American Journal of Plant Sciences, 2016, 7, 547-561
Published Online March 2016 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2016.73048

Effects of Nitrogen Addition on the Mixed Litter Decomposition in *Stipa baicalensis* Steppe in Inner Mongolia

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Received 26 January 2016; accepted 20 March 2016; published 23 March 2016

Abstract

During the past two centuries, global changes (*i.e.*, enhanced nitrogen deposition) have exerted profound effects on ecological processes of steppe ecosystems. We used litterbag method and mixed litters of three different plant species tissues (*Stipa baicalensis*: Sb, *Leymus chinensis*: Lc and *Artemisia frigid*: Af), endemic to *Stipa baicalensis* Steppe, and measured the mass loss of mixtures over 417 days under the N addition treatment. We studied the effect of N addition (N0: no N addition; N15: 1.5 g N/m^2·a; N30: 3.0 g N/m^2·a; N50: 5.0 g N/m^2·a; N100: 10.0 g N/m^2·a; N150: 15.0 g N/m^2·a) on the rate of mixed litter decomposition and nutrient dynamics change. The decomposition constant (k) of leaf mixtures was higher than that of root mixtures. The k values of leaf mixed combinations were 0.880 (Sb + Lc), 1.231 (Lc + Af), 1.027 (Sb + Lc + Af), respectively. The k value of stem was 0.806 (Lc + Af) and the root mixed combinations were 0.665 (Sb + Lc), 0.979 (Lc + Af) and 1.164 (Sb + Lc + Af), respectively. The results indicated that N addition had significantly effect on the mixed litter decomposition and nutrient releasing. The rate of plant tissues litter decomposition had different response to N addition. In the context of N addition, litter decomposition rate and nutrient dynamics were changed by synthetic effect of decaying time, specie types and N addition dose. Our findings suggested that prairie plants may adapt to environmental change by adjusting litter quality, thus retaining the stability of the steppe ecosystem.

Keywords

Grassland, Litter Decomposition, Mixed Effect, N Addition, Mass Loss, Nutrient Dynamics

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How to cite this paper: Li, W.Y., Yu, W.C., Bai, L., Liu, H.M. and Yang, D.L. (2016) Effects of Nitrogen Addition on the Mixed Litter Decomposition in *Stipa baicalensis* Steppe in Inner Mongolia. *American Journal of Plant Sciences*, **7**, 547-561.
http://dx.doi.org/10.4236/ajps.2016.73048

1. Introduction

As an important phenomenon of global change, atmospheric nitrogen deposition leads to a series of ecological problems, and affects elements circulation on broad spatial scales potentially [1] [2]. Natural grassland, as the largest green vegetable layers of terrene, is easily affected by the global change, meanwhile, it has important functions in maintenance of global carbon, nitrogen balance and water cycling [3] [4]. Plant litters are produced by the vegetable metabolism, and litter decomposition is a key process of nutrients cycling and energy flowing in the steppe ecosystem and plays a critical role in plant productivity and ecosystem carbon storage [5] [6]. Soil nutrient has long been considered as predominant factor which affects the dynamic change process of litter decomposition, therefore nitrogen deposition can affect the rate of litter decomposition directly and indirectly, and eventually influence nutrient cycling and productivity of steppe ecosystem [7] [8].

Litter decomposition keeps material transformation and nutrient cycling operation expedite [9], has irreplaceable impact on carbon and nitrogen circulation in ecosystems [10], and has caused an extensive concern of ecologists, especially in steppe ecosystem. The process of litter decomposition contains leaching, mechanical disruption, transformation of organic material, digestion of soil animals and enzymatic action of soil microbies. It is controlled by many factors including climate, the microenvironment around the litter, decomposer activity and litter quality [11]-[13]. Litter quality parameters, especially initial N content, have correlation with the rate of litter mass loss. Aerts [14] manifested that initial chemistry differences among litters, especially nitrogen and lignin concentrations, are primarily controlled from early to late stages of decomposition, respectively. In the complexity of real ecosystems, mixed litter from different species with varying litter quality and plant structure changes the chemical environment and physical character could drive interaction among adjacent litters from different species during decomposition. Gartner *et al.* [15] stated that litter mixtures often increased mass loss and had greater nutrient concentrations than predicted from single-species dynamics, meanwhile enhanced nutrient content could reflect less nutrient loss and enhanced import from surrounding litter and soil. Mixtures decaying can influence the litter decomposition rate potentially, may deviate from expectation values calculated as the additive mean of component litters decaying alone, and have important effect on litter mass loss dynamic, nutrient content, decomposers abundance and activity. In the process of litter decomposition, the microenvironment of mixtures decomposition (physical and chemical environment) differs from single-species litter decomposition environment [16] [17]. Due to leaching or biological effects, specific nutrients or secondary metabolites of litter transfer among different types influence the rate of litter decomposition and nutrient dynamic process. Almost 70% of the literatures, both domestic and international, on litter mixtures show mixed-litter decomposition in accordance with a non-additive model, but have inconsistent conclusions: synergistic or antagonistic and is insensitive to changes in mixtures [18] [19]. Gatner *et al.* [12] summarized that 76% mixed litters performed non-additive effect during the litter decaying among 123 kinds of specie types. Mixed litter could predict litter decomposition in natural ecosystem more accurately, and is of great significance on the biogeochemical cycles.

So far, due to agriculture activities, power plant and vehicle emissions, large areas have excessive nitrogen deposition, have side effects on aeschynomenous phytocoenosium and sensitive species components, which are far higher than thresholds of ecosystem (Carly *et al.* 2012) [20]. It is estimated that global nitrogen deposition amount was 103 Tg/a (1 Tg = 1012 g) in 1990, three times of 1860, and was expected to reach 195 Tg/a by 2050 [21]. In the late 80s, international start to pay close attention to the impact of global change (*i.e.* nitrogen deposition) on the dynamic change process of litter decomposition [22]. Atmospheric nitrogen deposition to the soil surface can improve the soil N content and then promote the plant growth and impact litter production and decaying rate directly. Numerous studies of nitrogen addition control tests verified simulated nitrogen deposition could reduce vegetation species richness and change the dominant species of settlement, it can also lead to the loss of rare species and the succession of species [23] [24], finally these will affect the rate of litter decomposition. Stevens *et al.* [25] found each additional 0.25 $g/m^2 \cdot a$ nitrogen deposition would reduce a plant species within 4 m^2 in 68 acid grasses. Xia *et al.* [26] stated the gramineae was more sensitive to nitrogen and easier to lose than grass. Therefore, nitrogen deposition leads to the decrease of species richness and different functional group of plants (*i.e.* legumes and grasses) transform, which will affect litter decomposition and cause long term chronic effects on the nutrient cycling in the ecosystem.

