



Species Diversity Induces Idiosyncratic Effects on Litter Decomposition in a Degraded Meadow Steppe

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Litter decomposition is a fundamental path for nutrient cycling in a natural ecosystem. However, it remains unclear how species diversity, including richness and evenness, affects the decomposition dynamics in the context of grassland degradation. Using a litter bag technique, we investigated the litter-mixing effects of two coexisting dominant species (*Leymus chinensis* Lc and *Phragmites australis* Pa), as monocultures and mixtures with evenness (Lc:Pa) from M1 (30:70%), M2 (50:50%), and M3 (70:30%), on decomposition processes over time (60 and 365 days). The litter bags were placed on the soil surface along a degradation gradient [near pristine (NP), lightly degraded (LD), and highly degraded (HD)]. We found that 1) mass loss in mixture compositions was significantly and positively correlated with initial nitrogen (N) and cellulose contents; 2) litter mixing (richness and evenness) influenced decomposition dynamics individually and in interaction with the incubation days and the degradation gradients; 3) in a general linear model (GLM), nonadditive antagonistic effects were more prominent than additive or neutral effects in final litter and nutrients except for carbon (C); and 4) in nutrients (C, N, lignin) and C/N ratio, additive effects shifted to nonadditive with incubation time. We speculated that the occurrence of nonadditive positive or negative effects varied with litter and nutrients mass remaining in each degraded gradient under the mechanism of initial litter quality of monoculture species, soil properties of experimental sites, and incubation time. Our study has important implications for grassland improvement and protection by considering species biodiversity richness, as well as species evenness.

Keywords: litter mixing, nutrient cycling, litter quality, evenness, non-additive effects, incubation time, grassland

INTRODUCTION

Grasslands cover approximately 40% of earth's land surface (Huang et al., 2012) and are of critical importance to the maintenance of biogeochemical cycles in grassland ecosystems (Rumpel et al., 2015). However, most grasslands now suffer from unprecedented changes (i.e., degradation or desertification) in biodiversity, ecosystem functions, and structure across the globe (Maxwell et al., 2016). In grassland, litter decomposition is a pivotal process that controls the balances of nutrient

cycling and energy flow (Zeng et al., 2018). Like other ecosystems with higher plant diversity, the litter on grassland floor naturally decomposes together (Mao and Zeng, 2012). Usually, biodiversity in an area is dependent on two dimensions, species richness (number of plant species) and evenness (relative abundance of plant species) (Munoz et al., 2018). Nonetheless, previous studies have focused on the contribution of species richness (Ball et al., 2008; Lecerf et al., 2011; Wu et al., 2013; Mao et al., 2015), and the contribution of species evenness in the decomposition process remains understudied.

Litter decomposition is a process in which 50–99% of the above-ground biomass becomes part of the detritus food chain and decompose into nutrients (Gessner et al., 2010; Jacobson et al., 2015). Plant litter decay is controlled by abiotic (prominently soil moisture, temperature, and soil C or N contents) and biotic (species diversity and composition, litter quality, and decomposers community) factors (Meier and Bowman, 2008; Gessner et al., 2010; García-Palacios et al., 2016). For instance, favorable humidity and temperature can enhance litter decomposition by reducing moisture and thermal limitations on the activities of decomposers, i.e., bacteria and fungi (Liski et al., 2003; Smith et al., 2010). Abiotic drivers often influence decomposition at a larger scale, while the impacts of biotic factors become more noticeable as the scale decreases (Zhang et al., 2013). Biotic factors, such as the quantity and quality of plant species, regulate the mass dynamics within a given climatic condition (Austin et al., 2014). In many grasslands, a litter with lower carbon to nitrogen (C/N) ratios tends to have an accelerated decomposition process, as N contents in plant tissues can improve litter decay (Penner and Frank, 2018). On the other hand, recalcitrant C and lignin in plant litter hinders the decay process (Gessner et al., 2010). Some studies showed that plant diversity impacted litter decomposition through changing microclimatic conditions, rhizosphere processes, and complimentary nutrient use (Hättenschwiler and Gasser, 2005; Santonja et al., 2018; Yang et al., 2019). The interactions between litter quality and ecological processes together explain approximately 70% of global litter decay (García-Palacios et al., 2013).

Litter mixing effects are usually calculated as the deviation of observed decomposition rates of mixed litter from the predicted values based on the arithmetic means of individual species decomposing alone (Liu et al., 2007). However, it has been pointed out that the decaying of plant species in the mixture is difficult to predict by a simple arithmetic mean of species decomposing in isolation (Lecerf et al., 2011; Zhang et al., 2019). The reasons of relative unpredictability may include variations in climatic conditions, experimental duration, and selection of litter component (Lecerf et al., 2007; Srivastava et al., 2009; Lecerf et al., 2011). Initial nutrient contents (litter quality) of the component species are the critical aspects affecting the decomposition (Penner and Frank, 2018; Luai et al., 2019). The litter that possesses high nutrient concentrations, low recalcitrant compounds, and low C/N ratios is considered as high-quality litter. When the litter of several plant species are mixed under natural conditions, C and other beneficiary nutrient contents can be transformed from high-to low-quality litter. This occurs

because of the effects of secondary metabolites and microbial activities alteration (Pérez-Harguindeguy et al., 2007; Chen et al., 2019). As a consequence, the decomposition rate of low quality can be suppressed or enhanced depending upon the inhibitory or stimulating nature of the compounds transformed (Wu et al., 2013). Changes in species evenness can alter litter decomposition depending on the dominancy of plant species in the mixture (Li et al., 2013). A high proportion of nutrient-rich species in a mixture has been shown to intensify microbes' activities and thereby enhance the decay rate (Chen et al., 2019). Ward et al. (2010) found that plant richness with varying ratios increased decomposition rates when dominant species identity was the prime factor, suggesting that the evenness effect could be important.

Generally, grassland has a variety of biomes ranging from near-pristine to degraded lands due to negative changes (e.g., degradation) in vegetation and soil due to overgrazing (Wang and Ba, 2008; Peng et al., 2020), resulting in widescale of soil salinization (Huang et al., 2012). The decomposition process varies with the degrees of degradation and how plant and soil interact with each other (López-Pujol et al., 2011). In the meadow steppe ecosystem, plant diversity at different sites should not be neglected in litter decomposition experiments because richness and evenness both impact the magnitude and direction of litter mixing (Chen et al., 2019). Despite this, the relationship between plant diversity and degradation gradients has received insufficient attention in previous studies (Yi et al., 2012; An et al., 2019). Furthermore, a better understanding of their association may provide important information for effectively restoring or conserving the degraded grasslands (Yang et al., 2020).

