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SHORT COMMUNICATION

Nonadditive effects of litter mixtures on decomposition and correlation with initial litter N and P concentrations in grassland plant species of northern China

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Abstract We studied the occurrence of nonadditive effects of litter mixtures on the decomposition (the deviation of decomposition rate of litter mixtures from the expected values based on the arithmetic means of individual litter types) of litters from three plant species (i.e., *Stipa krylovii* Roshev., *Artemisia frigida* Willd., and *Allium bidentatum* Fisch. ex Prokh. & Ikonn.-Gal.) endemic to the grassland ecosystems of Inner Mongolia, northern China and the possible role of initial litter N and P on such effects. We mixed litters of the same plant species that differed in N and P concentrations (four gradients for each species) in litterbags and measured mass losses of these paired mixtures after 30 and 80 days under field conditions. We found the occurrence of positive, nonadditive effects of litter mixtures and showed that the magnitude of the nonadditive effects were related to the relative difference in the initial litter N and P concentrations of the paired litters.

Keywords Decomposition · Grassland ecosystems · Litter mixture effects · Litter N · Litter P · Inner Mongolia

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Introduction

Litter decomposition controls nutrient and C cycling in most ecosystems (Swift et al. 1979; Berg and McClaugherty 1989; Sun et al. 2004). For a given ecosystem, the rate of litter decomposition is mostly affected by litter quality (Meentemeyer 1978; Aerts 1997; Huang et al. 1998; Liu et al. 2006). Many studies (e.g., Blair et al. 1990; Briones and Ineson 1996; McTiernen et al. 1997; Wardle et al. 1997; Robinson et al. 1999; Quested et al. 2002; Hoorens et al. 2003; Smith and Bradford 2003) have demonstrated that the observed decomposition rate of litter mixtures often differs from the expected values calculated as the arithmetic means of individual litter types—a phenomenon known as litter mixture effects or more specifically, nonadditive effects of litter mixtures on decomposition. Differences in the initial litter chemistry have been suggested to account for the litter mixture effects (McTiernen et al. 1997; Hättenschwiler and Vitousek 2000; Hector et al. 2000).

Litter chemistry especially N is known to affect the decomposition of individual litter types (Berg 1986; Fog 1988; Taylor et al. 1989; Kemp et al. 1994). Under many conditions, litter P may also affect decomposition, especially in P-deficient ecosystems (Gijsman et al. 1997; Moretto et al. 2001; Liu et al. 2006). Smith and Bradford (2003) tested the hypothesis that differences in N concentration between litter types could cause positive, nonadditive effects of litter mixtures. However, their study did not yield clear evidence to support this hypothesis. A similar study by Hoorens et al. (2003) also found no apparent linkage of the difference in initial single litter chemistry of component species with the nonadditive effects of litter mixtures. How common are the nonadditive effects of litter mixtures on decomposition across different plant species? Do key chemical compounds of plant structure and biological significance contribute to the litter mixture effects? Information is generally lacking to address these questions.

The grasslands of Inner Mongolia in northern China contain many plant species with diverse growth forms and litter qualities. Our previous study based on the litter of single types revealed marked differences in the rates of decomposition between two contrasting grassland plant species (Liu et al. 2006). However, information on the occurrence of litter mixture effects and the contributing factors is lacking for this grassland ecosystem, which constrained our ability to understand better the regional C and nutrient cycling.

In this study, we examined the occurrence of nonadditive effects of litter mixtures on the decomposition of litters from three plant species, *Stipa krylovii* Roshev., *Artemisia frigida* Willd., and *Allium bidentatum* Fisch. ex Prokh. & Ikonn.-Gal., which are endemic to the grassland ecosystems of northern China; the possible role of litter N and P on such effects was also studied.