The continued increasing of nitrogen deposition could change the litter chemical constituents to affect the rate of litter decomposition and nutrient dynamics [27]. Previous simulation experiments of nitrogen deposition showed there were three cases of litter decomposition in response to nitrogen deposition: Vestgarden [28] demonstrated nitrogen deposition accelerate litter decomposition, especially in some N limited sites, while others found negative effects, especially high levels nitrogen deposition areas [29] [30], or have no significant effects [31]. Some research showed that litter decomposition showed different response to nitrogen from initial phase of accelerate to the later stages of inhibit. It looks like nitrogen deposition could decrease the decomposers composition and activity [32], or N element with some decomposes substance forming harder degradable polymeric substances, thus inhibit litter decomposition [33]. It implied that the effects of nitrogen deposition on litter decomposition depended on decomposition time, site condition, species types and levels of nitrogen addition. The inconsistency in the relationships with stimulated nitrogen deposition and litter decomposition, hence, it is necessary to carry out relative investigation, especially on the effect of nitrogen deposition on mixed litter decomposition and nutrient dynamic change.

The *Stipa baicalensis* steppe, an endemic grassland type in Central Asia, is one of the representative meadow steppe types. It is mainly located in Songliao Plain and the eastern part of Mongolia Plateau forest steppe in China [34], in which nutrient availability is a limiting factor of the vegetation growth. Therefore, *Stipa baicalensis* steppe is an ideal experimental platform to carry out the study of temperate grassland response to global change. The studies abroad of mixed litter decomposition are mainly concentrated on temperate forest ecosystem, while domestic studies on mixed litter decomposition in steppe ecosystem merely restrict to Wang *et al.* (2000) [35] in three different steppes (Meadow steppe, *Leymus chinensis* Steppe and *Stipa grandis* Steppe), Liu *et al.* [36] in *Stipa krylovii* Roshev. Steppe. Chen *et al.* [17] in the typical grassland of Inner Mongolia and Zhang *et al.* [30] in Hulun Buir Meadow Steppe, while there remains lack of the study about mixed litter decomposition in *Stipa baicaleninsis* Steppe and potential impact of nitrogen deposition on nutrient cycling in steppe ecosystem. This paper will compare and analyze the effect of N addition, simulated nitrogen deposition experiment, on the mixed litter decomposition rate of mixed litter and nutrient dynamic changes of different species tissues litter (leaf, stem and root) among *Stipa baicalensis* (perennial bunch grass, *Sb*), *Leymus chinensis* (perennial rhizomatous grass, *Lc*) and *Artemisia frigid* (small semishrub, *Af*), which are the main grasses in the *Stipa baicalensis* steppe ecosystem. The main objective of this study is to assess the effect of N addition on litter decomposition in *Stipa baicalensis* steppe by comparing three species mixed litter decomposition rates, and the chemical composition change to adapt global change, which influences ecosystem nutrient cycling. Our study postulated N addition would have significant influence on mixed litter decomposition, and three different species with contrasting initial chemical composition and functional groups have diverse response to N addition. We aimed to provide data support for nutrient cycle in the steppe ecosystem in the context of global changes, and serve theoretical basis and scientific guidance rational utilization of natural grasslands, biodiversity conservation and degraded grassland restoration.

2. Materials and Methods

2.1. Study Area

The study area (119˚35'E - 119˚41'E, 48˚27'N - 48˚35'N, 760 - 770 m above sea level) was located in Ewenke County, Hulun Buir, Inner Mongolia. It was fenced to protect against domestic animals. The site had an annual average temperature of −1.6 degrees Celsius and an annual average rainfall of 328.7 mm. The annual accumulated temperature (≥0 degrees Celsius) and annual evaporation was 2567.5 degrees Celsius and 1478.8 mm, respectively. The study area has a northern Mid-temperate continental monsoon climate. The soil at the site was Dark Chestnut. The plant community was composed of *Stipa baicalensis* as the dominant species, as well as *Leymus chinensis*, *Artemisia frigida*, *Achnatherum sibiricum*, *Serratula yamatsutanna*, *Carex pediformis*, *Filifolium sibicum*, *Koeleriacr istata*, *Pocockia ruthenica*, *Carex duriuscula, and Cleistogenes squarrosa.*

2.2. Experimental Design

We chose smooth terrain and a vegetation representative area as an experiment site in *Stipa baicalensis* grassland. From 2010, we sprayed ammonium nitrate (NH_4NO_3) twice, at June 15th and July 15th, respectively. We designed six N addition controlled levels: 0, 1.5, 3.0, 5.0, 10.0, 15.0 g $N/m^2 \cdot a$, abbreviate to N_0, N_{15}, N_{30}, N_{50}, N_{100} and N_{150} respectively, with 3 repetitions and in total of 18 blocks were get，each block was 8 m × 8 m at intervals of 2 m, using a randomized complete block design [37]. N concentration was calculated by the percentage of months' average precipitation in annual total precipitation multiplied by N addition levels. NH_4NO_3 was dissolved in 8 L water (This corresponded to an additional 1.0 mm rainfall as annual rainfall), and sprayed uniformly on the each plot. CK was sprayed equal water to avoid the error of external water in each block. The same N concentration was used in each block per year.

