



Moss removal facilitates decomposition and net nitrogen loss of monospecific and mixed-species litter in a boreal peatland

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Abstract Litter decomposition plays an important role in biogeochemical cycling in boreal peatlands, where mosses, especially *Sphagnum* species, are a determinant. In recent decades, these peatlands have experienced a decline in moss cover due to abrupt climate warming and atmospheric nitrogen (N) deposition. To reveal the effect of the reduction in moss cover on litter decomposition, we adopted a field living moss removal experiment (with the senesced tissues remaining) in a *Sphagnum*-dominated boreal peatland, and investigated litter mass loss and net N

loss of three deciduous woody species decomposing in monocultures and mixtures over 3 years. Based on the observed and predicted mass loss and net N loss of litter mixtures, we divided litter mixing effects into additive (no significant difference), synergistic (observed value greater than predicted value), and antagonistic (observed value lower than predicted value) effects. Across 3 years of decomposition, moss removal increased litter mass loss and net N loss, irrespective of single- or mixed-species compositions. Moss removal generally changed litter mixing effects on mass loss from antagonistic to additive effects in the initial 2 years, but from synergistic to additive effects after 3 years of decomposition. Regarding net N loss of litter mixtures, moss removal often resulted in a shift from additive to synergistic effects or from antagonistic to additive effects after 2 and 3 years of decomposition. Our observations suggest that the declines in living moss cover can accelerate litter decomposition and nutrient release, and highlight that living moss loss makes litter mixture decomposition predictable by reducing non-additive effects in boreal peatlands. Given the widespread occurrence of reduced moss cover in boreal peatlands, the mechanisms explaining living moss controls on litter decomposition and N cycling should receive significant attention in further studies.

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Introduction

Boreal peatlands contain 20–30% of the global soil organic carbon (C) stock, which are still widely considered to be a net sink for atmospheric CO₂ (Roulet et al. 2007; Treat et al. 2019). Despite low primary productivity, the slow decomposition rate has led to a net accumulation of organic C in these ecosystems (Roulet et al. 2007; Yang et al. 2023). In addition to low temperature, poorly drained land, and limited nutrient availability, the presence of mosses, especially *Sphagnum* mosses, is also believed to be responsible for the slow decomposition rate (Verhoeven and Toth 1995; van Breemen 1995; Turetsky 2003). Mosses, a dominant and ubiquitous plant functional group in boreal peatlands, can not only produce recalcitrant litter, but also exert inhibiting effects on microbial decomposition of plant litter (Lang et al. 2009; Zhang et al. 2019; Asif et al. 2021). Moss species, especially *Sphagnum* species, are observed to secrete antimicrobial metabolites such as phenolic compounds and other low-molecular-weight soluble phenolics (Verhoeven and Liefveld 1997; Tetemadze et al. 2018; Fudyma et al. 2019), although the concentration of phenolics in living moss tissues does not appear to inhibit microbial activity (Mellegård et al. 2009). Moreover, there is a growing recognition that mosses can inhibit microbial decomposition indirectly by creating acidic conditions and reducing nutrient availability in boreal peatlands (Hajek et al. 2011; Fudyma et al. 2021; Koranda and Michelsen 2021).

For decades and continuing today, boreal peatlands have been subjected to abrupt climate warming and atmospheric nitrogen (N) deposition, which support woody species with a competitive advantage over moss species, especially in the southern edge of these peatlands (Dieleman et al. 2015; Robroek et al. 2017; Ma et al. 2022). Previous studies have revealed that both experimental warming (e.g., Dieleman et al. 2015; Antala et al. 2022) and N enrichment (e.g., Wieder et al. 2019; Shi et al. 2022) reduced the cover of moss species, together with increased cover of vascular plants, especially woody plants in boreal peatlands. In these ecosystems, the reduction in living moss cover may alter litter decomposition dynamics by reversing acidification, improving nutrient availability, and reducing the inputs of moss secondary metabolites, but the significance of this altered plant

composition on litter decomposition has not been examined. Considering the great importance of litter decomposition to soil organic C accumulation (Yang et al. 2023), it is imperative to clarify the effect of reduced living moss cover on plant litter decomposition in boreal peatlands.

In boreal peatlands, plant litter from diverse species often decomposes in mixed assemblages (Ward et al. 2010; Palozzi and Lindo 2017; Zhang et al. 2019, 2022). During litter mixture decomposition, the complex interactions among component species often accelerate (i.e., synergistic effects) or decelerate (i.e., antagonistic effects) decomposition of litter mixtures or component litter (Hättenschwiler et al. 2005; Gessner et al. 2010). Synergistic effects are generally attributed to resource complementarity and nutrient transfer, whereas antagonistic effects are mainly caused by the release of secondary compounds from component species during decomposition (Hättenschwiler et al. 2005; Gessner et al. 2010; Barantal et al. 2014; Liu et al. 2020). In recent decades, the consequences of moss disappearance on litter mixture decomposition have been assessed by mixing moss litter with vascular plant litter (e.g., Ward et al. 2010; Palozzi and Lindo 2017; Zhang et al. 2019, 2022; Asif et al. 2021). However, the existing studies cannot fully describe the consequences of reduced living moss cover on litter decomposition, because the influences of living mosses have often been ignored. In general, the disappearance of living mosses occurs during the initial phase of reduced moss cover. Moreover, living mosses can lower pH, decrease nutrient mineralization, shape decomposer community, and secrete antimicrobial compounds (Verhoeven and Liefveld 1997; Hajek et al. 2011; Tetemadze et al. 2018; Fudyma et al. 2019; Hamard et al. 2019; Koranda and Michelsen 2021), which may alter the magnitude and even the direction of litter mixing effects during decomposition. Therefore, assessing the consequence of living mosses on litter mixing effects during decomposition will contribute to our understanding of the effect of reduced living moss cover on biogeochemical cycling in boreal peatlands.