Previous studies in both field and laboratory microcosms have reported positive or neutral effects of plant diversity on decomposition due to interactive effects of litter assemblage (King et al., 2002; Dickson and Wilsey, 2009; Paudel et al., 2015), but the underlying mechanisms are not well understood. Specifically, we considered the following questions: 1) the differences in initial nutrient contents and C/N ratio between species across degraded grasslands, 2) mass and nutrients dynamics in monoculture and mixture treatments among incubation time and degradation gradients, and 3) effects of litter quality and soil properties on decomposition along with degradation gradients. The main question we sought to address was whether litter mixing induces additive or nonadditive effects on litter and nutrients mass along degradation gradients. If nonadditive, will these effects be antagonistic or synergistic? On the basis of the literature reviewed (Pérez-Harguindeguy et al., 2007; Wu et al., 2013; Chen et al., 2019), we hypothesized that nonadditive, antagonistic effects would prevail and that additive effects would shift to nonadditive effects with incubation time.

MATERIALS AND METHODS

Study Site

The litter decomposition experiment was conducted at a meadow steppe in the Grassland Ecological Research Station

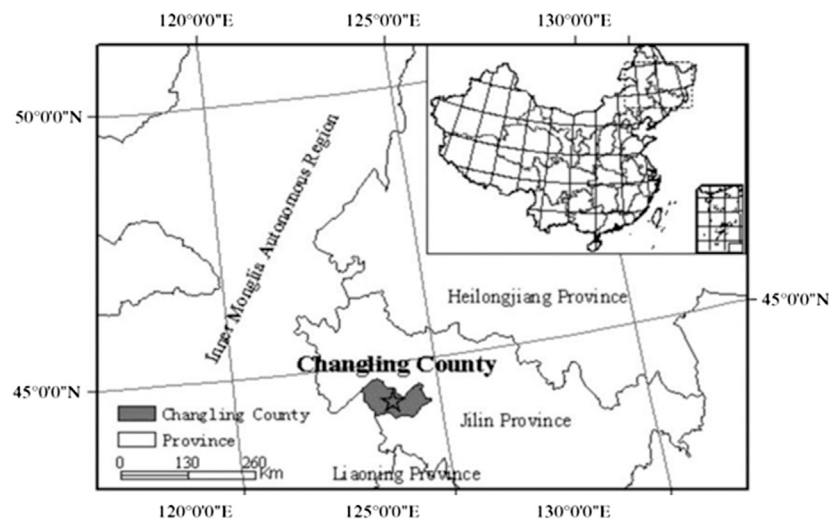


FIGURE 1 | Geographical map of study site.

(44°31'–44°46' N, 123°32'–123°57' E, elevation of 130–160 m above sea level) of Northeast Normal University, Changling County, Jilin Province, PR China (**Figure 1**). The climate of the study site is semiarid monsoon with mean annual temperature ranging from 4.6 to 6.4°C. Mean annual precipitation ranges 280–400 mm, and 70–80% of precipitation occurs during June–August. Annual potential of evaporation is 840–1,200 mm, and about 225 days are frosty or snowy. Soil is saline, and alkaline, with pH ranging from 8.2 to 10.6 (Mei et al., 2019). The soil in the study area possesses low N, phosphorus, calcium, and magnesium but high C (Wang et al., 2018). The dominant grass is *Leymus chinensis* (Trin.) Tzvel followed by other subdominant plants including *Phragmites australis* (Cav.) Trin. ex Stued., *Calamagrostis epigejos* (Linn.) Roth., and *Chloris virgata* Sw. Forbs include *Potentilla flagellaris* Willd, *Kalimeris integrifolia* Turcz., and *Carex duriuscula*, while legumes include *Lespedeza davurica* (Laxm.) Schindler and *Lathyrus quinquenervius* (Miq.) Litv.

Experimental Design

A fractional factorial experiment was conducted for 1 year (July 2018–July 2019) on near-pristine, lightly or moderately degraded, and highly degraded (NP, LD, and HD, respectively) sites by following a litterbag technique. Soil degradation in the studied area is due to alkaline soil and overgrazing (Wang and Ba, 2008). The three sites had different soil properties (**Supplementary Table S1**). Although many grasslands have experienced harsh conditions like cold climate and poor soils, some species can still cope with such environmental stresses and thrive (Mei et al., 2019). At all microsites, dominant and codominant plants include perennial grasses *L. chinensis* and *P. australis*, which often co-occur (Wang et al., 2018). *L. chinensis* is a dominant grass species of meadow steppe, while *P. australis* tolerates a variety of climatic conditions (Zhang et al., 2018). Nine plots (25 × 25 m) in total, three for each site, were set up and fenced in summer, 2017. Each

plot contains three randomly selected 5 × 5 m subplots separated by 50 × 250 cm wide buffers.

Soil and Plant Sampling

In July 2019, we collected topsoil (0–10 cm) using a soil corer (diameter = 2.5 cm) in the fenced plots. Samples were brought to a laboratory, powdered, sieved (mesh size = 2 mm), and divided into two halves. One half was oven dried (65°C for 48 h) to estimate total C (TC%), total organic C (TOC%), and total N (TN %) by an elemental analyzer (Vario EL cube, Elementar Analysensysteme GmbH, Hanau, Germany), while the other half was retained at 4°C for analyzing soil pH, electrical conductivity (EC μs/cm), particle density (PD g/cm³), and moisture content (MC%) later. For the assessment of soil pH and EC, the soil/water (1: 5) suspension technique was followed (Wang et al., 2015). Soil MC was determined by an ECH₂O soil moisture sensor (EC-5, Decagon Ltd., Pullman, WA, United States). A picnometric method was used to estimate soil PD (Zygmunt et al., 2014). Soil total C/N was calculated on the basis of atomic mass as **Eq. 1**:

$$\text{Soil C/N} = \left(\frac{\text{TOC}}{12} \right) / \left(\frac{\text{TN}}{14} \right) \quad (1)$$

where TOC is total organic carbon, and TN is total nitrogen.

Freshly senesced leaves of both species were manually collected in early July 2018 from each site. The litter used in this study consisted only of leaves, while shoots and stems were removed carefully. Samples were brought to a laboratory, cleaned from debris, washed lightly with deionized water, oven dried (65°C for 48 h or until it reached to constant weight), and placed separately in an incubator. Leaf litter samples were divided into two halves. The first half was finely powdered in a ball mill grinder (MM 400, Retsch GmbH, Hanau, Germany) to estimate initial C and N concentrations (%) with the same elementar used for soil C and N analyses. The other half was ground with a

mortar and pestle to make thread-like particles for the estimation of lignin and cellulose contents (%) by an FT 121 Fibertec™ 8,000 analyzer (FOSS). We also calculated the C/N and lignin/N ratios by simply dividing C % or lignin % by N %.

