and the N content of the W15N50 plot was lower than that of the W15N0 plot by 3.34%.

Table 3
Models (y = b-kx) of the relationship between the remaining litter mass (y) and time (x).

Treatment		Simulation function	R^2	*P*	No.	T50% (yr)	T95% (yr)
Unwatered	W0N0	y = 97.99-0.053x	0.86	< 0.01	6	2.48	4.81
	W0N25	y = 99.07-0.048x	0.89	< 0.01	6	2.80	5.37
	W0N50	y = 97.13-0.053x	0.89	< 0.01	6	2.44	4.76
	W0N100	y = 97.82-0.055x	0.84	< 0.01	6	2.38	4.62
Watered	W15N0	y = 98.26-0.058x	0.87	< 0.01	6	2.30	4.41
	W15N25	y = 95.48-0.051x	0.81	< 0.01	6	2.44	4.86
	W15N50	y = 97.60-0.058x	0.88	< 0.01	6	2.25	4.37
	W15N100	y = 97.58-0.058x	0.89	< 0.01	6	2.25	4.37

T50%, time to 50% mass loss; T95%, time to 95% mass loss.

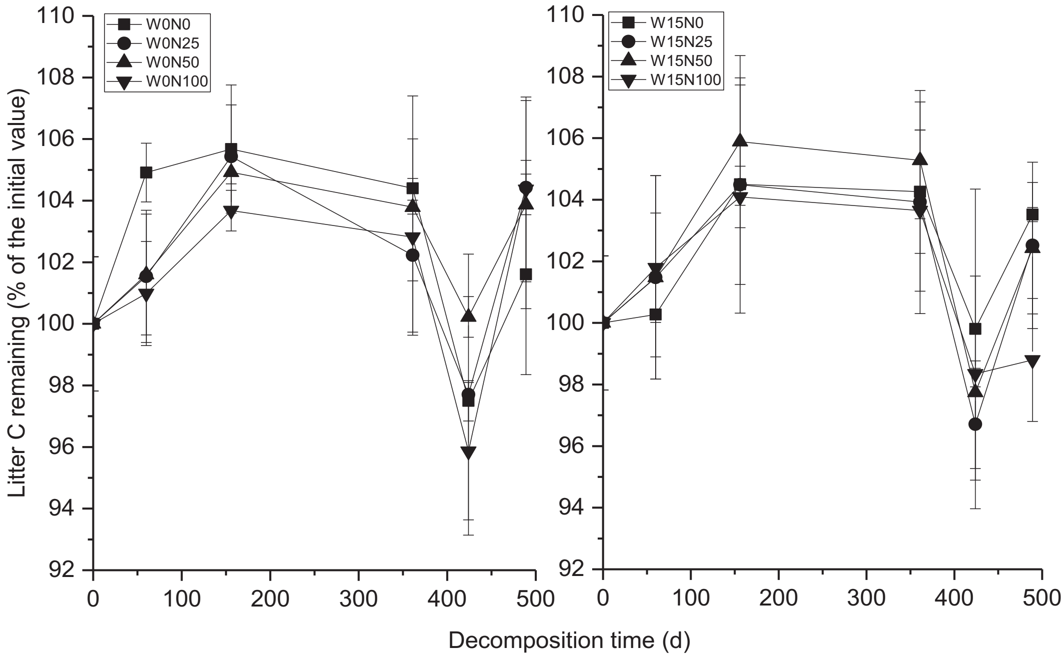

Figure 3. Remaining C in litter under different water and N treatments over the course of the experimental period. Filled symbols with error bars indicate the mean ± standard deviation ($n = 3$).

Analysis of Variance and Correlations Among Litter Residual Mass and Litter C and N Concentrations

The repeated measures ANOVA showed that litter mass loss differed significantly ($P < 0.001$) among the water treatments, with the plots that received additional water showing faster mass loss rates than those of the controls (Table 4). The effect of N fertilization on litter mass loss changed with time. As a result, the repeated measures ANOVA revealed no significant effect of N fertilization on mass loss ($P > 0.05$), but there was a significant water–N interaction ($P < 0.05$) throughout the experiment for at least the first 489 d. The organic C concentration was affected only by the combined effects of time, water, and N addition ($P < 0.05$). The results of the repeated measures ANOVA indicated that N addition had a significant effect on the N concentration of litter ($P < 0.01$), and a significant interaction was observed between precipitation and N application ($P < 0.05$). In conclusion, high N treatment (100 kg·N·ha^{-2}yr^{-1}) increased the concentration of N in the litter.