Peatlands are widely distributed in the Great Hing'an Mountains of northeast China, which lie in the southernmost region of the Eurasian permafrost (Jin et al. 2007). In these ecosystems, mosses, mainly the genus *Sphagnum*, are a critical functional group in plant communities, and play an important role in

constructing ecosystems and driving ecological processes (Mao et al. 2018; Zhang et al. 2018, 2022). In recent decades, the southern edge of boreal peatlands has been predicted to experience a substantial decline in the cover of mosses with the rising temperature and N enrichment (Ma et al. 2022; Shi et al. 2022). In these ecosystems, moss litter is observed to inhibit microbial decomposition and alter litter mixing effects (Zhang et al. 2019, 2022), but little is known about the effect of living mosses on litter decomposition. Here, using a field living moss removal (with the senesced tissues remaining) experiment, we investigated the changes in litter mass loss and net N loss of three deciduous woody species decomposing in monocultures and mixtures after living moss removal. The main objective was to assess how reduced living moss cover alters monospecific and mixed litter decomposition in boreal peatlands. Considering the importance of living mosses to shape harsh environments for decomposers by lowering pH, reducing nutrient availability, and secreting antimicrobial compounds (Verhoeven and Liefveld 1997; Hajek et al. 2011; Tetemadze et al. 2018; Fudyma et al. 2019; Koranda and Michelsen 2021), we hypothesized that living moss removal would (1) increase litter mass loss and net N loss, and (2) decrease the magnitude of antagonistic effects on mixed litter mass loss and net N loss.

Materials and methods

Study site description

The study was conducted in a permafrost peatland (52° 56' N, 122° 51' E) located in the north of the Great Hing'an Mountains in northeast China. The mean annual temperature (1980–2010) is -3.9 °C, and the mean annual precipitation is 450 mm. The active layer of permafrost is about 70 cm in depth, and peat depths range from 40 to 100 cm. The peatland embodies small-scale hummock-hollow microtopographic features, with the water table depths ranging from 11.6 to 32.0 cm beneath the peat surface in the hummock. The moss carpets are ubiquitous in the hummock-hollow complexes, with *Sphagnum* species, such as *S. magellanicum*, *S. perichaetiale*, *S. palustre*, and *S. girgensohnii*, accounting for more than 90% of the moss biomass (Zhang et al. 2018). Vascular plants such as deciduous shrubs, evergreen

shrubs, sedges, and grasses often grow on moss carpets, and the relative cover of vascular plants is about 50%. The annual litter inputs are about 78 g m^{-2} , more than 50% of which are derived from deciduous shrubs. The dominant deciduous woody species are *Betula platyphylla*, *Betula fruticosa*, and *Vaccinium uliginosum*. In autumn, senesced leaves of deciduous shrubs fall on the moss carpet and decompose there. In this peatland, climate warming and N enrichment have increased the growth of deciduous woody plants at the cost of moss species (Chen et al. 2017; Shi et al. 2022).

Leaf litter collection and chemical analyses

In August 2016, fresh leaf litter of *B. platyphylla* (Bp), *B. fruticosa* (Bf), and *V. uliginosum* (Vu) was collected from four 10×10 m plots with a distance of 15–30 m in the peatland. Only the intact freshly senesced leaves were sampled for litter decomposition experiment. About 200 g of fresh litter per species was collected in each plot to ensure representativeness. For each species, leaf litter sampled from the same plot was mixed carefully, oven-dried at 65 °C, and then divided into two subsamples. One subsample was used in the field decomposition experiment, and another subsample was milled (<0.15 mm) to measure chemical properties. Organic C concentration was measured by the dry combustion method (Multi N/C 2100 Analyzer, Analytik Jena, Germany), and lignin concentration was analyzed with the gravimetric method on a fiber analyzer (FT350, FOSS, Denmark) (Möller 2009). Following digestion with concentrated H_2SO_4 at 365 °C in the presence of mixed catalyst (K_2SO_4 : CuSO_4 = 10: 1), total N and P concentrations of leaf litter were respectively determined with the sodium salicylate-sodium hypochlorite and the molybdenum blue methods (Temminghoff and Houba 2004) on a continuous-flow autoanalyzer (AA3, Seal, Germany). The initial chemical properties of leaf litter are shown in Table 1.