Litter Bag Preparation

Senescent leaves after laboratory treatment were used for litter bag preparation. Nylon litter bags of 15 × 15 cm with a 1-mm mesh size were used. In each microsite, five compositions (two sets of monoculture species and three sets of their evenness mixtures) with triplicate were analyzed. Evenness mixtures included M1 with 30% *L. chinensis* and 70% *P. australis* (*P. australis* dominated or uneven mixture), M2 with 50% *L. chinensis* and 50% *P. australis* (even mixture), and M3 with 70% *L. chinensis* and 30% *P. australis* (*L. chinensis* dominated or uneven mixture). Each litterbag was filled with 5.00 ± 0.02 g oven-dried leaf litter of each composition. A total of 90 bags (5 compositions × 3 replications × 3 site types × 2 incubation dates) were prepared, of which 45 bags were collected after 60 days and the rest collected after 365 days. All the bags were deployed above the soil surface to give them natural conditions and on controlled sites to avoid any kind of disturbance. The litter was collected individually from each site, and their litter bags were also placed within each specific site.

Data Analyses

After collection, all litterbags were brought to a laboratory for cleaning and then transferred into paper bags. The litter was oven dried (65°C for 48 h) to measure mass and nutrient contents. Each bag sample was weighed to assess the final mass (MR) by percentage over the time of incubation in monocultures and mixtures:

$$MR\% = \left(\frac{M_o}{M_i} \right) \times 100 \quad (2)$$

where M_o and M_i are the observed and initial mass contents in grams. The final nutrient (NR) was determined based on (Bockheim et al., 1991):

$$NR\% = \left(\frac{N_o \times M_o}{N_i \times M_i} \right) \times 100 \quad (3)$$

where N_o and N_i are the observed and initial nutrient concentrations, while M_o and M_i are the same as in Eq. 2. The expected final mass (MR_{exp}) of mixture compositions was measured as (Hoorens et al., 2003):

$$MR_{exp}\% = \frac{[(M_a \times O_a) + (M_b \times O_b)]}{M_a + M_b} \quad (4)$$

where M_a and M_b are initial mass of the species in the mixture litter bags, and O_a and O_b are the final mass of the mixed litter species in the single-species litter bag. The expected final nutrients (C, N, lignin, or cellulose) were calculated same as Eq. 4 by using appropriate substitutions. We also measured the expected C/N ratio by dividing CR_{exp} with NR_{exp} . Mass loss rate by 60 and 365 days were calculated by:

$$MLrate \text{ mg g}^{-1} \text{ day}^{-1} = \frac{[(M_o - M_i)]}{M_o} \times 1000 \quad (5)$$

Statistical Analyses

Initial nutrient contents (%) and final mass or nutrients (%) in monoculture litter were statistically evaluated by *t* test. We used one-way ANOVA followed by Tukey's honestly significant difference (HSD) test to determine 1) differences among degradation gradients in initial nutrient contents of each species; 2) final litter or nutrient mass between monoculture litters; 3) final mass (%) or nutrients concentration (%) among mixture compositions; and 4) difference among pooled monoculture litters ($L_c + P_a$), M1, M2, and M3. Paired *t* test was applied to compare final mass or nutrient concentration between incubation times of mixture compositions and also for the comparison of litter decomposition between monoculture and mixture within each site type. Redundancy analysis (RDA), a constrained multivariate model, was conducted to quantify how soil properties of each site responded to litter dynamics along degradation gradients over 60 and 365 days. Pearson correlation was used to assess the relationship between initial nutrient contents and mass loss or final nutrient contents in monoculture and mixture compositions by 365 days. Moreover, multiple regression analysis was applied to assess the relationship between soil properties and litter decomposition dynamics in mono and (even or uneven) mixture treatments. Significance was calculated in all tests at $p < 0.05$.

Litter mixing effects of evenness treatments were analyzed by using two procedures. First, we applied a general linear model (GLM) Type I sums of squares (SS) to test additive or nonadditive effects on litter dynamics. In model, final litter and nutrients mass were added as a response variable to estimate the incubation days, species mixing, evenness, and site effects. Incubation days (T, two levels: 60 and 365 days), mixing term (M, two levels: observed and predicted values), evenness term (E, three levels: M1, M2, and M3), and site term (S, three levels: NP, LD, and HD) were added in the GLM model as main and two-way interaction effects. Nonsignificant values indicate additive effects, while significant values represent nonadditive effects. Second, a traditional method [i.e., O-P (observed-predicted)] was used to specifically test the data in terms of synergistic or antagonistic nonadditive effects. Classifications of litter mixing effects in the O-P method followed Gartner and Cardon (2004): O-P > 0 indicates nonadditive synergistic, O-P < 0 indicates nonadditive antagonistic, and if there is no significant difference between O and *p*, this would indicate additive effects. The one-sample *t* test was used to assess the values differing from zero.

Redundancy and multiple regression analyses were conducted in R version 3.6.1, and the significance of RDA correlations was evaluated by Monte Carlo permutation test. All the remaining statistical analyses were evaluated by using SPSS version 23.0 (SPSS Inc., United States) and presented by GraphPad prism.

TABLE 1 | Initial leaf litter nutrient contents and C/N ratio of plant species, *Leymus chinensis* and *Phragmites australis*.

Nutrients	Species	NP	LD	HD
Carbon (%)	<i>Leymus chinensis</i>	47.03 ± 0.13a **	47.57 ± 0.55a *	47.64 ± 0.28a **
	<i>Phragmites australis</i>	45.68 ± 0.27a	45.78 ± 0.16a	44.62 ± 0.43a
Nitrogen (%)	<i>Leymus chinensis</i>	2.69 ± 0.01a **	2.13 ± 0.02c *	2.35 ± 0.01 b ns
	<i>Phragmites australis</i>	2.11 ± 0.08b	1.96 ± 0.04b	2.49 ± 0.12a
Lignin (%)	<i>Leymus chinensis</i>	16.64 ± 0.05c ***	19.59 ± 0.09b ***	20.60 ± 0.12a ***
	<i>Phragmites australis</i>	15.34 ± 0.05b	15.48 ± 0.06b	16.47 ± 0.07a
Cellulose (%)	<i>Leymus chinensis</i>	41.24 ± 0.06b ***	36.83 ± 0.10c **	43.51 ± 0.13a **
	<i>Phragmites australis</i>	34.56 ± 0.09c	37.51 ± 0.06b	44.43 ± 0.08a
C/N ratio	<i>Leymus chinensis</i>	17.49 ± 0.01c **	22.37 ± 0.38a ns	20.24 ± 0.05b *
	<i>Phragmites australis</i>	21.71 ± 0.76a	23.34 ± 0.45a	17.96 ± 0.72b

Data are means ± SEM (n = 3). Lowercase letters indicate significant difference in nutrients among site types confine into each species (p < 0.05, Tukey's HSD test). Asterisks indicate significant difference between species within each site types (*p < 0.05; **p < 0.01; ***p < 0.001; and ns, not significant, t test). NP, near-pristine site; LD, light degraded site; and HD, highly degraded site.