No correlation was found between the litter mass remaining and litter C concentration. Both residual litter mass and litter C concentration had significant negative correlations with litter N concentration ($P < 0.01$).

Discussion

Water availability may influence the rates of litter decomposition and nutrient release directly by affecting the activity of decomposer communities (Clein and Schimel, 1994; Berg and Laskowski, 2005) and indirectly by altering the litter quality in terms of the lignin and nutrient concentrations of plants (Austin and Vitousek, 2000). A previous

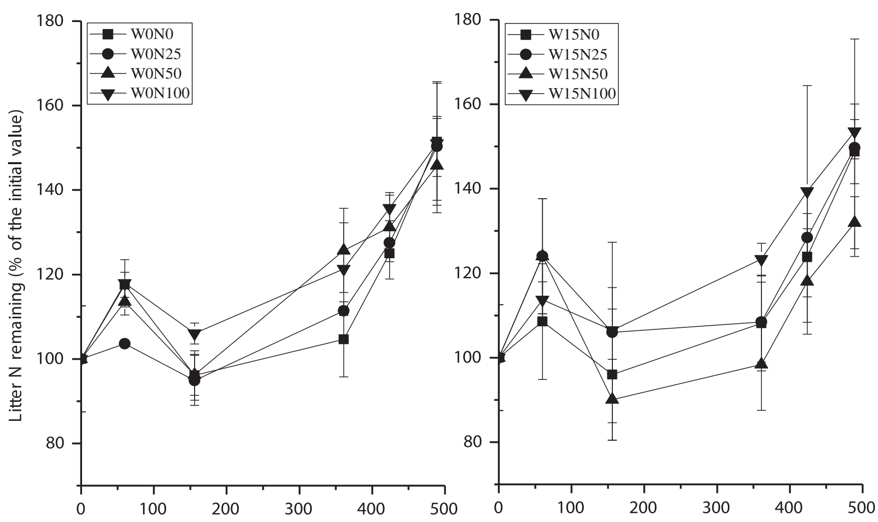

Figure 4. Remaining N in litter under different water and N treatments over the course of the experimental period. Filled symbols with error bars indicate the means ± standard deviation ($n = 3$).

Table 4
Repeated measures analysis of variance of the impact of time (T), water (W), and nitrogen (N) and their interactions on litter residual mass, litter C content, and litter N content (*F* and *P* values).

Source of variation	df	Litter residual mass		Litter C content		Litter N content	
		F	*P*	*F*	*P*	*F*	*P*
W	1	17.389	< **0.001**	0.017	0.896	1.089	0.312
N	3	2.276	0.119	1.965	0.160	5.545	< **0.01**
T	4	217.105	< **0.001**	31.399	< **0.001**	66.141	< **0.001**
W × N	3	3.850	< **0.05**	0.230	0.874	3.976	< **0.05**
T × W	4	0.374	0.826	1.206	0.317	0.444	0.776
T × N	12	0.493	0.912	0.349	0.976	0.546	0.876
T × W × N	12	0.586	0.846	2.252	< **0.05**	0.660	0.782

df = degrees of freedom (in full model). Significant effects ($P < 0.05$) are given in bold.