Experimental design and litter decomposition experiment

In September 2016, we randomly selected one pair of moss carpets (about 1.0 m^2) with moss covers of 70–90% in each plot where litter samples were collected, and a platform was set up around each moss

Table 1 Initial litter chemical properties of the selected three species in this study

Species	C (mg g ⁻¹)	N (mg g ⁻¹)	P (mg g ⁻¹)	Lignin (mg g ⁻¹)	C:N ratio	C:P ratio	N:P ratio	Lignin:N ratio	Lignin:P ratio
<i>Betula platyphylla</i>	517 (3) ^a	5.69 (0.15) ^a	2.49 (0.10) ^a	156 (6) ^a	90.8 (2.6) ^b	208 (9) ^b	2.29 (0.07)	27.4 (1.0) ^a	62.7 (1.1) ^a
<i>Betula fruticosa</i>	490 (4) ^c	5.36 (0.09) ^b	2.43 (0.06) ^a	110 (5) ^b	91.4 (1.6) ^b	202 (5) ^b	2.21 (0.09)	20.5 (1.2) ^b	45.2 (1.6) ^b
<i>Vaccinium uliginosum</i>	504 (2) ^b	3.37 (1.10) ^c	1.48 (0.13) ^b	98.7 (5.9) ^c	150 (5) ^a	342 (29) ^a	2.28 (0.25)	29.4 (2.3) ^a	66.9 (6.4) ^a

The stoichiometric ratios in this table are mass ratios. Data are mean values with standard deviations in parentheses ($n=4$). Different letters indicate significant differences between species ($P<0.05$)

carpet to reduce disturbance during sampling. In each plot, we carefully removed all living photosynthetic tissues of mosses on one carpet according to Meng et al. (2014) and Ward et al. (2015), and used another carpet without living moss removal as the control. Moss removal was performed every year during the process of litter decomposition. The temperature on the soil surface was determined with a portable digital thermometer (JM 624, Jinming Instrument CO., Ltd, Tianjin, China) in each treatment at 9:30–10:00 AM on the 20th day of every month during the growing season (i.e., from May to September) from 2017 to 2019.

Litter decomposition was measured with the litterbag method. Three monospecific litter treatments (Bp, Bf, and Vu) and four mixed-species litter treatments (Bp+Bf, Bp+Vu, Bf+Vu, and Bp+Bf+Vu) with equal mass proportion were set up in this experiment. For each treatment, 2.0 g of monospecific or mixed-species litter was placed into 20×15 cm litterbags with a mesh size of 1.2×1.0 mm at the upper facing side and 0.3×0.3 mm at the lower facing side. In this study, there were 168 litterbags, with seven litter compositions×two decomposing habitats (i.e., moss removal and the control)×four replicates×three sampling dates. In early October 2016, litterbags were horizontally placed at the base of the litter layer, in contact with the surface of moss carpet in the control treatment or directly on the top of peat layer in the moss removal treatment. After 1–3 years of decomposition, one litterbag per litter treatment was retrieved from each decomposition habitat. After retrieval, the remaining litter was carefully separated from attached debris, and the fresh weight was recorded. Subsequently, the remaining litter within litter mixtures was

sorted into species according to litter morphology and texture, oven-dried at 65 °C, and weighed. Litter moisture content (%) was expressed as the percentage of litter dry weight, which was used to reflect the influence of moss removal on the moisture condition of decomposition habitat. The remaining litter was milled (<0.15 mm) to measure total N concentrations with the method described above. In terms of litter mixtures, component species were re-mixed before being milled because the remaining amount of each component species was not enough for N analysis. The amounts of N in the litter were calculated based on the litter mass and total N concentration. Litter mass loss (%) was calculated from the difference in initial and remaining litter mass, and net N loss (%) was calculated based on the initial and remaining amounts of N in the litter.

Calculation and statistical analyses

The expected mass loss ($Exp_{mass\ loss}$, %) of whole litter mixture was calculated as follows: $Exp_{mass\ loss} = \sum_{i=1}^n Obsm_i \times P_i$, where $Obsm_i$ is the observed mass loss of species i decomposing in the monoculture, and P_i is the initial proportion of species i in the mixture. The expected net N loss ($Exp_{net\ N\ loss}$, %) of whole litter mixture was calculated as follows: $Exp_{net\ N\ loss} = 100 - (\sum_{i=1}^n ObsN_i \times M_i \times P_i) / N_{initial}$, where $ObsN_i$ and M_i are the observed N concentration and remaining mass of species i decomposing in the monoculture, respectively, P_i is the initial proportion of species i in the mixture, and $N_{initial}$ is the initial N amount of the whole mixture. The mixing effects (%) were calculated as $Obs-Exp$, where Obs is the observed mass loss or net N loss of the whole

mixture. One-sample *T* test was used to assess the difference between zero and mixing effects. No significant difference between zero and mixing effects indicates additive effects, and positive and negative values, respectively, indicate synergistic and antagonistic effects. Individual species response (%) to mixture was calculated as *Mixture—Alone*, where *Mixture* and *Alone* are the mass loss (%) of component species decomposing in the mixture and the monoculture, respectively. One-sample *T* test was used to assess the difference between zero and individual species responses. There was no significant response when the response was equal to zero, while there was a positive (i.e., accelerated mass loss) or negative (i.e., decelerated mass loss) response when the value was greater or lower than zero, respectively.