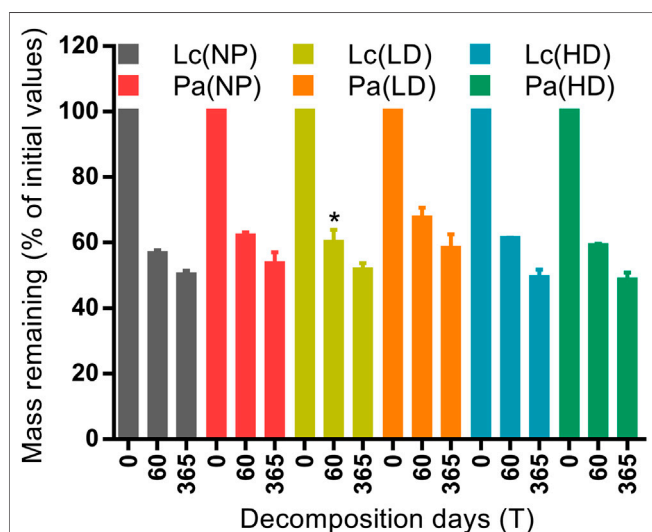


FIGURE 2 | Final mass in monoculture litters of plant species, *Leymus chinensis* (Lc) and *Phragmites australis* (Pa), over 60 and 365 days of decomposition. NP, near-pristine site; LD, light-degraded site; HD, highly degraded site. Data are means ± SEM (n = 3). Asterisks indicate significant difference between species within each site and confine into each incubation date (*p < 0.05, t test).

RESULTS

Initial Nutrient Contents

Initial nutrient contents and C/N ratio varied between monoculture litters, *L. chinensis* and *P. australis*. Nutrient concentrations including C, N, lignin, and cellulose in *L. chinensis* litter were higher than in *P. australis* litter (p < 0.001) with few exceptions (Table 1). The C/N ratio was lower in *L. chinensis* than in *P. australis*. Lignin contents were ≤20% in both species, and cellulose contents were almost twice as much as lignin. Among sites, initial nutrient contents, except for C, were statistically different. N contents in *L. chinensis* and *P. australis* were highest in NP and HD with the order of NP > HD > LD and HD > LD = NP, respectively. In contrast, lignin and cellulose concentrations increased with the level of degradation and

reached a maximum at HD site (Table 1). Lowest C/N contents were recorded at NP and HD in *L. chinensis* and *P. australis*, respectively (p < 0.05). Overall, *L. chinensis* litter was richer in nutrients than *P. australis* litter at almost all degraded sites (Table 1).

Monoculture Species

At the end of the experiment, final litter and nutrients mass in monoculture litter showed differences with a few significant cases between species (Figure 2 and Supplementary Figure S1). Litter mass declined rapidly at first, and then, the decomposition slowed down toward the last incubation day. The final mass was 50.01, 51.50, and 49.13% for *L. chinensis* and 53.49, 58.17, and 48.46% for *P. australis* at NP, LD, and HD sites, respectively, while the only significance recorded between species was at LD by day 60 (p < 0.05). Final mass was consistently lower at the HD site (Figure 2). Litter C and N contents declined with decomposition time, and final C and N contents differed significantly at LD site by day 60 (Supplementary Figures S1A,B). Lignin mass decreased in the beginning and then accumulated through time in a few cases and differed statistically at LD by 365 days (p < 0.01) and also at HD by both incubation periods (Supplementary Figure S1C). However, cellulose mass decreased with time in both species and was different between species at NP by 60 days and HD by 365 days (Supplementary Figure S1D). Among the degraded gradients, final litter mass and nutrients contents did not show any discernible difference.

Mixture Species

In line with final mass in monoculture litter, there was no significant difference recorded among mixture treatments of *L. chinensis* and *P. australis* species, except for final mass at HD by 60 days (Figure 3). In M1, M2, and M3 mixtures, the final mass averaged 48.61, 49.60, and 46.85% at NP; 54.68, 57.03, and 49.89% at LD; and 43.89, 41.47, and 41.23% at HD, respectively. The order of final mass across sites was NP = LD > HD (Figure 3). On the other hand, nutrient dynamics were statistically different by 365 days except for C concentrations at NP and lignin or cellulose concentrations at LD (Supplementary Figure S2). A linear downward pattern was shown in C contents at each site with incubation time, and N contents were

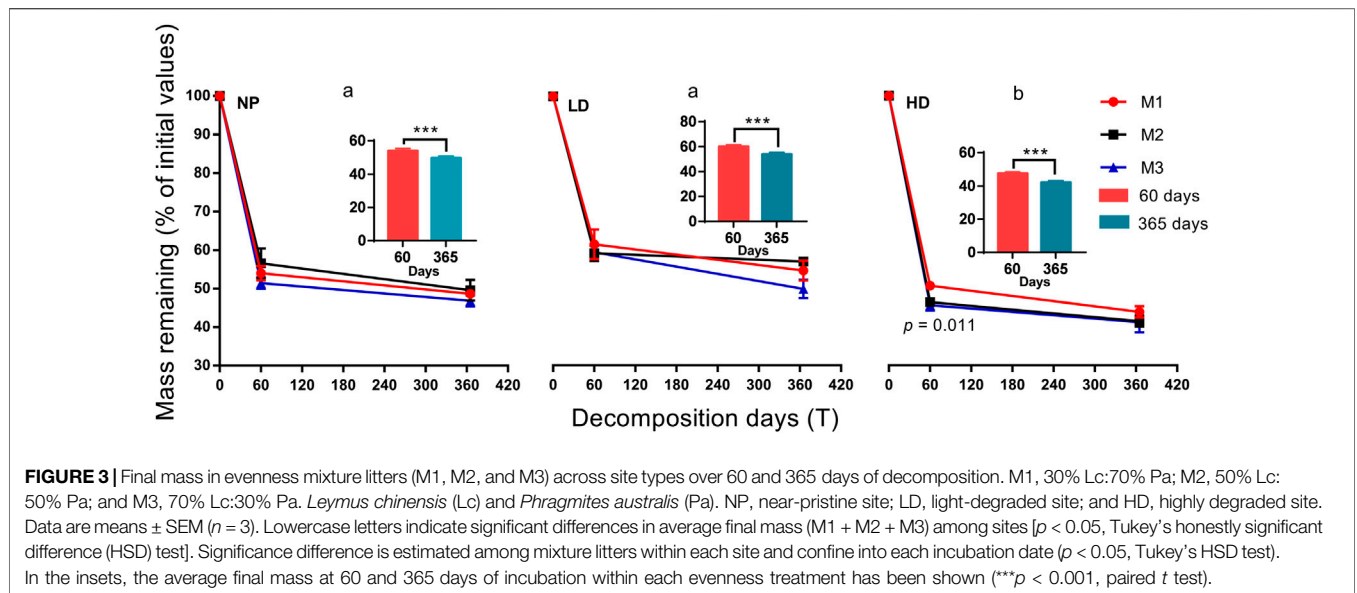


FIGURE 3 | Final mass in evenness mixture litters (M1, M2, and M3) across site types over 60 and 365 days of decomposition. M1, 30% Lc:70% Pa; M2, 50% Lc: 50% Pa; and M3, 70% Lc:30% Pa. *Leymus chinensis* (Lc) and *Phragmites australis* (Pa). NP, near-pristine site; LD, light-degraded site; and HD, highly degraded site. Data are means ± SEM (n = 3). Lowercase letters indicate significant differences in average final mass (M1 + M2 + M3) among sites [p < 0.05, Tukey's honestly significant difference (HSD) test]. Significance difference is estimated among mixture litters within each site and confine into each incubation date (p < 0.05, Tukey's HSD test). In the insets, the average final mass at 60 and 365 days of incubation within each evenness treatment has been shown (**p < 0.001, paired t test).