2.3. Litter Preparation

We collected the litter of *S. baicalensis, L. chinensis and A. frigid* outside of the experiment treatment sites, and air dried the samples to constant mass. The naturally dried samples were divided into leaf, stem and root and cut into 10 cm fragments. We used the litter-bag technique with a nylon mesh bag, which was used to quantify litter decomposition [14]. We placed leaf, stem and root litter into 15 cm × 25 cm of 0.2 mm litterbags. Each bag was filled with 8 g of single litter and the equivalent amount of mixed litter by three plant species tissues (**Table 1**). We prepared three bags for each species litter combination, and in total of 45 litterbags were placed on every treatment block in 29th August 2011, using metal pins to fix litterbags on the ground surface to prevent being blown away by the wind. 810 litterbags were retrieved randomly after litter decaying 295d, 356d and 417d respectively. The retrieved materials were cleaned from soil particles and picked out other plants, roots and shoots. The cleaned materials were then oven-dried for 48 h at 70 degree Celsius. The dry litter materials were weighed to determine the remaining mass.

The oven dried litter was ground and sieved through a 1mm mesh and analyzed for C, N, P, K and lignin contents. Organic carbon content was determined using the potassium dichromate oxidation-concentrated sulfuric acid external heating method. For the determination of N, P and K content powdered samples were digested in a H_2SO_4-H_2O_2 solution. Total N was determined using Auto Analyzer 3 [38]. Phosphorus (P) was determined by ammonium molybdate colorimetry [39]. Potassium was determined by flame photometer method [40]. Lignin content was determined by ultraviolet spectrophotometer method [41].

2.4. Statistical Analyses

The remaining mass loss of the three species plant tissues litter was calculated by the following equation: R (%) = $M_t/M_0 \times 100\%$. Where R is litter mass remaining, M_t is the measured litter mass remaining in the litterbag at any time (t) of decomposition; M_0 is the mass of initial litter before decomposition.

Equal amounts of fragment litter from the three different species were mixed together in the litterbags, thus the expected mass remaining (M_e) can be calculated using the equation [42]: $M_e = [M_A/M_A + M_B + M_C] \times R_A + [M_B/M_A + M_B + M_C] \times R_B + [M_C/M_A + M_B + M_C] \times R_C$. Where R_A, R_B and R_C is the mass remaining in the single species litterbag of species A, B and C respectively; M_A, M_B and M_C is the initial litter mass of each fragment from the three species in the mixture.

All statistical analyses were performed by SPSS 16.0. The data of litter decomposition were subjected to analysis of variance (ANOVA), and three-way variance was used to test the effect of N addition level, species type and decomposing time on litter decomposition. T-test was used to test differences between the observed

Table 1. Litter quality combinations.

Plant tissues	Richness level	Percentage of each litter quality (%)	Litter composition
	1	100	Sb, Lc, Af
Leaf	2	50	Sb + Lc, Lc + Af
	3	33	Sb + Lc + Af
Stem	1	100	Lc, Af
	2	50	Lc + Af
	1	100	Sb, Lc, Af
Root	2	50	Sb + Lc, Lc + Af
	3	33	Sb + Lc + Af

Note: Sb, *Stipa baicalensis*; Lc, *Leymus chinensis*; Af, *Artemisia frigid.* "1" represents only one species in one bag, "2" represents two different species were mixed in one bag, "3" represents three different species were mixed in one bag.

and expected mass remaining of mixed litter.

3. Results

3.1. Chemical Parameters of Initial Litter

Comparing the different plant tissues litter of *S. baicalensis*, *L. chinensis* and *A. frigid*, the initial chemical contents were different (**Table 2**). For the different tissue litters of each plant, the N content of leaf litter was significantly higher than stem and root litter, but C/N lower than stem and root litter ($P < 0.05$). There was no significant difference of lignin content between tissue litters of three plants. For the different plants litter, the leaf litter of *A. frigid* had the highest C, N, P, K contents and lowest C/N, followed by *L. chinensis*, *S. baicalensis* was opposite, and three plant leaf litters had similar lignin content. The stem litter of *A. frigid* had lowest C, N, P, K contents than *L. chinensis*. The root litter of *L. chinensis* had highest C contents than *S. baicalensis* and *A. frigid*, but had lowest N concentrations.

3.2. Effects of N Addition on the Mixed Litter Mass Losing

Without N addition treatment, after 417d, the leaf mixture litter residual rate was 79.8%, 80.2%, 80.2% (Sb + Lc, Lc + Af, Sb + Lc + Af) respectively, stem mixture litter residual rate was 85.6% (Lc + Af), root mixture litter residual rate was 82.1%, 79.5%, 80.3% (Sb + Lc, Lc + Af, Sb + Lc + Af) respectively. After N addition treatment, as time goes by, the mixture litter residual rate presented the tendency of continuous declination, from the day of 356, the mass losing became faster until the day of 417 (**Figure 1**).

3.3. Effects of N Addition on the Rate of Mixed Litter Decomposition

Under the six levels of nitrogen addition treatments, the mixed litter remaining mass of different plant tissues were on the decline with the litter continuous decomposing (**Table 3**). For the leaf mixed litter, the remaining mass (%) under nitrogen addition treatments were higher than N0 at 295d after decaying. When decaying to 417d, the mixed litter remaining mass of plant tissues turned to opposite side, N addition accelerated mixtures decomposing except for N150. For the stem mixed litter, the remaining mass of the litters under nitrogen addition treatments lower than N0. During root mixed litter decomposing, the remaining mass of the litters under nitrogen addition treatments higher than N0. In general, the plant tissues litter remaining mass of three grasses, with different initial chemical composition, had different response to N addition, but no consistent increasing or decreasing were found with elevated levels of N addition. N150 significantly inhibited leaf mixtures mass loss, while other levels showed different degrees of promotion. N30 significantly accelerated stem mixtures mass loss, but N100 and N150 were opposite. N addition significantly inhibited root mixtures, only N100 and N150 transitorily promoted *L. chinensis* and *A. frigid* (Lc + Af) root mixtures decomposing ($P < 0.05$).

Control plot with non-nitrogen addition could simulate the natural condition to predict the rate of mixed litter decomposition and non-additive effect of mixed plant tissues litter (**Table 3**). After 417d, we found the leaf and

Table 2. Initial chemical compositions of three litter from various plant species (mg/g).