The data were statistically analyzed in the R (v. 4.0.5), with an accepted significance level of $P < 0.05$. Data were checked for normality with the Shapiro–Wilk test, and were log-transformed before analysis if necessary. Repeated measures analysis of variance (ANOVA) within the general linear model was adopted to assess the effect of species composition, moss treatment, and sampling date on litter mass loss and net N loss, and mixing effects during decomposition. One-way ANOVA, followed by Tukey’s multiple comparison test, was used to determine the difference in initial chemical properties among the selected three species. At each sampling date, independent-sample *T* test was performed to compare the differences in soil surface temperature, litter moisture, litter mass loss, and net N loss between the moss removal and the control treatments. In terms of litter mixture decomposition, we determined the effects of moss removal on mixing effects and individual species responses

mainly based on the qualitative results, such as synergistic vs. additive effects (for mixing effects) and positive vs. negative responses (for individual species responses).

Results

Soil surface temperature and litter moisture content

Throughout the experiment period, soil surface temperature did not differ significantly between the control and moss removal treatments across the growing season (all $P > 0.05$, Fig. S1). Irrespective of sampling date and litter type, litter moisture contents during decomposition were nearly always above 120%, with no significant differences between the control and the moss removal treatments (all $P > 0.05$, Fig. S2).

Litter mass loss and net N loss

Both moss removal and species composition significantly affected mass loss and net N loss, and such effects varied with sampling date (Table 2). Litter mass loss in the moss removal treatment was always greater than in the control treatment across the 3 years of decomposition, irrespective of species composition (Fig. 1a–c). Compared to the control treatment, litter mass loss was 4.92–8.10% and 5.51–11.8% greater in the moss removal treatment after 1 and 3 years of decomposition, respectively (Fig. 1a–c). Concomitantly, moss removal also significantly increased net N loss of all litter treatments, with increment magnitudes of 16.2–22.5%, 20.5–37.7%, and 13.8–22.1%

Table 2 Results (*F*-values) of repeated measures ANOVA indicating effects of species composition (C), moss treatment (M), and sampling date (D) on decomposition dynamics and mixing effects across 3 years of decomposition

	Decomposition dynamics			Mixing effects		
	<i>df</i>	Mass loss	Net N loss	<i>df</i>	Mass loss	Net N loss
Within-subject effect						
D	2	7201**	7402**	2	46.2**	47.3**
D×C	12	4.59**	13.4**	6	4.51**	3.16**
D×M	2	22.1**	63.4**	2	31.3**	5.02**
D×C×M	12	4.11**	2.14*	6	4.14**	2.83*
Between-subject effect						
C	6	195**	204**	3	1.88 ns	11.4**
M	1	1753**	1267**	1	4.26*	10.1**
C×M	6	2.21 [§]	5.08**	3	0.398 ns	0.739 ns

ns $P > 0.06$,

[§] $0.05 < P < 0.06$, * $P < 0.05$,

** $P < 0.01$

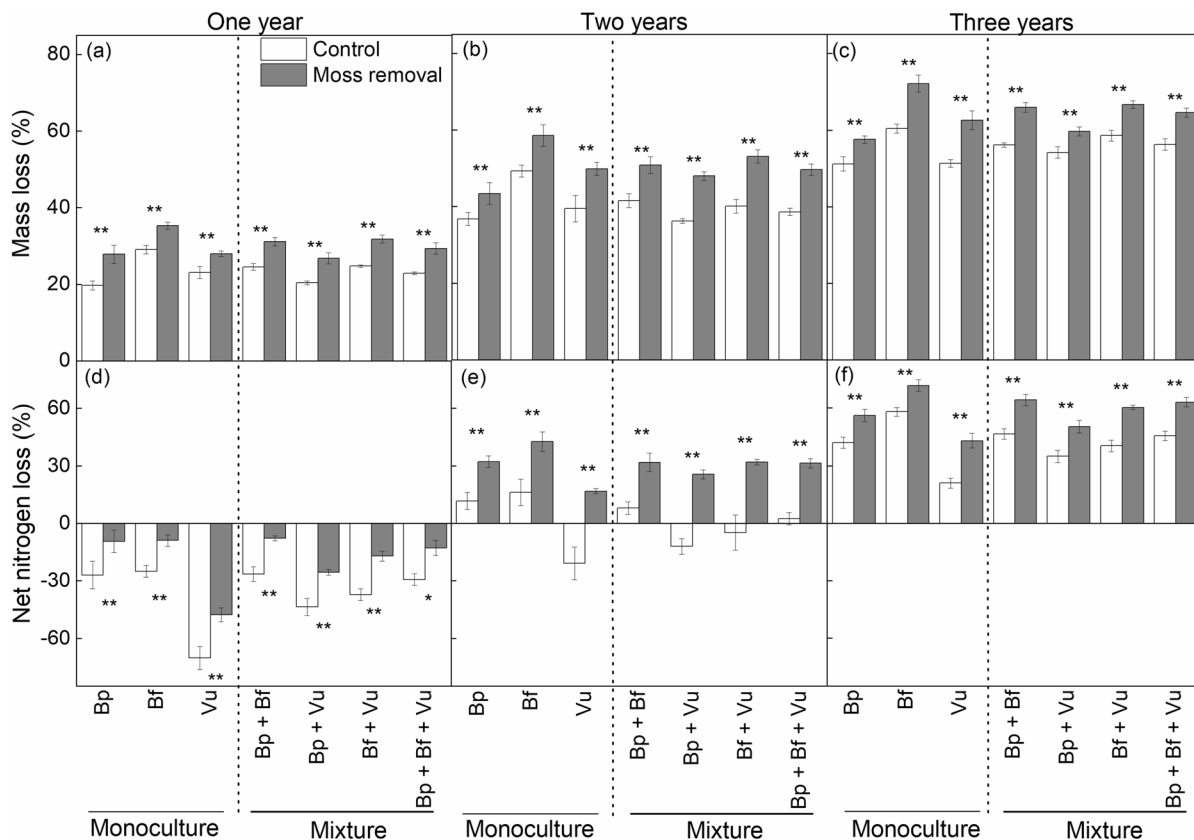


Fig. 1 Mass loss (a–c) and net nitrogen loss (d–f) of litter monocultures and mixtures during 3 years of decomposition. Data are mean values ($n=4$) and error bars represent the standard deviation. The significant differences between control and