Materials and methods

This study was conducted at an experimental field site (latitude, 42°02′N; longitude, 116°16′E; altitude, 1344 m a.s.l.) of the Duolun Restoration Ecology Experimentation and Demonstration Station of the Institute of Botany, the Chinese Academy of Sciences, in Inner Mongolia. The area is in a semiarid and temperate zone with a typical continental climate. Long-term mean of annual precipitation is 385 mm, and mean annual, minimum, and maximum air temperatures are 1.6, −18.3, and 18.7°C. Soils of the region are classified as chestnut (FAO-UNESCO 1974). The surface 10-cm soil layer is slightly alkaline (pH=7.2) and contains 20.4 g kg^{-1} total C, 1.63 g kg^{-1} total N (with available N at 10.35 mg kg^{-1}), and 0.31 g kg^{-1} total P (unpublished data). The soil bulk density is ~1.3 g cm^{-3}.

The three plant species used in this study, *S. krylovii*, *A. frigida*, and *A. bidentatum*, are common plants of the local plant communities but with contrasting growth forms, with *S. krylovii* being a perennial bunchgrass, *A. frigida* a semishrub, and *A. bidentatum* a perennial forb. Our study design was paired-mixing of litters with four different N and P concentrations within each of the three species. Litters were collected as senescent leaves in mid-August 2004 from a fertilization experiment with plots differing in addition rates of mixed N and P fertilizers (i.e., A, 0 addition; B, addition of 8 g N and 4 g P m^{-2}; C, addition of 16 g N and 8 g P m^{-2}; and D, addition of 32 g N and 16 g P m^{-2}). Nitrogen was added as granular urea and P in the form of granulated triple superphosphate, both being applied in early July 2004. Litters of the same plant species were separated by the addition rate of mixed N and P fertilizers and differentiated as four individual litter qualities within each species (Table 1). Litter chemistry was analyzed on five oven-dried subsamples (50°C to constant mass), using the semimicro Kjeldahl method for total N, and the molybdenum blue colorimetric method for total P at the time of litterbag deployment in the field.

Litter decomposition was determined using the litterbag method under field conditions in a randomized block design with five replicated plots. Each litterbag (10×15 cm

Table 1 Initial litter N and P concentrations for the three grassland plant species of Inner Mongolia, China

Species	Quality	N	P	Mass remaining (%)	
		(mg g^{-1})	(mg g^{-1})	30 days	80 days
Stipa krylovii	A	4.79±0.03 l	0.42±0.04 l	95.1±1.23	87.6±1.92
	B	6.92±0.04 k	0.88±0.04 k	93.9±1.70	85.8±2.40
	C	7.79±0.06 j	1.04±0.05 j	93.4±0.87	85.3±0.77
	D	8.58±0.05 i	1.13±0.04 i	92.6±1.69	83.7±1.52
Artemisia frigida	A	19.39±0.11 f	2.07±0.06 g	86.1±2.92	72.9±2.58
	B	23.09±0.18 c	3.37±0.05 e	83.7±4.20	70.3±1.31
	C	28.53±0.15 b	5.04±0.08 b	81.7±3.50	68.5±1.07
	D	34.10±0.10 a	5.75±0.06 a	78.3±3.84	67.6±1.72
Allium bidentatum	A	15.17±0.09 h	1.92±0.07 h	87.2±4.01	76.3±3.68
	B	17.62±0.11 g	2.85±0.09 f	85.6±5.89	74.3±4.36
	C	20.37±0.09 e	3.56±0.08 d	83.0±5.11	72.1±3.46
	D	22.94±0.12 cd	4.22±0.06 c	81.8±5.97	71.2±1.86

Each species includes four distinct litter qualities (designated as A, B, C, and D) with different N and P concentrations. Data in the same column followed by the same letter are not significantly different ($P<0.05$). Litter mass remaining after 30 and 80 days of decomposition are also shown. Values are means ± SE ($n=5$).

dimension), made of a 1×1-mm polyethylene mesh, contained a total 3 g of air-dried litter sample, either pure or in a 50:50 ratio of paired mixture (mass basis) of two litters in a well-mixed state. The paired-mixing of four individual litters within each species produced six litter mixtures as A + B, A + C, A + D, B + C, B + D, and C + D. The filled litterbags were placed and secured on a soil surface by wire hooks in a designated area in the field on 4 July 2005 with each sample type in duplication on each of the five 4×4-m plots. Duplication of litter samples was for the purpose of retrieval at two different times after being deployed. In the laboratory, extraneous matter such as other plant materials, rocks, and soil animals were handpicked from the decomposed litters, and the clean samples were then oven-dried at 50°C to a constant mass. The initial air-dried litter mass was converted to an oven-dried mass based on previously determined conversion factors for the litter samples.