Chemical composition		Carbon	Nitrogen	Phosphorus	Potassium	C/N	Lignin
Stipa baicalensis	Leaf	436.9 ± 13.3 a	14.4 ± 0.2 a	0.89 ± 0.2 a	10.8 ± 0.2 a	30 ± 0.6 a	119.6 ± 6.3 a
	Root	349.9 ± 82.0 b	8.9 ± 0.8 b	0.63 ± 0.2 a	4. 9 ± 1.0 b	50 ± 6.3 b	117.3 ± 1.2 a
Leymus chinensis	Leaf	451.7 ± 2.4 a	16.5 ± 1.2 a	0.89 ± 0.1 a	11.9 ± 0.4 a	27 ± 2.1 b	120.7 ± 7.6 a
	Stem	420.5 ± 36.7 a	7.9 ± 0.6 b	0.83 ± 0.1 a	10.2 ± 0.5 b	53 ± 5.1 a	119.3 ± 5.0 a
	Root	396.2 ± 40.3 a	7.9 ± 0.7 b	0.63 ± 0.1 b	4.9 ± 1.0 c	50 ± 8.8 a	113.0 ± 6.23 a
Artemisia frigida	Leaf	460.7 ± 75.2 a	21.1 ± 0.6 a	1.78 ± 0.2 a	18.5 ± 1.6 a	22 ± 1.3 b	121.8 ± 5.4 a
	Stem	354.9 ± 54.0 ab	7.2 ± 1.3 c	0.68 ± 0.1 b	10.2 ± 0.3 c	49 ± 10.4 a	111.9 ± 1.9 a
	Root	313.0 ± 39.2 b	9.0 ± 0.3 b	0.67 ± 0.1 b	14.0 ± 0.1 b	35 ± 7.9 b	109.4 ± 1.0 a

Note: Values within the same column with different small letters mean significant difference at $P = 0.05$ level, the same as follows.

Table 3. The effect of N addition on different tissues mixture litter remaining mass (%) after decaying 417 d.

N addition levels		Leaf			Stem	Root		
		Sb + Lc	Lc + Af	Sb + Lc + Af	Lc + Af	Sb + Lc	Lc + Af	Sb + Lc + Af
	Obs	59.7 ± 1.05	53.2 ± 0.68	57.4 ± 0.41	65.6 ± 0.67	66.0 ± 0.30	57.5 ± 0.51	54.5 ± 0.68
N_0	Exp	63.8 ± 2.05	58.0 ± 2.13	60.8 ± 1.78	61.6 ± 0.99*	60.9 ± 1.17*	54.5 ± 2.38	58.6 ± 2.29
	k	0.880 a	1.231 bc	1.027 c	0.806 b	0.665 c	0.979 c	1.164 a
	Obs	58.8 ± 0.89	52.3 ± 1.10	54.8 ± 0.35	65.5 ± 0.61	63.3 ± 0.40	59.1 ± 0.41	60.6 ± 0.56
N_{15}	Exp	61.3 ± 1.13	55.6 ± 0.63*	57.6 ± 0.74*	61.6 ± 1.19*	61.7 ± 1.98	57.1 ± 1.23	60.0 ± 1.26
	k	1.008 a	1.347 ab	1.181 b	0.774 b	0.791 a	0.951 cd	0.821 bc
	Obs	56.0 ± 0.27	55.1 ± 0.43	54.4 ± 0.22	61.8 ± 0.31	65.1 ± 1.02	60.2 ± 0.29	61.2 ± 0.82
N_{30}	Exp	60.3 ± 0.56*	53.8 ± 0.42	56.7 ± 0.58*	60.9 ± 1.51	68.6 ± 0.74*	64.1 ± 0.57*	66.1 ± 0.58*
	k	1.125 b	1.158 c	1.077 c	0.945 a	0.651	0.917 d	0.806 c
	Obs	60.0 ± 0.77	50.9 ± 0.62	57.9 ± 0.20	66.8 ± 0.14	67.2 ± 0.52	60.0 ± 0.29	59.2 ± 1.53
N_{50}	Exp	61.2 ± 1.12	52.6 ± 2.11	55.9 ± 1.72	61.0 ± 0.86*	61.7 ± 2.78	55.6 ± 1.46*	59.0 ± 0.25
	k	0.922a	1.449 a	1.048 c	0.757 b	0.668 c	0.995 c	0.861 b
	Obs	62.1 ± 0.72	55.8 ± 0.44	52.1 ± 0.54	65.6 ± 0.54	63.9 ± 0.26	56.3 ± 0.16	60.5 ± 0.25
N_{100}	Exp	59.4 ± 1.04	55.1 ± 0.91	56.2 ± 0.70*	61.3 ± 0.83*	58.7 ± 3.03	56.8 ± 2.08	58.6 ± 2.31
	k	0.944 a	1.076 cd	1.396 a	0.818 b	0.746 ab	1.095 b	0.743 d
	Obs	60.6 ± 0.31	59.9 ± 0.16	61.9 ± 0.30	65.4 ± 0.66	67.9 ± 0.44	53.0 ± 0.17	61.6 ± 0.72
N_{150}	Exp	61.6 ± 0.48	54.5 ± 0.59*	57.4 ± 0.56*	63.8 ± 1.29	59.0 ± 1.52*	54.3 ± 1.50	55.4 ± 1.92*
	k	0.848 a	0.946 d	0.810 d	0.812 b	0.690 bc	1.232 a	0.724 d

Note: Values with star means significant difference between Obs and Exp at $P = 0.05$ level. Obs means the values of experiment observed, Exp means the values calculated means of mixtures component of single litter from three plants. k was litter decomposition constant, and the litter letter in the same line behind the values mean significant difference among six nitrogen addition treatments.

root litter of *S. baicalensis*, *L. chinensis* and *A. frigid* (Sb + Lc + Af) showed synergistic effect, which the observed residual rate was lower than expected values 3.3% and 4.1%, respectively. *L. chinensis* and *A. frigid* (Lc + Af) stem mixtures , *S. baicalensis* and *L. chinensis* (Sb + Lc) root mixtures showed antagonism effect, which the observed residual rate was higher than expected values 4.0% and 5.1%, respectively. N addition significantly affected mixed effect, and different mixture combination had various responses to N addition.