moss removal treatments are indicated by * ($P<0.05$) and ** ($P<0.01$). Bp *Betula platyphylla*, Bf *Betula fruticosa*, Vu *Vaccinium uliginosum*

after 1, 2, and 3 years of decomposition, respectively (Fig. 1d–f).

Mixing effects on litter mass loss and net N loss

During 3 years of decomposition, moss removal significantly affected mixing effects on mass loss, and such effects varied with sampling date rather than species composition (Table 2). In the control treatment, litter mixtures, except for *B. platyphylla*+*B. fruticosa* mixtures, exhibited antagonistic effects on mass loss in the initial 2 years of decomposition, but synergistic effects after 3 years of decomposition (Fig. 2). However, additive effects on mass loss were observed for all mixtures during decomposition in the moss removal treatment (Fig. 2).

Both moss removal and species composition significantly affected mixing effects on net N loss

during 3 years of decomposition, and such effects varied with sampling date (Table 2). For *B. fruticosa*+*V. uliginosum* and *B. platyphylla*+*B. fruticosa*+*V. uliginosum* mixtures, synergistic effects on net N loss were observed in both control and moss removal treatments after 1 year of decomposition (Fig. 2d). After 2 years of decomposition, antagonistic effects on net N loss were observed for *B. platyphylla*+*B. fruticosa* and *B. platyphylla*+*V. uliginosum* mixtures in the control treatment, but such mixing effects changed to additive effects with moss removal (Fig. 2e). After 3 years of decomposition, synergistic effects on net N loss were found for the three-species mixture in the moss removal treatment, while antagonistic effects were found for *B. platyphylla*+*B. fruticosa* mixture in the control treatment (Fig. 2f).

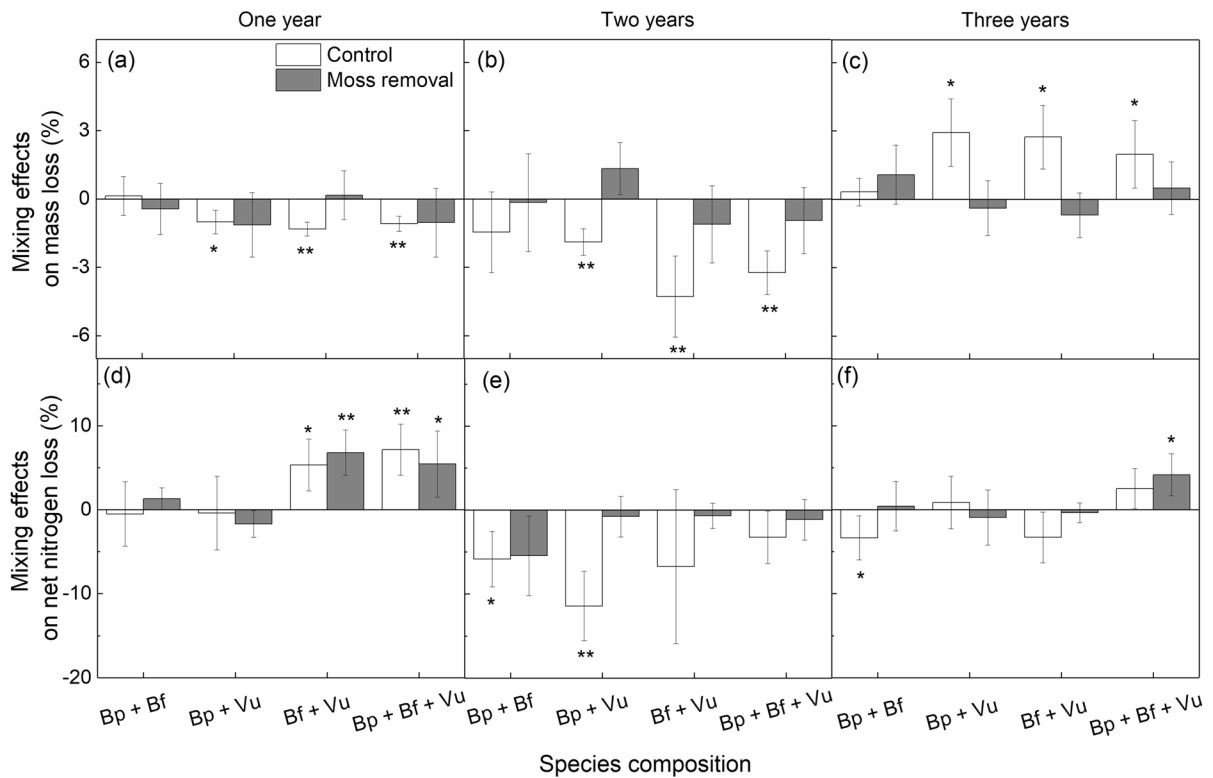


Fig. 2 Mixing effects on mass loss (a–c) and net nitrogen loss (d–f) of whole litter mixtures during 3 years of decomposition. Data are mean values ($n=4$) and error bars represent the standard deviation. The significant differences between the lit-

ter mixing effects and zero are indicated by * ($P<0.05$) and ** ($P<0.01$). Bp *Betula platyphylla*, Bf *Betula fruticosa*, Vu *Vaccinium uliginosum*