TABLE 2 | Matrix of Pearson correlation between initial nutrient contents and mass loss or final nutrient contents observed in monoculture and mixture compositions after 365 days of decomposition.

	Initial C	Initial N	Initial lignin	Initial cellulose	Initial C/N	Initial lignin/N
Mono litter						
Mass loss	-0.058 ns	0.224 ns	0.329 ns	-0.006 ns	-0.272 ns	0.090 ns
C contents	0.624**	0.305 ns	0.723**	0.054 ns	-0.149 ns	0.368 ns
N contents	0.306 ns	0.786**	0.288 ns	0.624**	-0.807**	-0.473*
Lignin contents	-0.575*	-0.279 ns	-0.731**	-0.006 ns	0.193 ns	-0.432 ns
Cellulose contents	-0.242 ns	0.230 ns	-0.542*	0.493*	-0.337 ns	-0.732**
Mixture composition						
Mass loss	-0.225 ns	0.634**	0.346 ns	0.743**	-0.655**	-0.256 ns
C contents	0.471*	-0.432*	-0.248 ns	-0.743**	0.477*	0.176 ns
N contents	0.058 ns	0.197 ns	-0.739**	-0.575**	-0.183 ns	-0.578**
Lignin contents	0.013 ns	-0.675**	0.171 ns	-0.324 ns	0.648**	0.577**
Cellulose contents	-0.073 ns	-0.620**	-0.473*	-0.303 ns	-0.146 ns	-0.397*

Data are means ± SEM (n = 3). Asterisks indicate significant difference between mass or nutrient contents and initial nutrient contents (*p < 0.05; **p < 0.01; and ns, not significant, Pearson correlation).

significantly higher at NP followed by HD and LD sites (Supplementary Figures S2A,B). Unlike C, lignin contents showed linear upward trend at all sites, and cellulose contents showed irregular patterns (Supplementary Figures S2C,D). Among sites, C, N, and cellulose contents decrease with the degree of degradation (Supplementary Figures S2A,B,D); however, lignin contents were higher at LD site than at the other two sites (Supplementary Figure S2C). Additionally, final mass and nutrient concentrations (except cellulose) were highly significant between incubation days (Supplementary Figure S2).

Overall mass loss was higher in M3, intermediate in M2, and lower in M1 with significant differences between monoculture species (*L. chinensis* + *P. australis*) and M3 mixture by day 60 (Supplementary Figure S3). Between the monoculture litters, we observed that mass loss in M3 mixture increased when mixed with *L. chinensis* monoculture litter in both periods, but the effect

was not statistically significant (p > 0.05). In contrast, *P. australis* monoculture litter had great influence on the mass loss of M3 mixture by day 60 (Supplementary Figure S4). Along degraded sites, we found a significant difference only at HD site between monoculture and mixture final mass, C contents, and lignin contents (Supplementary Figures S5A,B,E). At the LD site, monoculture litter was different from mixture litter in N contents, lignin contents, and C/N ratio (Supplementary Figures S5C–E).

Influence of Litter Quality on Monospecific and Mixture Species Mass Loss

Mass losses after 365 days in the monoculture litter were not correlated with litter quality or initial nutrient contents (Table 2). For all nutrients, negative correlation was observed more frequently than positive correlation between initial and final

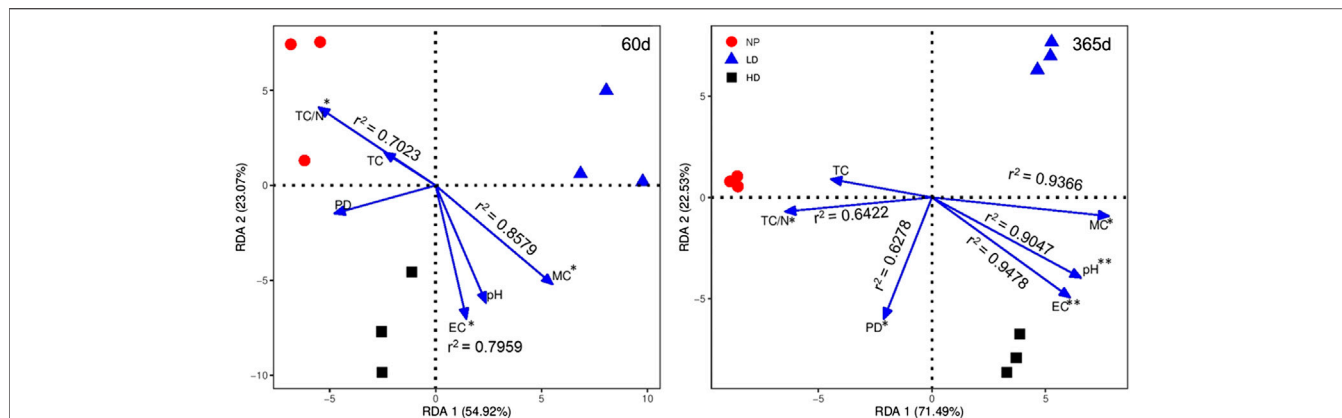


FIGURE 4 | Redundancy analysis (RDA) for analyzing the effect of soil properties on final mass and nutrient concentrations in monoculture and mixture litters along degradation gradients over 60 and 365 days of decomposition. Final mass and nutrient contents coordinates are not displayed for clearer results. EC, electrical conductivity; TC, total carbon; MC, moisture contents; PD, particle density; and TC/N, total C/N. NP, near-pristine site; LD, light-degraded site; and HD, highly degraded site. Significance between compositions and soil properties variables has been shown (* $p < 0.05$ and ** $p < 0.01$, Monte Carlo permutation test).

concentrations. Initial lignin contents showed highly significant relationships with final C (positive), N (positive), lignin (negative), and cellulose (negative). Initial cellulose contents were positively correlated with final N and cellulose. Initial C was positively related with final C, while initial lignin/N was negatively correlated with final cellulose. Finally, initial C, C/N ratio, and lignin/N ratio were negatively related to final N (Table 2).

Mass losses after 365 days in mixture compositions were positively correlated with initial nutrient contents of N or cellulose and negatively correlated with initial C/N ratio (Table 2). Initial lignin, cellulose, and lignin/N ratio were negatively related to final N and cellulose. Positive relationship was found between initial C/N and lignin/N ratios, on the one side, and final lignin on the other. Initial N showed negative relations with final N and lignin. Finally, significant relationships between initial cellulose (negative), C (positive), and C/N (positive), on the one side, and final C, on the other, were observed.