Three-way ANOVA showed the effect of N addition on mixed litter decomposition rate changed with the decomposition time (Table 4). Decomposing time, species types and N addition all had significantly influence on the rate of aboveground mixed-litter decomposition ($P < 0.01$), and had interaction effect except for decomposing time and species types. Due to belowground litter undergo different decayed microenvironment, specie types played dominant role in controlling belowground mixed-litter decomposing under N addition treatment.

3.4. Effects of N Addition on the Nutrient Dynamic Change of Mixed Litter Decomposition

Due to the difference of plant tissues litter types, the response of mixed litter nutrient dynamics to N addition were different, and the response of aboveground and belowground litter was opposite (Table 5 and Table 6). At the early stage of litter decomposing to 295d, N addition significantly inhibited leaf and stem C element release, oppositely N addition accelerated root C element release ($P < 0.05$). At the end to 417d, the C element releasing of different mixed combination performed differently under the N addition treatments. Individual nitrogen levels accelerated N element releasing of leaf mixed litter, N_{50}, N_{100} and N_{150} significantly enhanced stem mixed litter the enrichment capacity of N element, retarded root mixed litter N element releasing. The ability of P element releasing of mixed litter was more sensitive than N element. High levels of nitrogen addition significantly inhibited P element releasing of aboveground mixed litter, N_{15} and N_{30} accelerated *S. baicalensis*, *L. chinensis* and *A.*

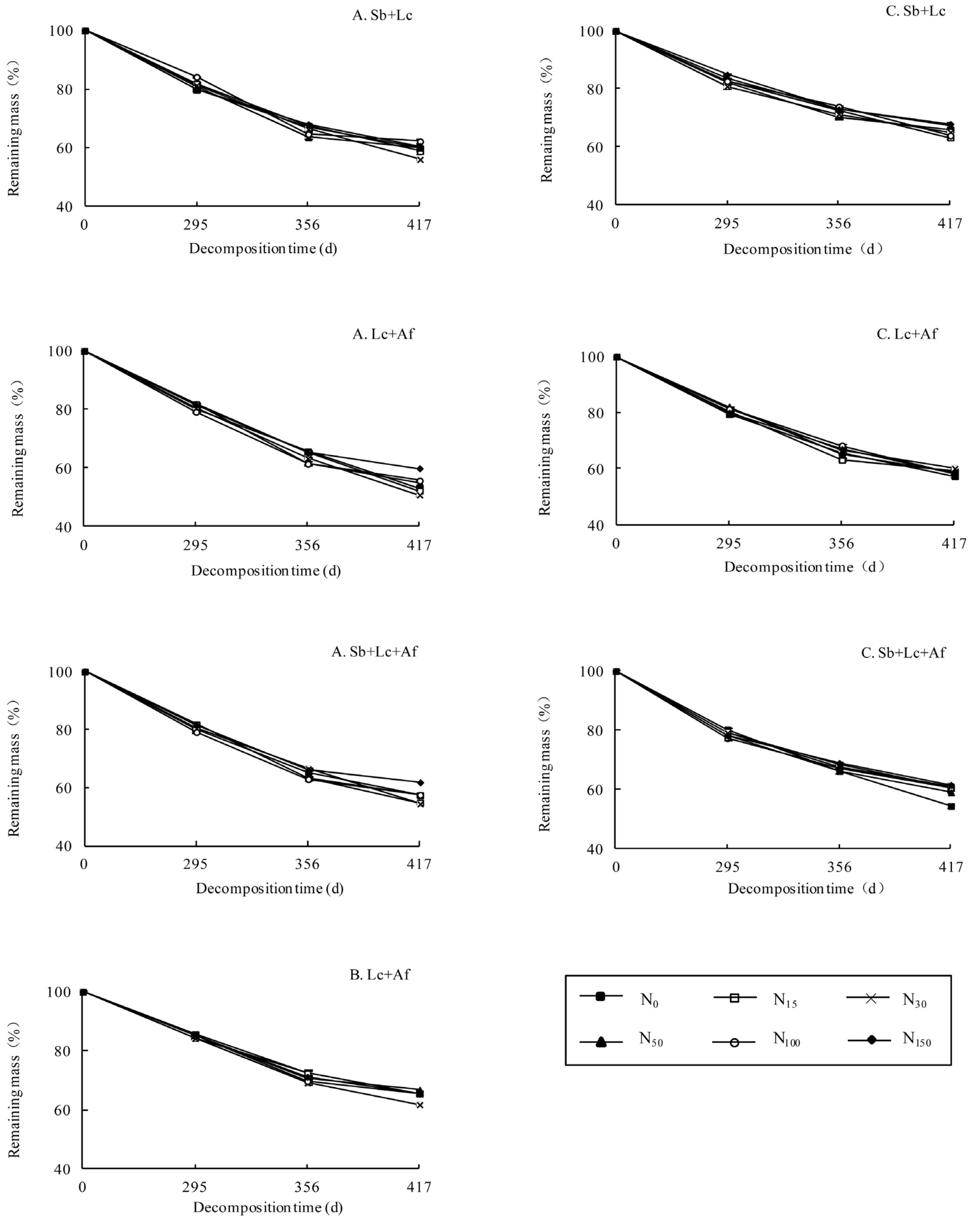

Figure 1. The effects of N addition on mixed litter remaining mass (%) after decaying to 417d. Sb, *S. baicalensis*; Lc, *L. chinensis*; Af, *A. frigid*. A, Leaf; B, Stem; C, Root.

frigid (Sb + Lc + Af) leaf mixtures and *L. chinensis*, *A. frigid* (Lc + Af) stem mixtures P element releasing. Comprehensively, N addition was incline to inhibit each plant tissues mixed litter nutrient releasing, especially high N addition levels. For the aboveground mixtures, the nutrient releasing of mixed combination of *S. baica-*

lensis and *L. chinensis* (Sb + Lc), which was the same family plant, was less sensitive than the mixed combination with *A. frigid*. Compared with aboveground mixtures, the nutrient dynamics of belowground mixtures was more readily affected by N addition.