The expected mass remaining of the composite litters was calculated as (Hoorens et al. 2003):

$$\text{Expected mass remaining}(\%) = [M_1/(M_1+M_2)] \times R_1 + [M_2/(M_1+M_2)] \times R_2$$

where R_1 and R_2 are the remaining mass (%) for the litter type 1 and 2, respectively, and M_1 and M_2 are the estimated initial dry mass of these litters in the mixture. R_1 and R_2 were determined from the single-litter bags.

Bivariate correlations were performed to determine the relationship between the decomposition rate and the initial litter N and P concentrations in the single-type litter bags for each retrieval time. Differences between observed and expected mass remaining of litter mixtures were tested by one-way analysis of variance. Linear regression and correlation analyses were used to determine whether the relative difference between the expected mass remaining (MR_{Exp}) and the observed mass remaining (MR_{Osb}), $(MR_{Exp} - MR_{Obs})/MR_{Exp}$, were related to the differences in the initial N and P concentrations between the litter in the mixture across all plant species. These statistical analyses were performed using procedures of SPSS (v. 11.0), and the accepted significance level was $P<0.05$.

Results

The litter samples in this study had a wide range of initial N and P concentrations owing to the differences between species and the fertilizer treatments (Table 1). The litter of *A. frigida* had the highest levels of both N and P, and *S. krylovii* had the lowest levels. The three species differed

Table 2 Observed and expected mass remaining of the litter mixture samples after 30 and 80 days of decomposition for the three grassland plant species of Inner Mongolia, China

Species	Litter mixture	30 days		80 days	
		Observed	Expected	Observed	Expected
Stipa krylovii	A + B	93.0±1.1	94.5±1.5	84.0±1.3	86.7±0.9**
	A + C	93.0±0.6	94.3±0.9*	84.0±0.9	86.4±0.6**
	A + D	91.3±1.4	93.8±1.2*	82.5±1.5	85.6±1.6*
	B + C	92.0±1.4	93.6±1.1	83.6±1.6	85.6±1.5
	B + D	91.6±1.2	93.2±1.4	82.1±1.4	84.7±1.3*
	C + D	91.6±1.0	93.0±0.8*	82.3±0.9	84.5±0.5**
Artemisia frigida	A + B	82.1±1.6	84.9±1.8*	67.9±1.2	71.6±1.5**
	A + C	80.7±1.6	83.9±2.4*	66.3±1.1	70.7±1.2***
	A + D	78.2±1.2	82.2±2.0**	65.9±1.0	70.3±1.5**
	B + C	80.4±2.6	82.7±2.3	65.6±1.5	69.4±0.8**
	B + D	78.0±2.2	81.0±3.4	64.7±1.6	69.0±1.4**
	C + D	77.8±2.1	80.0±3.3	65.1±1.6	68.0±1.2*
Allium bidentatum	A + B	83.1±4.1	86.4±4.9	71.6±3.1	75.3±3.5
	A + C	81.9±3.0	85.1±3.5	70.7±2.1	74.2±1.7*
	A + D	81.3±3.7	84.5±3.9	69.7±2.4	73.8±2.2*
	B + C	82.2±2.8	84.3±4.5	69.3±1.6	73.2±1.8**
	B + D	80.1±4.2	83.7±4.8	69.1±2.3	72.8±2.3*
	C + D	80.4±3.2	82.4±3.7	68.6±2.6	71.7±2.3

Values shown are means ± SE (n=5). Asterisks indicate levels of significance for differences between observed and expected mass remaining of the same retrieval point.

*$P<0.05$

**$P<0.01$

***$P<0.001$

greatly in the rate of litter decomposition, which was apparently related to the litter quality in terms of the initial N and P concentrations (Table 1). The correlation coefficients (r) of the litter mass remaining were −0.98 and −0.96 with the initial N concentration and −0.96 and −0.92 with the initial P concentration after 30 and 80 days of decomposition, respectively, across all species ($P<0.01$).