Influence of Soil Properties on Monospecific and Mixture Species Mass Loss

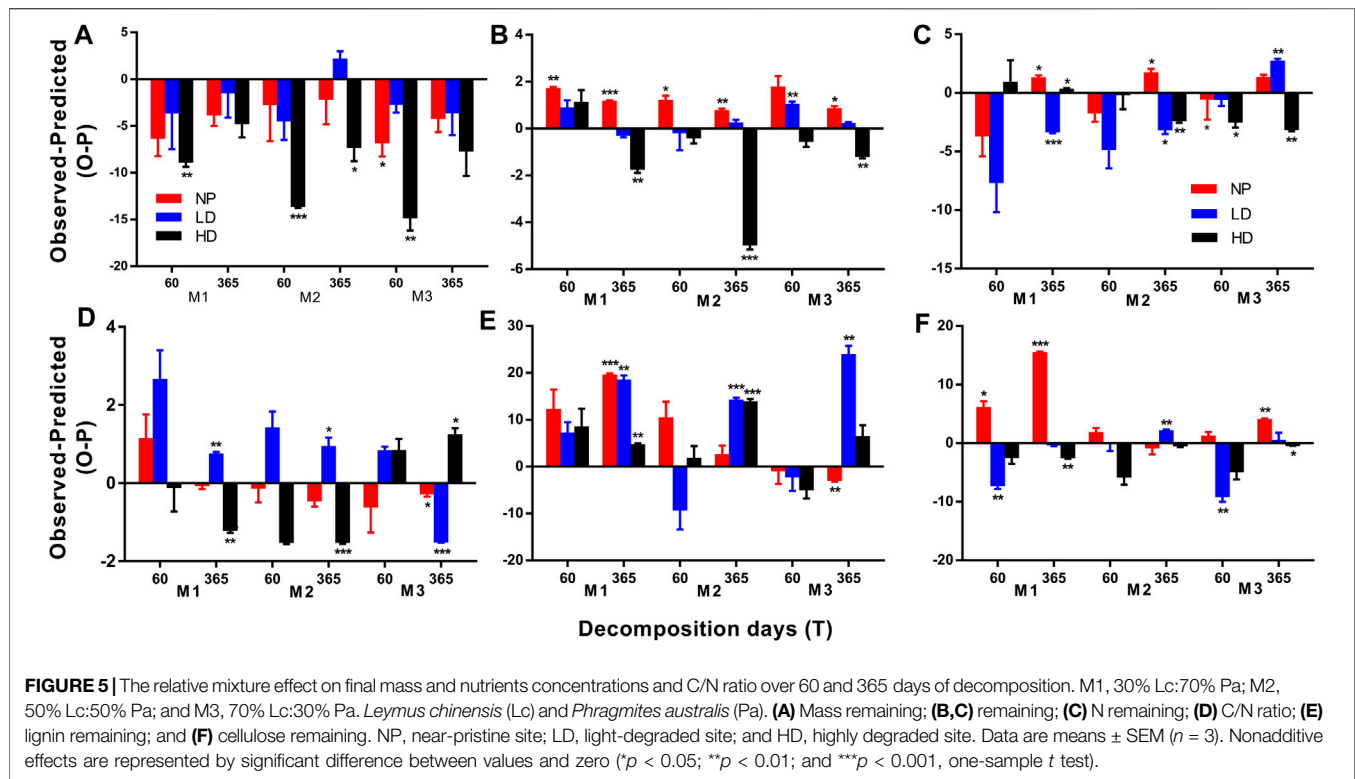
According to multiple regression and RDA analyses, soil properties significantly impacted the litter decomposition dynamics among grassland degraded degrees (Figure 4 and Supplementary Table S2). The soil attributes were constrained to the first and second axes, explaining 54.92 and 23.07% by 60 days and 71.49 and 22.53% by 365 days of the total variance in final mass and nutrient concentrations of all treatments on all sites. Litter decomposition dynamics of mixture treatments were positively correlated with EC, MC, TC/N, while pH and PD were minor factors affecting litter dynamics by 365 days only (Figure 4). Regarding degradation gradients, HD site was strongly regulated by pH, EC, and MC over 365 days. Multiple regression analysis showed that single-

TABLE 3 | Summary of the general linear model (GLM) using Type I sum of squares (SS) for testing the evenness and diversity effects of litter mixing on final litter mass and nutrients and C/N ratio at different degraded gradients during decomposition days.

	df	F	p	F	p	F	p
		Mass remaining (%)		C Remaining (%)		N remaining (%)	
Days (T)	1	234.787	<0.001	305.884	<0.001	242.702	<0.001
Mixing (M)	1	122.081	<0.001	0.026	0.871	5.120	0.027
Evenness (E)	2	7.558	0.001	8.204	0.001	24.975	<0.001
Site (S)	2	100.859	<0.001	125.939	<0.001	288.430	<0.001
T × M	1	12.596	<0.001	1.143	0.288	2.091	0.152
T × E	2	0.301	0.741	0.614	0.544	0.570	0.568
T × S	2	1.054	0.354	0.342	0.711	3.940	0.024
M × E	2	1.673	0.195	0.296	0.745	0.610	0.546
M × S	2	19.503	<0.001	1.537	0.222	1.433	0.245
E × S	4	1.044	0.390	2.529	0.047	2.424	0.055
		C/N ratio		Lignin remaining (%)		Cellulose remaining (%)	
Days (T)	1	45.714	<0.001	351.463	<0.001	130.704	<0.001
Mixing (M)	1	3.222	0.077	44.042	<0.001	0.047	0.829
Evenness (E)	2	21.230	<0.001	57.218	<0.001	22.776	<0.001
Site (S)	2	241.780	<0.001	120.754	<0.001	175.454	<0.001
T × M	1	19.489	<0.001	17.497	<0.001	13.662	<0.001
T × E	2	1.160	0.319	7.547	0.001	0.338	0.714
T × S	2	12.208	<0.001	9.901	<0.001	2.446	0.093
M × E	2	3.984	0.023	6.097	0.004	2.601	0.081
M × S	2	10.835	<0.001	1.025	0.364	17.287	<0.001
E × S	4	22.663	<0.001	3.863	0.007	8.227	<0.001

F values of the terms were sensitive to the order in which they were added. p values are highlighted in bold.

species litters were not significantly affected by soil properties (Supplementary Table S2). In uneven mixtures, positive correlations were found in M1 by MC and pH while in M3 by EC, MC, TOC, and TN. In even mixture, M2 was strongly affected by all soil properties except soil pH (Supplementary Table S2).