Three-way ANOVA showed N addition had significant effect on mixed litter nutrient releasing (**Table 6**). Dynamic change process of C element similar to the tendency of litter residual rate could be regarded as the principal part of litter decomposition, affected by decomposing time, species types and N addition simultaneously.

4. Discussion

In comparison with the condition of single litter decomposition, the mixtures of various specie types or initial chemical composition, altered litter quality and spatial structure, increase the complexity of the litter decomposition, and the change of decomposition environment had influence on the rate of mixed litter decomposition and nutrient dynamics [43] [44]. The studyon mixed litter decomposition of four kinds of functional groups at the initial stage found that mixed effects had important influence on litter decaying rate, which was not the simple additive mass loss arithmetic from single litter [45]. Previous studies on mixed litter decomposition stated that there would be synergism effect [18], antagonism effect [46] and additive effect [47] during the mixed litter decomposition. In our study, at the end to 417d, the rate of individual mixed litter decomposition was deviated from its components single litter decomposition. Compared the actual residual rate of mixed litter decomposition with its expected values, we found the leaf and root litter of *S. baicalensis*, *L. chinensis* and *A. frigid* (Sb + Lc + Af) showed synergistic effect, and *L. chinensis* and *A. frigid* (Lc + Af) stem mixtures and *S. baicalensis* and *L. chinensis* (Sb + Lc) root mixtures showed antagonism effect, while other combinations showed additive effect. Meanwhile, there was a slight tendency of the remaining two leaf mixed litter combination (Sb+Lc and Lc+Af) showed synergism effect, *L. chinensis* and *A. frigid* (Lc + Af) root mixtures showed slightly antagonism effect. Whether this trend would reverse as the decomposing time increasing or had a significant enhancement to perform non-additive effect still needed long-term tracking and investigation. Antagonism or additive effects could be responsible for the inhibit effect of transfer secondary metabolism substance (*i.e.* phenolic) between adjacent mixed litters [48]. In our study, as the duration of litter decomposition, mixed effects would in flux. Under the

Table 4. Three-way ANOVA for decomposition rate of mixed-litter under N addition after decomposing 295 d, 356 d and 417 d.

Source of variation	*df*	F	*P*
Aboveground			
Time (T)	2	53.24	<0.01
Specie types (S)	3	8.36×10^3	<0.01
N addition (Ni)	5	1.52×10^3	<0.01
T*S	6	2.43	0.27
T*Ni	10	13.97	<0.01
S*Ni	15	935.56	<0.01
T*S*Ni	30	4.55	<0.01
Belowground			
Time (T)	2	0.16	0.854
Specie types (S)	2	295.93	<0.01
N addition (Ni)	5	12.57	<0.01
T*S	4	6.38	<0.01
T*Ni	10	1.88	0.052
S*Ni	10	30.60	<0.01
T*S*Ni	20	0.96	0.514

Table 5. The nutrient releasing rate of belowground mixture after decaying 417 d.

Species	Fertilization level	Carbon	Nitrogen	Phosphorus
Leaf				
Sb + Lc	N0	50.8 ± 2.5 a	31.2 ± 0.4 a	38.1 ± 1.5 a
	N15	40.8 ± 3.8 a	31.9 ± 1.4 a	34.1 ± 3.6 a
	N30	49.6 ± 2.6 a	33.0 ± 0.4 a	38.0 ± 1.0 a
	N50	44.7 ± 2.0 a	35.1 ± 3.3 a	34.4 ± 1.6 a
	N100	42.4 ± 1.6 a	31.2 ± 1.4 a	30.9 ± 3.0 a
	N150	46.7 ± 2.8 a	30.7 ± 1.3 a	30.5 ± 2.4 a
Lc + Af	N 0	55.4 ± 2.2 a	30.6 ± 1.6 ab	53.9 ± 2.6 d
	N15	50.8 ± 1.8 a	32.1 ± 2.8 b	49.7 ± 3.6 cd
	N30	57.8 ± 4.1 a	29.8 ± 0.4 ab	43.9 ± 5.1 bc
	N50	53.2 ± 2.4 a	34.7 ± 1.5 b	50.1 ± 1.1 cd
	N100	52.3 ± 1.1 a	30.3 ± 1.9 ab	36.8 ± 1.0 ab
	N150	51.7 ± 1.8 a	25.3 ± 0.9 a	31.9 ± 1.3 a
Sb + Lc + Af	N0	51.3 ± 1.3 b	37.1 ± 0.5 bc	37.8 ± 0.6 bc
	N15	53.3 ± 4.3 b	33.9 ± 1.4 b	50.1 ± 5.9 c
	N30	57.2 ± 1.0 b	34.6 ± 1.7 b	48.0 ± 5.9 c
	N50	51.0 ± 1.8 b	35.6 ± 0.9 b	32.1 ± 0.8 ab
	N100	55.7 ± 1.6 b	40.5 ± 0.9 c	40.2 ± 1.5 bc
	N150	43.3 ± 0.9 a	26.0 ± 1.6 a	24.1 ± 4.1 a
Stem				
Lc + Af	N0	37.9 ± 2.8 a	-5.5 ± 2.3 bc	42.6 ± 0.9 b
	N15	40.0 ± 4.6 a	-4.1 ± 2.8 bc	46.6 ± 1.2 b
	N30	44.0 ± 4.1 a	-0.5 ± 3.0 c	45.2 ± 1.1 b
	N50	37.7 ± 5.0 a	-10.7 ± 0.4 ab	34.5 ± 1.3 a
	N100	31.9 ± 2.9 a	-16.3 ± 2.9 a	30.3 ± 4.2 a
	N150	33.1 ± 4.0 a	-13.9 ± 3.2 a	33.6 ± 0.9 a
Root				
Sb + Lc	N0	52.2 ± 2.3 ab	22.8 ± 1.8 b	58.0 ± 0.5 bc
	N15	55.2 ± 1.8 b	33.5 ± 3.2 c	59.1 ± 0.3 c
	N30	56.8 ± 2.9 b	17.7 ± 3.3 b	57.3 ± 1.7 bc
	N50	48.2 ± 0.9 a	18.4 ± 2.3 b	48.7 ± 1.2 a
	N100	56.5 ± 1.4 ab	19.1 ± 0.9 b	56.6 ± 1.7 bc
	N150	54.1 ± 1.9 ab	15.2 ± 4.4 a	54.6 ± 1.0 b
Lc + Af	N0	50.0 ± 2.2 a	23.3 ± 2.9 a	33.3 ± 0.9 b
	N15	46.5 ± 5.5 a	21.3 ± 1.2 a	25.0 ± 1.0 a
	N30	49.2 ± 3.6 a	24.3 ± 7.5 a	31.3 ± 4.7 ab
	N50	48.2 ± 5.6 a	21.3 ± 3.7 a	42.6 ± 1.7 ab
	N100	45.6 ± 1.2 a	19.8 ± 3.0 a	32.2 ± 2.6 c
	N150	48.0 ± 1.9 a	17.1 ± 2.9 a	36.8 ± 2.0 bc
Sb + Lc + Af	N0	57.4 ± 1.4 b	30.6 ± 2.8 c	50.9 ± 3.5 b
	N15	53.9 ± 3.7 ab	24.0 ± 1.3 bc	46.2 ± 3.6 b
	N30	55.1 ± 1.3 ab	25.0 ± 2.8 bc	47.1 ± 2.3 b
	N50	52.9 ± 4.4 ab	26.4 ± 3.1 bc	44.0 ± 2.7 ab
	N100	47.4 ± 3.7 a	20.9 ± 3.5 b	40.3 ± 1.9 b
	N150	48.5 ± 1.5 ab	12.1 ± 0.6 a	36.2 ± 2.2 a