study (Bontti et al., 2009) suggest that with homogeneous litter quality, root litter decomposition is best predicted by the mean annual precipitation, which also suggests that high soil moisture accelerates root litter decomposition. In a tallgrass prairie, the decomposition rates of mixed litter were decreased by approximately 29% on average under-reduced precipitation (Reed et al., 2009). Increased rainfall variability can enhance the environmental conditions that mediate litter decay, but the implications of the projected changes in rainfall for litter dynamics remain poorly understood (Schuster, 2016). Specifically, total precipitation is one of the major drivers of the effects of soil fauna on litter decomposition rates on the global scale, and these effects have been shown to be more positive at warmer and wetter sites (Garcia-Palacios et al., 2013). A manipulative experiment with rainout shelters in the semiarid Patagonian steppe also found that litter decomposition was linearly related to water input (Yahdjian et al., 2006). Consistent with previous work on precipitation-induced leaching, physical fragmentation of litter enhances litter mass loss (Brandt et al., 2010). The results from this study support that water addition significantly increased litter decomposition at the initial stage of the decay process ($P < 0.001$; see Table 4). It is generally accepted that N fertilization among pastures increases the size and quality of soil organic matter pools (Malhi et al., 1997) and the supply of soil N, which sustain plant productivity and litter decomposition (Gill et al., 1995; Hatch et al., 2000). However, many previous studies have also shown that increased N deposition has no effect (Johannesmh et al., 2007; Knorr et al., 2005; Liu et al., 2007) or a negative effect on the rate of litter decomposition (Aerts et al., 2003; Knorr et al., 2005; Hobbie, 2008). Moreover, Berg and Matzner (1997) found a positive response to N in the initial decomposition phase, but a negative response in later stages. Specifically, N generally reduced the decomposition rates of substrates with high lignin concentrations but stimulated the decomposition rates of substrates with low lignin concentrations (Hobbie, 2008). Finn et al. (2015) observed that the extent of decomposition of pasture and crop residue was primarily dependent on plant species and the soil C/N ratio, but the addition of urea-N to soils with low C/N ratios had no effect on the decomposition of plant material (Finn et al., 2015). In our study, the soil C/N ratio was 10.54 ± 0.58, and the results of our work generally agree with previous results in that we did not find significant differences in litter decomposition rates under different N treatments. This result may be explained by differences in the effects of N addition on the production of decomposer enzymes, which are also partially regulated by the magnitude of N deposition relative to historical N availability. In addition, litter chemistry and decomposition rates may not respond to N addition as strongly under dry conditions (Schuster, 2016). Moreover, the main factor limiting litter decomposition often varies by decomposition stage (Liu et al., 2007). In a California annual grassland, N addition was found to interact with water addition to increase the decomposition of grass litter (Henry et al., 2005). We found that only the W0N25 treatment resulted in a slight decrease of 4.32% in the rates of litter decomposition compared with that of the controls, and the other treatments resulted in nearly the same rates as those of the ambient control plots. This finding is generally consistent with those of many other studies showing small or no responses of litter decomposition to increasing soil N input (Pastor et al., 1987; Theodorou and Bowen, 1990; Downs et al., 1996). One of the possible explanations could be that other nutrients, such as phosphorus, were more limiting for decomposer microbes as found by other investigators (Ostertag and Hobbie, 1999; Cleveland et al., 2002). This study also demonstrates that the addition of both water and N can lead to interactive effects on litter decomposition ($P < 0.05$; see Table 4).

In the Central Grassland region of North America, the C/N ratio in litter increased with increasing precipitation (Murphy et al., 2002). In the semiarid Patagonian steppe, in plots that received ambient control and 30% rainfall interception, the litter initially immobilized N and then shifted to releasing N after 7 months. However, no N immobilization was recorded in the 55% and 80% rainfall interception plots, and the amount of N released was lower in the 80% rainfall interception plot than that in the 55% interception plot, although the differences were not statistically significant (Yahdjian et al., 2006). Consistent with the findings of previous studies that C loss in decomposing litter was not affected by exogenous N addition (Peng et al., 2014), the C content in our study was not affected by water and N addition, at least during the first 2 yr. The effect of chronic N addition on C losses from ecosystems through decomposition is poorly understood. Indirect evidence suggests an important role for N restriction on decomposition as litter often immobilizes N during the early stages of decomposition, suggesting that fresh litter contains insufficient N to meet the growth and maintenance requirements of decomposers (Gosz et al., 1973; Staaf and Berg, 1982; Parton et al., 2007), although contrasting results have also been found (Pastor et al., 1987; Aerts, 1997). Consistent with our hypothesis, the N concentration was increased by N fertilization ($P < 0.01$; see Table 4). The reason may be that although the C:N ratio of microorganisms is not constant, fungi can have broad C:N ratios ranging from 4.5: 1 to 15:1, and bacteria usually have a range of 3:1 to 5:1 (Paul and Clark, 2014), but the C:N ratio (~89) of the litter in our study was much higher, suggesting that the N concentration in litter is not sufficient to satisfy microbial requirements. Accordingly, many studies have shown that microorganisms import N from the soil to the decomposing litter (Hart and Firestone, 1991; Frey et al., 2000) and that N fertilization increased the available N in soil for microorganisms, leading to increased N accumulation in litter.