Influence of Litter Mixing on Litter and Nutrients Mass Remaining

Due to our nonfully factorial experiment design, there was a caution needed to unveil the results of GLM. Despite this, litter-mixing-induced nonadditive effects of mixing (M), evenness (E), site (S), and days (T) on final mass occurred in six out of 10 cases (Table 3). The two-way interaction as $T \times M$ and $M \times S$ strongly affected final mass, while remaining (two-way) interactions produce additive results. In final nutrient contents and C/N ratio, two-way ANOVA indicated that nonadditive effects prevailed over additive effects in 31 out of 50 cases of the mixture compositions. Nonadditive litter mixing effects were more prominent in lignin, cellulose, and C/N ratio as compared to C and N contents. Almost all the main or single terms showed significant nonadditive effects on litter and nutrients dynamics (Table 3).

Unlike GLM model results, additive effects were prevalent, pooling together with litter and nutrient dynamics, in 57% of all mixtures (62 out of 108 cases, Figure 5). Nonadditive antagonistic effects on final mass were observed in 22% of all mixture litters (4 out of 18 cases; three mixtures with two incubation dates, Figure 5A). In final nutrients, litter mixing induced both antagonistic and synergistic effects in evenness treatments along the decomposition period. In final C, 50% (9 out of 18 cases) showed more synergistic effects than antagonistic effects (6 vs. 3 out of 18 cases, Figure 5B). Overall, 56% of all mixtures in final N were nonadditive in which antagonistic effects were slightly more prominent than synergistic effects (6 vs. 4 out of 18 cases, Figure 5C). Concerning C/N ratio, nonadditive

antagonistic and synergistic effects were found in 17 and 22% of all mixtures, respectively (3 vs. 4 out of 18 cases, Figure 5D). Nonadditive synergistic effects masked the antagonistic effects in 33 and 6% of all mixtures, respectively, in final lignin (6 vs. 1 out of 18 cases, Figure 5E). Regarding cellulose, antagonism and synergism were recorded at the same rate (22%, 4 vs. 4 out of 18, Figure 5F). Among mixtures, the highest nonadditive litter mixing effects were recorded in M3 mixture followed by M1 and M2 mixtures (17 vs. 16 vs. 13 out of 108 cases, respectively) on mass and nutrient dynamics throughout the decomposition process. Meanwhile, litter mixing effects were significant among degraded gradients in mixtures over incubation days with few exceptions. However, more cases were recorded at HD site followed by NP and LD sites (18 vs. 15 vs. 13 out of 108 cases, respectively, Figure 5).

DISCUSSION

Some previous studies in temperate grasslands experimentally investigated litter mixing effects of different plant species with various proportions and found nonadditive effects being either antagonistic or synergistic. In the real world, however, it is complicated to explain these effects due to variations in vegetation properties, habitats, and their associated climatic conditions. In the current experiment, we tried to test the effect of litter mixing on monoculture and mixture species in degraded meadow steppe and also found antagonistic and synergistic effects in the same mixtures, but additive effects

shifted to nonadditive effects in final nutrients concentrations with time.

The two contrasting species chosen in this study cover a wide range of nutrient contents that elucidate the differences detected for decomposition rates in single- and mixture-species litter bags (Figures 2, 3 and Table 1). There was a small difference in initial C and N contents and C/N ratios between *L. chinensis* and *P. australis* examined in the experiment (Table 1). Both plants are perennial, rhizomes, and resistant to alkaline stress (Zhang et al., 2018). Additionally, they belong to the same Gramineae family and coexist in the study area for a long time (home effect), but their richness varies with site conditions (Wang et al., 2018). Because of this reason, we found ample evidence for host advantage in our experiment with consumers decomposing litter more rapidly in their home plots (Luai et al., 2019). In the light of this, leaf litter of *L. chinensis* decomposed faster than that of *P. australis* (although not statistically different, Figure 2). However, the presence of *L. chinensis* litter did not affect mixture compositions significantly over both incubation days (Supplementary Figure S4), and final mass of pooled monoculture litter (*L. chinensis* + *P. australis*) was higher than final masses of mixture treatments (Supplementary Figure S3). Besides, soil properties, especially total organic C and N contents, were strongly correlated with mass loss in *L. chinensis*-dominant mixture (Supplementary Table S2). Soil chemical properties affect the activities of microbial communities and, in turn, stimulate mass loss (Frouz, 2018). We found rapid mass loss in monoculture and mixture litter compositions regardless of site effect ranging from 50 to 60% throughout the incubation process (Figures 2, 3), indicating that rapid mass decay is common in grasslands (Moore and Fairweather, 2006). Nearly 50% of the litter in monoculture and mixture decomposed in the first 60 days, which is comparable with other studies (Hector et al., 2000; Bengtsson et al., 2011; Berg and McClaugherty, 2014). The initial litter decay was due to the outcome of the more labile components in the litter (Table 1). In addition, our study began in July, a warm season with high precipitation, followed by November–May, a cool dry season. Given that microbial activities are higher during warm and humid seasons than during cold and dry seasons, litter decomposition was highly dependent on the intensity of microbial activities (Yang et al., 2011; Yao et al., 2011). Although the microbial abundance was not estimated, we noticed that mixture litters were more heavily fed upon by soil animals than monoculture litters.

We also found a wide range of nutrients dynamics in the leaf litter of monoculture and mixture specific species (Supplementary Figures S1, S2). In our study, the species with C/N < 20 showed continuous C and N contents release, while the litter with a C/N greater than the critical level accumulated these nutrients (Supplementary Figures S1A,B, S2A,B and Table 1). This result is aligned with that of Lu et al. (2011), who reported the same finding and stated that N is immobilized above the threshold level of C/N, and its dynamics can be explained based on the critical threshold level of the C/N ratio of the litter. The threshold level of C/N, in general, is 20–30 (Liu et al., 2012). Since fungi and bacteria are the main drivers of the decomposition process, at the beginning of the decay, the

litter generally contains less nitrogen according to the needs of decomposers. Therefore, decomposer communities immobilize initial plus mineralized nitrogen from the neighboring environment and use litter carbon as the primary source of energy (Berglund and Ågren, 2012). On the other hand, lignin contents showed a constant increase in monoculture and mixture species at all sites (Supplementary Figures S1C, S2C); as a result, decomposition slowed down after the first incubation date (Figures 2, 3). Our findings support the fact that lignin is a resistant nutrient in litter compared to other chemical substrates (Taylor et al., 1989). The clear increase in lignin contents during litter nutrient dynamics is due to other labile contents as C or cellulose that have probably rapid loss rates (He et al., 2019). Loss in cellulose contents was twice as fast as loss in lignin contents during the last days of the experiment (Supplementary Figures S1C,D, S2C,D), although cellulose accumulation was detected by 60 days. Consistent with the hypotheses of a past study (Berg and McClaugherty, 2014), cellulose contents release from litter increased during later decomposition phase because the labile material of litter in plant cell walls was confined by recalcitrant C and lignin. These results are reasonable as litter is considered one of the main source of soil nutrient cycling. Furthermore, the analysis of two species mixtures of similar functional plant groups gives knowledge about the litter mixing effects on nutrient dynamics that can be helpful when scaling up the nutrient fluxes at grassland ecosystem levels.