Note: Values were mean ± SE, litter letter in the same line behind the values for each litter mean significant difference at $P < 0.05$ level.

Table 6. Three-way ANOVA for C, N and P releasing rate of mixed-litter under N addition after decomposing 417 d.

Source of variation	*df*	Carbon		Nitrogen		Phosphorus	
		F	*P*	F	*P*	F	*P*
Aboveground							
Time (T)	2	954.343		1.80×10^3		423.303	
Specie types (S)	3	129.325		968.98		17.649	
N addition (Ni)	5	5.415		5.628		17.465	
T*S	6	1.999	0.067	62.782		16.951	
T*Ni	10	1.281	0.242	3.875		7.692	
S*Ni	15	1.521	0.099	2.464		2.914	
T*S*Ni	30	0.853	0.690	4.116		3.856	
Belowground							
Time (T)	2	369.592		944.323		812.960	
Specie types (S)	2	14.953		19.938		283.533	
N addition (Ni)	5	2.801	0.019	4.469		6.786	
T*S	4	0.763	0.550	2.052	0.090	10.441	
T*Ni	10	3.064		6.440		3.084	
S*Ni	10	3.171		1.020	0.429	5.663	
T*S*Ni	20	2.261		2.949		4.119	

Note: Vacancy in the table means had significant difference at $P < 0.01$ level.

interaction of adjacent single litters, mixed litter would conserve, enrich or release nutrient element to adapt the environmental changes, and thus maintain the stability of the entire ecosystem. N addition had no significant effect on the litter decomposition because that N addition may have a long-term impact on litter decomposition.

In the similar studies on N addition experiments, Liu *et al.* [49] found N addition had significant effect on the rate of mixed litter decomposition, while Zhang *et al.* [30] found no significant effect. In our previous speculation, we found N addition affected the rate of mixed litter decomposition significantly. The different conclusions were due to the discrepancy of N addition does. Zhang *et al.* [30] was focus on low N addition dose, they were 0, 1.0 and 2.0 $g/m^2 \cdot a$ nitrogen addition levels, which was significantly lower than our study. Liu *et al.* [16] designed three N addition levels on typical steppe which was 8.0, 16.0, 32.0 $g/m^2 \cdot a$, respectively. In addition, the impact of N addition on decomposition rate was affected by decomposition phase, experimental field condition, soil nitrogen availability and natural factors. We found significant interaction effect between species and N addition on the rate of mixed litter decomposition, which would change over decomposing time. Aboveground and belowground litter decomposition rate had different response to species types and N addition. The promote effect of N addition on aboveground mixed litter decomposition rate was stronger than belowground, whilst belowground was more sensitive to specie types. Due to aboveground and belowground litter were subject to different decomposition microenvironment. Therefore they may experience different decomposing patterns and exhibit opposite decomposition result. We found that N addition accelerated leaf and stem mixed litter decaying except N_{150}, significantly inhibited leaf mixtures decaying. High dose of N addition would promote *S. baicalensis* and *L. chinensis* (Sb + Lc) and *L. chinensis* and *A. frigid* (Lc + Af) root mixtures decaying, but N addition retarded *S. baicalensis*, *L. chinensis* and *A. frigid* (Sb + Lc + Af) root mixtures decaying. The effect of N addition on decomposition were relate to some aspects of litter chemistry [50]. Previous studies suggested litter initial chemical composition could better predict litter decomposition rate such as N content, C/N and lignin/N [51]-[53]. The higher initial N content may lead to faster litter decomposition rate, excessive lignin content would retard litter decay [53]. Some prior studies elucidated that N addition was resistant to decomposition may produce microbial decomposers synthesize phenolic compound or break down litter lignin and other polyphenolic compounds into compounds that react with inorganic N to decomposition [54]. Hobbie *et al.* [55] found residual rate of litter was strongly influenced by the interaction of plant species and N addition, which was the same to our study. N addition altered decomposer community composition in steppe ecosystem [30]. He *et al.* (2010) [56] demonstrated N addition decreased microbial abundance and microbial activities, high level of N addition could disrupt balanced relationships in plant-microbial symbiosis system. Tanya *et al.* [10] manifested decomposer organisms with low functional diversity slowed the litter decomposition and the cycling of carbon and nitrogen. N addition, increased soil nitrogen availability, had effect on root mixed litter decomposition directly. Therefore, in the further trial, we would measure decomposer communities and relative impact factors to explore the mechanism of effect of N addition on mixed litter decomposition.