Litter C and N concentrations are key predictors and regulators of decomposition worldwide (Parton et al., 2007). Instead of using initial litter chemistry to predict decomposition rates, changes in litter quality should be analyzed throughout the process of decomposition to account for the effects of litter chemistry complexity (Wickings et al., 2012). Furthermore, the litter decomposition rate has been negatively correlated to lignin/N and C/N ratios and positively correlated to N content (Houghton et al., 2001; Manzoni et al., 2008). Berg and Laskowski (2005) showed that the N content in litter samples increases with decomposition time. They argued that litter is colonized by decomposing organisms, and since N is usually a limiting nutrient for soil biota, it may be actively brought into the decomposing material through ingrowing fungal mycelia. As a result, the N content in the whole sample (including the litter substrate and the decomposers) increased. In semiarid environments, N accumulation during the decomposition of some plant materials may be attributed mainly to microbial activity (Chen and Stark, 2000). Portillo-Estrada (Portillo-Estrada et al., 2016) also reported that decomposition had almost no effect ($r^2 = 0.008$) on the C content in grass and confirmed the positive trend of N content over decomposition time ($r^2 = 0.55$) and the cumulative mass loss in litter ($r^2 = 0.63$). Consistent with part of our second hypothesis, our results (Table 5) indicated a positive significant relationship ($P < 0.01$; $r^2 = 0.605$) between litter mass loss and N content, but no relationship ($P > 0.05$; $r^2 = 0.106$) between litter mass loss and C content over decomposition time. Given the tight association between mass loss and litter N dynamics, the litter N concentration may reflect the stage of litter decomposition, although this remains to be demonstrated.

Table 5
Results of correlation analyses of the litter mass remaining, litter C concentration, litter N concentration, and C/N ratios under different water and N treatments over the course of the experimental period.

	Litter mass remaining	Litter C content	Litter N content
Litter mass remaining	1		
Litter C concentration	0.106	1	
Litter N concentration	−0.605[1]	−0.297[1]	1

[1] Significance at $P < 0.01$.

It should be noted that this study was conducted over a relatively short period and the results may only regionally apply to the early stage of litter decomposition. Different effects of water and N addition on litter decomposition at later stages may occur, warranting further research.

Implications

The 489-d field experiment carried out in this study showed that water addition increased litter mass loss significantly, whereas N addition did not inhibit litter decomposition but increased the N concentration of litter. Furthermore, a repeated measures ANOVA showed that the interaction effects of water and N were significant in terms of both mass loss and litter N content. The decomposition pattern under each treatment was well described by a linear decay function in the short term. Significant negative correlations were observed between residual litter mass and N content. The influence of precipitation on litter decomposition was greater than that of N addition. Moreover, the correlation of litter quality loss with N storage is greater than that with C content. Our results indicate that the long-term effects of climate and litter quality on litter decomposition in grasslands must be assessed to accurately predict litter decomposition under global climate change because the interactions between climate and litter quality result in complex decomposition processes, and it is difficult to evaluate the relative importance of each factor on litter decomposition. The other biotic and abiotic factors should also be evaluated and included in models to predict litter decomposition in grasslands, and a full understanding of C and N dynamics and the corresponding mechanisms require further research in the future, especially given the increased N deposition in recent years through human activities. Our results also highlight the necessity of long-term trials.

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