According to the GLM model, litter mixing (in interaction with other terms) and species evenness (individually) induced nonadditive effects on mass and nutrients dynamics (Table 3). In two-way interactions with mixing, time, and site, the evenness factor had no effect on final mass, C, and N contents (Table 3). A few previous studies discovered similar findings (King et al., 2002; Li et al., 2013), but some also found positive relationships between evenness and decomposition (Dickson and Wilsey, 2009; Ward et al., 2010). This may explain the lack of relationship between litter evenness and decay process. Our results suggest that evenness effects are time dependent, and our study is 365 days long, thus unable to predict evenness effect in the advanced humus near stage. Our findings about litter mixing effects on final mass and nutrient contents are consistent with published results (Ball et al., 2008; Wu et al., 2013; Castro-Diez et al., 2019). The assumption that alteration is biogeochemical functioning is probably challenged by nonadditive effects on litter decay dynamics under climate change (Wu et al., 2013). Wardle et al. (1997) reported that nonadditive effects of litter mixing are dominant when monoculture litters vary greatly in functional properties, but strong evidence provides unambiguous support for this phenomenon (Quested et al., 2002; Hoorens et al., 2003; Mao et al., 2015). In conflict, it has been stated that mixture having functionally similar litter types may decompose faster than the predicted mass decay (Maisto et al., 2011). Likewise, our chosen species also shared the same functional group and pattern of decomposition. Moreover, litter quality of individual species in the mixture strongly affected the functioning of the decomposition process (Scowcroft, 1997; Zhang et al., 2013). We also found that initial nutrient contents, especially N and C/N ratio, were positively correlated with final mass of mixture

species (**Table 2**). Species mixing and evenness can mediate the decay and most nutrient contents release, but plant species evenness per se may result in more stable decomposition processes (Mao et al., 2015). However, it is difficult to predict any directional effect of evenness on decay method as the number of species has been shown to be much less important than litter mixture's taxonomic composition in controlling decomposition (Lecerf et al., 2011; Chen et al., 2019).

Based on general exploratory method, ~57.4% additive, ~21.3% antagonistic, and ~21.3% synergistic effects were found in final mass and nutrients contents in a 1-year decomposition experiment (**Figure 5**). These results were different from reported results (~30% additive, ~50% antagonistic, and ~20% synergistic effects) by Gartner and Cardon (2004) and other renowned studies (Bonanomi et al., 2010; Li et al., 2013; Chen et al., 2019). Some researchers suggested that litter mixing could have mixed effects on litter decomposition (Kominoski et al., 2007; Butenschoen et al., 2014). In line with our hypothesis, we found antagonistic effects of litter mixing on final mass in the mixture treatments but no relation with time (**Figure 5A**). Regarding nutrients, contrary to our hypothesis, synergistic effects prevailed over antagonistic effects, but additive effects shifted to nonadditive effects with incubation time (**Figures 5B–F**). Nonadditive effects are inconsistent during different decomposition stages of a litter cohort (Chen et al., 2019). This is because of deviations in the contents of water-soluble minerals and fiber components during incubation (Lecerf et al., 2011; Berg and McLaugherty, 2014). The conflicting results are likely due to the differences in the methodology used in previous studies by Ball et al. (2008), Bonanomi et al. (2010), and the current study, including the variant effects of species selection, environmental conditions, and most importantly incubation time i.e., 3 years, 90 days, and 1 year, respectively. Additionally, our experiment ended soon after cool dry season, so leaching and decay of inhibitory compounds in litter reduced microbial activities (Trevathan-Tackett et al., 2020). Moreover, there are more neutral effects recorded in the current study in agreement with a previous study by Srivastava et al. (2009). This happens because nonadditive effects mainly took place in labile carbon and most of the labile carbon lost by 60 days; therefore, additive effects become more obvious in the final mass. These outcomes could be because no correlation was found between mass loss and initial lignin or lignin/N ratio (**Table 2**). Second, the pattern of lignin and cellulose release (**Supplementary Figures S1C,D**) hinders litter decay in the later phase of incubation and hence results in antagonistic effects. Third, rapid leaching of nutrient (C and N) contents into soil occurred at the start of experiment (**Supplementary Figures S1A,B, S2A,B**) and increased in secondary metabolites due to high precipitation (Li et al., 2013). In our study, 70–80% precipitation was concentrated during June–August; thereby, the nutrient transfer among species in mixtures by leaching was high during the early phase of incubation. Our results showed that climatic conditions should be accounted for in the prediction of frequency and magnitude of litter mixing effects, different from some previous research findings (Duan et al., 2013).

Shifts in litter mixing effects over time could have a substantial influence on biogeochemical functioning from a long-term perspective due to change in litter quality during the decomposition process.

Owing to the environmental stresses from livestock overgrazing and climate impacts like droughts, about one-third of the available grasslands have been degraded worldwide (Peng et al., 2020). Grassland degradation affects vegetation and soil properties and in turn affects litter decomposition. Our study found specific effects of degradation on litter and nutrient dynamics, as mass loss was high at the HD site in both monoculture and mixture litters (**Figures 2, 3**). Particularly, the significant nonadditive effects of litter mixing on final mass were more common at HD sites (**Figure 5**). Moreover, we found significant differences in final mass and C content at HD site between monoculture and mixture litters (**Supplementary Figure S5**). Cowan and Anderson (2019) did not discover any significant effect of site type on decomposition because of dense litter fall occurrence at degraded sites. Bengtsson et al. (2011) showed delay in the decomposition process in the degraded site compared with the pristine site. In contrast, another study reported an increase in mass with the degree of degradation in Chinese forest systems (Paudel et al., 2015). Such a conflict with previous studies is possibly due to that soil properties as pH, electrical conductivity, and moisture contents significantly affected mass and nutrient dynamics at HD site by 365 days (**Figure 4**). Moisture contents made the litter more favorable to microbial attack, leading to high decomposition (Brandt et al., 2010). Meanwhile, diverse litter fall can also increase litter decomposition at the degraded site (Naeem et al., 2017). Overall, the current study reveals a variable and peculiar response of plant litter decomposition dynamics to species diversity, especially for evenness.

CONCLUSION

In this study, litter mixing produced idiosyncratic response to mass and nutrients release in the decomposition process at variant degrees of degradation with incubation time. Although our experiment was limited to 1 year, we still found that decomposition was positively affected by *L. chinensis* and *P. australis* species, and almost every mixture showed significant results on litter dynamics in the meadow steppe. Nonadditive effects on litter and nutrients found in species mixing, evenness, site, and decomposition time are driven by litter quality. Our study suggests that the role of diversity, especially species evenness, is more important and critical to understanding the full scope of potential species loss and relative species composition in grasslands. Our study also provides insights into the quantification of nutrient cycling in the meadow steppe ecosystem.

Data Availability Statement

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

LW, DW, and IN designed the study; IN, TA, XW, and LY performed the research; TA, XW, IN, and NH conducted statistical analyses; IN, TA, HW, and DW wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2021.582409/full#supplementary-material>.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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