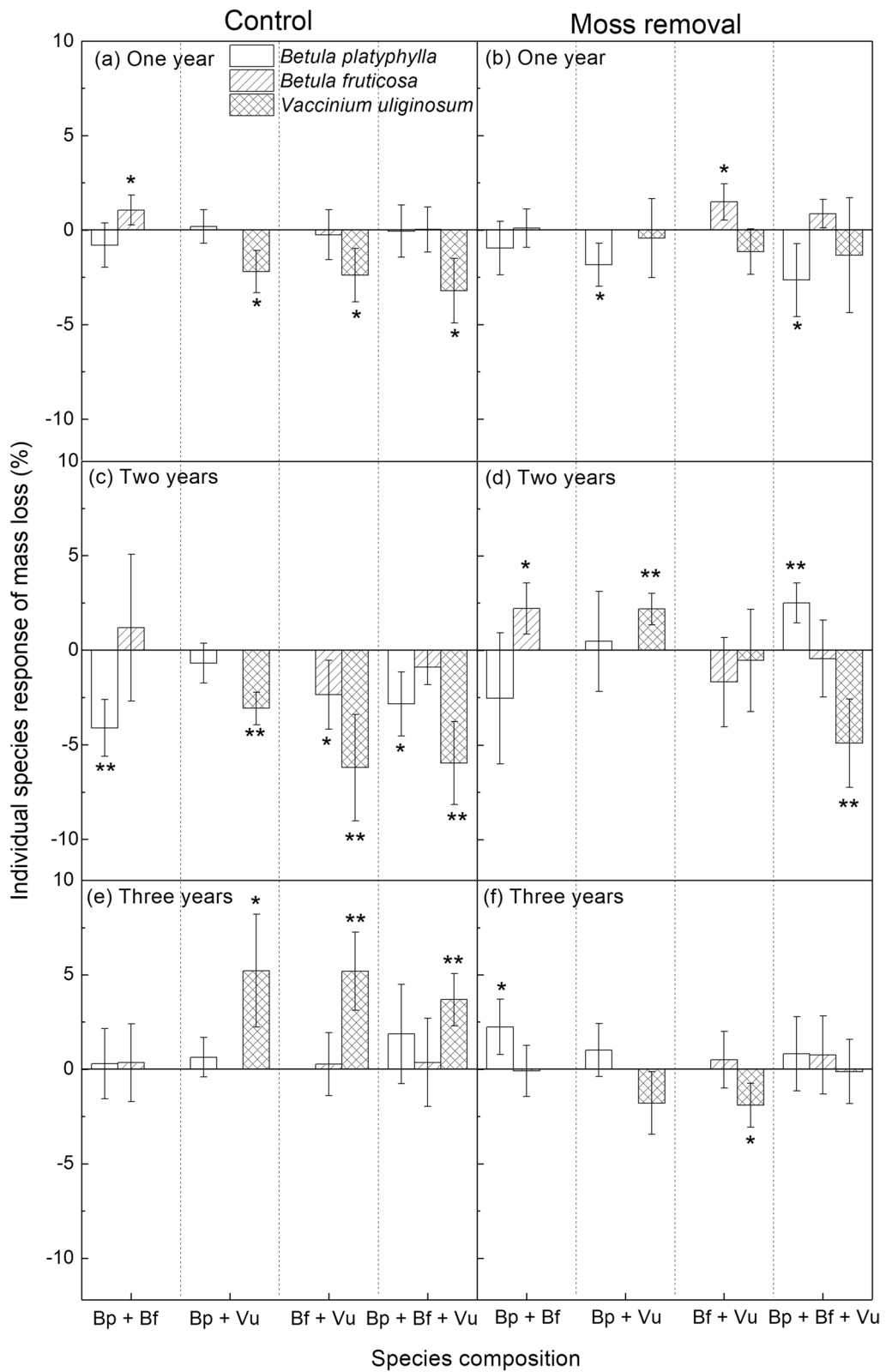
Individual species response of mass loss to mixture

The response of *V. uliginosum* to litter mixture ranged from -2.20 to -3.21% in the control treatment after 1 year of decomposition, but such a negative response disappeared with moss removal (Fig. 3a and b). Similarly, *B. platyphylla* exhibited a negative response after 1 year of decomposition in the *B. platyphylla* + *V. uliginosum* and *B. platyphylla* + *B. fruticosa* + *V. uliginosum* mixtures in the moss removal treatment (Fig. 3b). After 2 years of decomposition, *V. uliginosum* exhibited a negative response ($-3.08 \sim -6.20\%$) to litter mixtures, while *B. platyphylla* showed a negative response in *B. platyphylla* + *B. fruticosa* and *B. platyphylla* + *B. fruticosa* + *V. uliginosum* mixtures in the control treatment (Fig. 3c). In the moss removal treatment, *V. uliginosum* had a negative response in the three-species mixture, but had a positive response in the *B. platyphylla* + *V. uliginosum* mixtures after 2 years of decomposition (Fig. 3d). In contrast,