In the natural environment, mixed litter with different physical characters of shape, hardness and specific surface area had higher heterogeneity and beneficial environment to hold on more decomposers. Besides physical characters, nutrient substances and secondary metabolism transferred through the connection effect of leaching and fungal hyphae caused litter decomposition rate changing (Salamanca *et al*. 1998). It is established that the litter contained phenol and lignin in compound form decreasing the decomposition of the mixed litter [57]. Different physical and chemical properties were responsible for various litter decomposition rate, nutrient dynamics and had different response to N addition. Previous reports had proposed that faster decomposition rates and nutrient turnover rate in mixed litters were due to the transfer of nutrients between the litters and the slower decomposition was due to the release of inhibitory compounds [48]. Recent study [10] confirmed the existence of nitrogen transformation from nitrogen-fixing litter species type to fast decomposing litter species type. The shift of nutrient between mixtures may change the litter decomposition, even affect the carbon cycling in ecosystem.

In this study, we found the nutrient dynamic process of each plant tissues litter had different response to N addition. From the chemical composition perspective, the mixture combination within similar initial chemical composition was inclined to negative effect, while ones within discrepant initial chemical composition (*i.e*. *L. chinensis* and *A. frigid* leaf litters) were inclined to positive or non-additive effect. Litter initial chemical composition was the key factor for the mixed effects, and the difference of initial chemical composition between adjacent mixtures determined mixed effect direction. Litter with high nutrient contents could fix and support more microorganisms on the surface, and promoted the nutrient turnover rate of low litter quality [58]. The litter decomposition and nutrient turnover rate were deviated from single litter decomposition by the nutrient transfer and fungal hyphae [48], mixed litters decomposition dynamics were affected by mixed effect, and more sensitive to N addition, and then reacted corresponding variational mechanism to adaptation environmental change. From physical perspective, mixed litter of different plant families, compared to the same plant families' mixtures, was prone to releasing nutrient elements. Therefore, the richness of different plant families may give rise to litters variance response to N addition. But in our study, we only chose *S. baicalensis* (Gramineae), *L. chinensis* (Gramineae) and *A. frigid* (Composite), and did not consider more different families plant litters. It has been suggested that primarily chemical and physical interactions between litter species are the factors in non-additive effects [36]. In addition, identifying the key species or key properties (e.g. secondary chemistry) seems decisive for predicting the process of mixed litter decomposition. Longer-term study is needed to further investigate the effect of litter quality diversity and plant family richness on decomposition.

In our study, N addition had significant effect on litter nutrient dynamics and had interaction effect with decomposing time and specie types. N addition inhibited different plant tissues mixed litter nutrient releasing rate, especially high N addition level. P element releasing rate was more sensitive to N addition than C and N element, showed continuous releasing over litter decomposing. Due to soil N content replenished, litter could get more availability nitrogen, but limited the availability of P element [59]. Vivanco *et al.* [60] suggested N addition accelerated mixed litter decomposition rate and nutrient releasing, and converted non-additive effects into additive effect in temperate forest of South America. Qi *et al*. [61] stated that N addition was not direct factor to restrict litter decomposition, but to the growth of microbial and influenced litter decomposition rate and nutrient release indirectly. The effect of N addition on litter nutrient dynamics had relationship with decomposing time scale, the succession in decomposers species and community structure would be more complex and efficient, and had higher demand for nitrogen with exogenous nitrogen increasing (Berg and Staaf 1980) [39]. In our study, high levels N addition had inhibited effect on mixed litter nutrient releasing. N addition prolonged the nutrient releasing time and was conducive to the retention of nutrient and organic matter in the ecosystem. N addition, increased nitrogen input in soil and enhanced soil fertility directly, could provide more carbon and nutrient for microbial in the short term, which was advantageous to the grassland ecosystem balance, especially to recover degraded grassland. Excessive nitrogen reduced community structure and activity of decomposer and weaken decompose capacity for decaying litter of disintegrators. Hence, the inhibited effect of N addition or nitrogen deposition increased caused the decrease of nutrient turnover in long-term, in the lack of nutrient adverse to plant growth and microbial activity, and impedes the positive succession of ecosystem. As the main carbon source, litter decomposition provided plentiful nutrient for microbial activity. N addition, simulated nitrogen deposition increasing, accelerated the rate of leaf, stem mixed litters, *S. baicalensis* and *L. chinensis* (Sb + Lc) root mixed litter and *L. chinensis* and *A. frigid* (Lc + Sb) root mixed litter, but inhibited litters nutrient releasing rate. The possible explanation could be that litter quality of mixed litters in trial could not sheer meet decomposer microbial activity as absorbed nutrient from environment and reduced litter nutrient releasing [61]. Abby *et al.* [62] stated that the litter quality change associated with N deposition led to increasing litter decomposition. Knorr [63] indicated that N could affect the litter decomposition rate by altering the initial quality and decomposer community in chronic and high N deposition areas. As the process of litter decomposition, mixed litters mediate autologous chemical composition and improve litter quality constantly, conducive to microbial activity and promote litter decomposition, whilst N addition promoted the adaption mechanism of litter decomposition to environmental change.

5. Conclusion

In conclusion, our results demonstrated N addition had significantly effect on mixed litter decomposition rate and nutrient dynamics of plant tissues litter from three plants. We found mixed litter decomposition was influenced by the interaction of decomposing time, species types and N addition. N addition could alter mixed effects among different plant tissues litter, correspondingly different litter quality may lead to different response to N addition. N addition simulated nitrogen deposition tended to inhibit litter nutrient releasing and influenced the retention of nutrient in *Stipa baicalensis* steppe in Inner Mongolia.

Acknowledgements

The authors gratefully acknowledged the financial supported by the National Natural Science Fund of China (Project 31170435), “Twelfth five-year” national science and technology plan projects funding (2012 bad13b07), Central public welfare basic scientific research institutes were special funds (Agro-Environmental Protection Institute, Ministry of Agriculture, Tianjin) and Key laboratory of original Agro-environment quality of ministry of Agriculture and Tianjin Key laboratory of Agro-environment and Safe-product funding.

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