The mixed litter samples decomposed faster than the expected values as inferred from the data on the litter mass remaining, especially at the 80-day sampling point (Table 2). Linear regression analysis of the relative difference between expected and observed mass remaining with a difference between initial N and P concentrations of the paired litter in the mixture across the three plant species showed significant ($P<0.001$) and positive relationships, with the relationship being stronger at 80 days than at 30 days (Fig. 1).

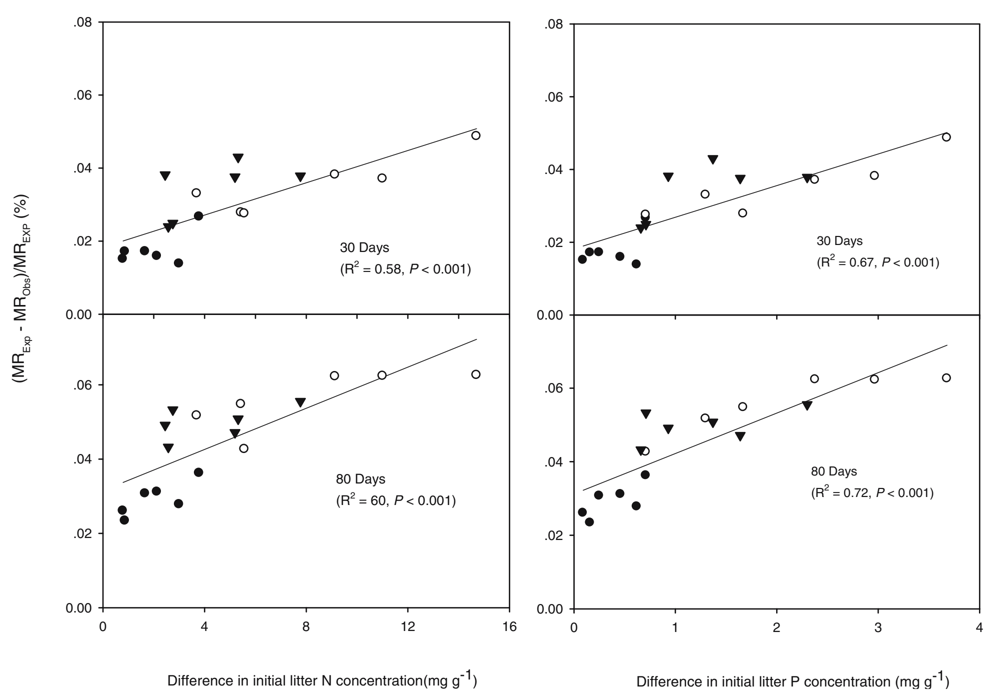
Discussion

Chemical control of nonadditive effects of litter mixtures has remained a controversial issue (e.g., Wardle et al. 1997; Hoorens et al. 2003; Smith and Bradford 2003). Hoorens et al. (2003) studied the decomposition of a wide array of two-species litter mixtures in two different ecosystems to test whether or not interactions in litter mixtures were driven by differences in initial litter chemistry. They found that the nonadditive effects (positive or negative) existed in many of the litter mixtures, and that although initial litter C, P, and phenolic concentrations were significantly correlated with decomposition rates, they were not related to the nonadditive effects of litter mixtures. It was suggested that the presence of interactions among multiple constituents hindered a clear interpretation of the effects from specific chemical properties of litter (Hoorens et al. 2003).

In our study, we mixed the litter of the same species with different N and P concentrations. Although we only measured N and P concentrations in response to soil N and P fertilizer applications, there has been clear evidence that N and P fertilization results in little changes in other chemical compounds critical to litter decomposition. For example, Kemp et al. (1994) found that after 6 months of N fertilization at either ambient or elevated CO_2, the content of soluble organic compounds was only slightly increased, while cellulose and lignin contents were slightly decreased in three tallgrass prairie leaf litters. Aerts et al. (2003) found that N and P fertilization for 12 years did not produce a detectable effect in the concentrations of phenolic compounds and lignin content of bulk litters from two grasslands differing in soil nutrient status.