B. platyphylla and *B. fruticosa* showed a positive response in the *B. platyphylla* + *B. fruticosa* + *V. uliginosum* and *B. platyphylla* + *B. fruticosa* mixtures after 2 years of decomposition in the moss removal treatment (Fig. 3d). After 3 years of decomposition, however, *V. uliginosum* exhibited a positive response in all mixtures in the control treatment (Fig. 3e). In the moss removal treatment, *B. platyphylla* exhibited a positive response in *B. platyphylla* + *V. uliginosum* mixture, whereas *V. uliginosum* exhibited a negative response in *B. fruticosa* + *V. uliginosum* mixture after 3 years of decomposition (Fig. 3f).

Discussion

Irrespective of single- or mixed-species litter assemblages, moss removal accelerated leaf litter decomposition throughout the experiment, providing supportive evidence for our first hypothesis. In previous



◀**Fig. 3** Individual species responses of mass loss to mixture during 3 years of decomposition. Data are mean values ($n=4$) and error bars represent the standard deviation. The significant differences between individual species responses and zero are indicated by * ($P<0.05$) and ** ($P<0.01$). Bp *Betula platyphylla*, Bf *Betula fruticosa*, Vu *Vaccinium uliginosum*

studies, *Sphagnum* litter was also observed to decelerate vascular plant litter decomposition (Verhoeven and Toth 1995; Zhang et al. 2019, 2022). However, Ward et al. (2015) observed that moss removal hardly affected litter decomposition in an ombrotrophic bog. The rationale behind such inconsistency may be attributed to the difference in the dominance of *Sphagnum* species. Contrary to Ward et al. (2015), *Sphagnum* mosses rather than feather mosses are the dominant group in the moss carpet in our study site (Zhang et al. 2018). In general, living *Sphagnum* mosses can inhibit microbial activity by lowering pH (Soudzilovskaia et al. 2010; Fudyma et al. 2021) and reducing nutrient availability (Häjek et al. 2011; Koranda and Michelsen 2021). Moreover, the accumulation of phenolic compounds released from *Sphagnum* mosses may suppress microbial decomposition (Verhoeven and Liefveld 1997; Tetemadze et al. 2018; Fudyma et al. 2019; Hamard et al. 2019), although the amount of phenolics in living moss tissues may not be elevated enough to support antimicrobial activity (Mellegård et al. 2009). Given the lack of observed difference in soil surface temperature (Fig. S1) and litter moisture content (Fig. S2), the accelerated litter decomposition rate after moss removal may be attributed to the reduction in acidic conditions, increased nutrient availability, and reduced inputs of antimicrobial compounds. Because senesced mosses were preserved in situ after living moss removal, the emerging results imply that the living mosses also exert a strong inhibiting effect on litter decomposition in *Sphagnum*-dominated boreal peatlands.

Consistent with our first hypothesis, moss removal increased litter net N loss throughout 3 years of decomposition. During the initial stage of decomposition, microbial decomposers can immobilize N from soils, litter, and microbial dead residues, leading to a net increase in litter N remaining (Manzoni et al. 2010; Kou et al. 2020). We also observed net increases in N content in all litter treatments after 1 year of decomposition, indicating microbial N

immobilization in decomposing litter. In this study, the positive effect of moss removal on litter net N loss may be associated with enhanced soil microbial activity stimulating N-cycling and potential use. In boreal peatlands, moss carpets can lower soil N availability by intercepting atmospheric deposited N and suppressing soil N mineralization (Lamers et al. 2000; Turetsky 2003). Therefore, moss removal could create suitable conditions for soil microbes and thus accelerate microbial mineralization of N from decomposition litter. Unfortunately, we did not determine soil microbial community and activity in this experiment, which limits our understanding of microbial-driven N dynamics in decomposing litter. Nevertheless, our results imply that reduced living moss cover contributes to net N loss from decomposing litter, which will increase available N for plant growth in boreal peatlands.

In the presence of living mosses, except for *B. platyphylla*+*B. fruticosa* mixture, antagonistic effects on litter mixture mass loss were observed after 1 and 2 years of decomposition, whereas synergistic effects on mass loss occurred after 3 years of decomposition (Fig. 2). Previous studies have also observed the occurrence of synergistic, additive, and antagonistic effects during decomposition of litter mixtures in boreal peatlands (e.g., Ward et al. 2010; Leory et al. 2018; Zhang et al. 2019, 2022, 2023). In this study, the additive effects on mass loss of *B. platyphylla*+*B. fruticosa* mixture throughout 3 years of decomposition were presumably due to the similar chemical quality of component species (Zhang et al. 2020). However, antagonistic effects on mass loss of litter mixtures in the initial 2 years may be related to the following mechanisms. First, living mosses, especially *Sphagnum* species, can inhibit litter decomposer growth and activity by shaping acidic conditions (Stalheim et al. 2009; Soudzilovskaia et al. 2010; Fudyma et al. 2021) and decelerate nutrient mineralization (Häjek et al. 2011; Koranda and Michelsen 2021). Second, soil microbes are well-adapted to utilize recalcitrant moss litter in the presence of living mosses (Hamard et al. 2019; Sytiuk et al. 2022), which may suppress complementary resource use. In addition, the accumulation of antimicrobial metabolites secreted from living mosses may suppress microbial decomposition of plant litter (Verhoeven and Liefveld 1997; Fudyma et al. 2019). As litter decomposition proceeds, lignin-related