We found that initial litter N and P concentrations were not only good predictors of decomposition rate but also significantly correlated with the nonadditive effects of litter mixtures. During decomposition, there is an obvious demand for N by active heterotrophs, and thus, litter with higher initial N concentration (lower C/N ratio) usually decomposed faster in the early stage of decomposition (Richards 1987). When being mixed together during decomposition, litters of high N and P concentrations might induce a priming effect on the litters of low N and P concentrations, facilitating faster decomposition of the low quality litters without necessarily

Fig. 1 Relationship of the relative difference between expected mass remaining (MR_{Exp}) and observed mass remaining (MR_{Obs}), expressed as (MR_{Exp} − MR_{Obs})/MR_{Exp}, of the mixed litter with the difference in initial N and P concentrations between the paired litter in the mixture after 30 and 80 days of decomposition in three plant species (*Stipa krylovii* [*closed circle*], *Artemisia frigida* [*open circle*], and *Allium bidentatum* [*closed triangle*] of grassland ecosystems in Inner Mongolia, China



retarding the decomposition of the high quality litters (Seastedt 1984). Mixing litters of different qualities could also change the quantity, composition, and activities of decomposer organisms, which partly contribute to the nonadditive effects of litter mixtures. Research by others has demonstrated greater numbers of microarthropods (Thomas 1968), higher litter heterotrophic activity (respiration), and greater numbers of selected invertebrate groups in litter mixtures than in single-litter types (Chapman et al. 1988; Salamanca et al. 1998). Blair et al. (1990) found that lengths of fungal hyphae, bacterial numbers, nematodes and microarthropod abundances of litter-mixture bags were different than those of single-litter bags.

In a study similar to ours, Smith and Bradford (2003) mixed grass litter of the same plant species that differed in initial N concentration to investigate whether initial N concentration accounted for positive, nonadditive effects of litter mixtures; they found inconsistent interaction patterns across different mixtures. In this study, we found a positive, nonadditive effect of initial litter N on mixed litter decomposition. The different outcomes between this study and that by Smith and Bradford (2003) may relate to differences in the litter decomposition phases. Although the study of Smith and Bradford (2003) only lasted for 60 days, after 30 days of decomposition, more than half of their selected litters had decomposed, and after 60 days, only about 30% were left. On the contrary, in our study, after 80 days of decomposition, more than 70% of litter mass of the three plant species was still undecomposed. It is generally believed that in the initial stages of decomposition, N can be a good predictor of decomposition rate, whereas in the later stages, chemical compounds such as lignin can play a more important role. Furthermore, excess N might react with chemical compounds such as the breakdown products of lignin degradation to form recalcitrant complexes, which retard litter decay (Berg 1986; Fog 1988).

In this study, the litter decomposition rates seemed a little faster than those of other similar studies in the grasslands of northern China. After 80 days of decomposition, the mass losses for leaf litter of *S. krylovii*, *A. frigida*, and *A. bidentatum* were around 15, 30, and 25%, respectively. In our previous study (Liu et al. 2006), litters of *S. krylovii* (mainly stems) and *A. bidentatum* (mainly leaves) lost about 12 and 27%, respectively, of their original weight after 100 days of decomposition under a natural environment. Chen and Huang (1992) found that there was only about 20% of initial litter mass decomposed after 100 days of decomposition. The fertilizer treatment of the sites where the litter were collected might have enhanced the overall quality of the litter samples used in this study, making them more prone to microbial colonization. The use of leaf litter might have also contributed to the faster litter decomposition in this study.

Our results also indicated that the nonadditive effects of litter mixture on decomposition varied with time (Table 2). While a comprehensive explanation is somewhat constrained by our study design, we may speculate that the biotic degradation of organic compounds could be responsible for the time dependence of the nonadditive effects of litter mixtures (Vossbrinck et al. 1979; Blair 1988).

In conclusion, results of this study showed the occurrence of mixture effects in litters of three grassland plant species with contrasting growth forms, and that the magnitude of the nonadditive effects of litter mixtures was related to the difference in the initial litter N and P concentrations of the two litters of the paired-mixing.

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