compounds often become selectively enriched in remaining decomposed litter (Berg and McClaugherty 2014). Compared to the *Betula* species, *V. uliginosum* leaves were less lignified, and thus, the preferential feeding by consumers would accelerate decomposition of the whole litter mixtures (Castro-Díez et al. 2019). Thus, the mixing effects on litter mass loss were switched to synergistic effects after 3 years of decomposition. According to the species performance within mixtures, we also observed that such a switch resulted from a reversion from a negative to a positive response of *V. uliginosum* litter. These observations imply that decomposition stage may partly account for the inconsistent litter mixing effects during litter mixture decomposition in boreal peatlands.

In accordance with the second hypothesis, moss removal initially changed antagonistic to additive effects on mass loss, but subsequently shifted synergistic to additive effects during litter mixture decomposition. Although the presence of moss litter often causes antagonistic effects on litter mixture decomposition (e.g., Ward et al. 2010; Zhang et al. 2019; Asif et al. 2021), the effect of living mosses on litter mixing effects and its underlying mechanisms have been rarely assessed. In this peatland, moss removal may increase microbial decomposition by creating favorable conditions for decomposers (Stalheim et al. 2009; Hájek et al. 2011; Koranda and Michelsen 2021) and reducing antimicrobial compound inputs (Verhoeven and Liefveld 1997; Fudyma et al. 2019). Moreover, increased microbial decomposition-induced increments in nutrient availability may reduce the incidence of non-additive effects by reducing trait dissimilarity of litter mixtures (Barantal et al. 2014; Zhang et al. 2023). Therefore, antagonistic effects were shifted toward additive effects after 1 and 2 years of moss removal. Litter chemical composition often converges during decomposition (Kou et al. 2020). Moss removal-accelerated litter decomposition may reduce litter chemical diversity after 3 years of decomposition, leading to the changes in litter mixing effects from synergistic to additive effects. These findings indicate that loss of living moss cover can make litter mixture decomposition predictable by reducing the incidence of non-additive effects during decomposition in *Sphagnum*-dominated boreal peatlands.

Despite unchanged litter mixing effects on net N loss after 1 year of decomposition, moss removal

often changed antagonistic to additive effects or additive to synergistic effects after 2 and 3 years of decomposition. Thus, these observations partly supported the second hypothesis. This changing trend of mixing effects on net N loss was different from that on mass loss, implying that the effect of moss removal on nutrient loss could not be mirrored by that on mass loss during litter mixture decomposition. During litter decomposition, N is often initially immobilized by decomposers because plant litter has much greater C:N ratio than decomposer organisms, and subsequently exhibits a net release of N when litter C:N ratio reaches a critical threshold value (Manzoni et al. 2010; Spohn and Berg 2023). As shown in Fig. 1, litter exhibited a net accumulation of N after 1 year of decomposition likely because of microbial N immobilization. Thus, moss removal did not alter the mixing effects on litter net N loss during the phase of N immobilization. However, after 2 and 3 years of decomposition, we speculated that the shifts in litter mixing effects on net N loss after moss removal could be attributed to enhanced litter N mineralization due to increased microbial activity (Hájek et al. 2011) and subsequent N transfer among component litter (Kou et al. 2020; Liu et al. 2020). These results indicate that altered mixing effect is an important factor driving the stimulating effect of reduced moss cover on net N loss of litter mixtures in boreal peatlands. Due to multiple pathways of microbial N immobilization and mineralization, further studies using ^{15}N -labelled trace experiments should be conducted to quantify the amount of N transfer and thus uncover the potential mechanisms.

Based on a 3-year field experiment, moss removal significantly facilitated litter mass loss and net N loss during monospecific and mixed litter decomposition, and changed the direction of mixing effects on mass loss and net N loss. These results suggest that living mosses play an important role in retarding litter decomposition and net nutrient loss in *Sphagnum*-dominated boreal peatlands. Although living mosses are reported to inhibit decomposer activity indirectly by causing acidification and reducing nutrient availability (Hájek et al. 2011; Fudyma et al. 2021; Koranda and Michelsen 2021), whether living moss-secreted secondary metabolites such as phenolic compounds suppress field litter decomposition is still unclear. In this study, we did not measure soil pH, phenolic concentration, nutrient availability, and microbial

biomass and activity, so our results do not provide evidence for the mechanisms controlling the stimulating effect of living moss cover reduction on litter decomposition. Therefore, further studies are needed to investigate these abiotic and biotic parameters to fully uncover the role of living mosses in regulating litter decomposition in boreal peatlands.

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Author contributions RM conceived and designed the experiments. XZ, XW, and RM performed the experiments. XZ and RM analyzed the data. XZ and RM wrote the manuscript. WC and WXC provided editorial advice.

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Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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