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Non-additive effects of litter diversity on greenhouse gas emissions from alpine steppe soil in Northern Tibet

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While litter decomposition is a fundamental ecological process, previous studies have mainly focused on the decay of single species. In this study, we conducted a litter-mixing experiment to investigate litter diversity effects on greenhouse gas (GHG) emissions from an alpine steppe soil in Northern Tibet. Significant non-additive effects of litter diversity on GHG dynamics can be detected; these non-additive effects were the result of species composition rather than species richness. Synergistic effects were frequent for CO₂ and N₂O emissions, as they were found to occur in 70.5% and 47.1% of total cases, respectively; antagonistic effects on CH₄ uptake predominated in 60.3% of the cases examined. The degree of synergism and antagonism may be significantly impacted by litter chemical traits, such as lignin and N, lignin:N ratio, and total phenols during decomposition ($P < 0.05$). In addition, the relationship between chemical traits and litter-mixing effects changed over incubation time. Our study provides an opportunity to gain insight into the relationship between litter diversity and soil ecological processes. The results indicate that higher plant diversity may generally enhance CO₂ and N₂O emissions while inhibiting CH₄ uptake; meanwhile, the direction and strength of non-additive effects appear to be related to litter chemical traits.

Over the past century, rates of species extinction have accelerated to 2–3 orders of magnitude higher than the ambient levels recorded in the fossil record¹. Changes in ecosystem functioning are a major consequence of decreasing diversity, because some ecosystem processes depend on the presence of a specific number of functional groups, species, and genotypes of organisms². Within the field of biodiversity-ecosystem functioning research, the majority of works have focused on how plant diversity affects above-ground ecosystem processes^{3,4}. However, the mechanisms by which plant diversity can affect other key ecosystem processes, such as litter decomposition and soil ecological processes, are still being examined^{5–7}.

Litter decomposition is a fundamental multitrophic process that supplies organic and inorganic elements to soil in natural ecosystems⁸. Most terrestrial ecosystems consist of a mixture of plant species, and litter-mixing studies indicate that litter decomposition processes in mixtures can be quite different from those of a single species^{2,9}. In the litter-mixing experiment, litters from at least two species are mixed together and the effects of the mixture are compared with what would be expected based on the additive effects of all the component species in monoculture¹⁰. Previous studies demonstrated that the

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effects of litter-mixing on decomposition rate were unpredictable, because additive and non-additive (synergistic and antagonistic) effects were observed, and non-additivity seemed to be predominant^{2,9,11,12}. If non-additive effects occur, results of litter effect in mixture cannot be predicted as simply the sum of single species results, thus, litter decomposition of a single species did not sufficiently represent litter decomposition processes at an ecosystem level.

Mixed litters from species with varying resource quality and structure change the chemical environment and physically alter the total litter surface where decomposition is occurring^{13,14}. These alterations can also affect soil ecological processes such as soil respiration, net N mineralization, and microbial activity. Although there have been some studies examining how litter mixing affects these soil ecological processes^{11,15,16}, the direction of the specific effects and the role that diversity itself plays in mediating the non-additivity of soil processes remains unclear, and our understanding of how soil ecological processes can be altered by litter diversity is still limited.

Carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) are three important GHGs contributing to global warming in the atmosphere¹⁷. Soils play an important role in the global budgets of these GHGs, as they are able to act both as sources and sinks for the GHGs¹⁸. Plant litter provides a source of readily available C, N, and other chemical components (e.g., condensed tannin and terpenes) into the soil during decomposition, and subsequently influences CO₂, CH₄, and N₂O emissions from soil^{19,20}. Nevertheless, the majority of litter mixing studies have focused on mass loss and nutrient dynamics^{6,21,22}, as well as decomposer community¹¹, with less information available on how GHGs respond to litter mixture decomposition. Considering the ecological significance of GHGs, a thorough understanding of the dynamics of the GHG response to plant litter diversity is, therefore, indispensable for an accurate comprehension of terrestrial ecosystem functioning and for climate change projects²³.

Litter diversity can be defined as species richness and composition or interactions among species⁵. In the present study, a full-factorial litter-mixing experiment was employed to investigate the effects of litter diversity on GHG emissions from an alpine steppe soil in Northern Tibet. Four litter species (*Stipa purpurea*, SP; *Carex moorcroftii*, CM; *Leontopodium pusillum*, LP; *Artemisia nanshanica*, AN) were incubated in monoculture and mixture with soil, and fluxes of CO₂, N₂O, and CH₄ were measured 16 times during a 61-day incubation period. The aim of our work was to (1) investigate the effects of plant litter additions from the alpine steppe soil in Northern Tibet on GHG (CO₂, CH₄, and N₂O) emissions; and (2) test the effects of litter species diversity (richness and composition) on soil GHG emissions during decomposition.

Results

Effects of litter addition on GHGs. Alpine steppe soil without litter was the “source” of CO₂ and the addition of litter to microcosms can significantly enhance CO₂ emissions (Fig. 1 a1–a3). After the 61-day incubation, cumulative CO₂ emission from soil was 59.19 ± 1.55 mg-C kg soil⁻¹ in the control treatment. In monospecific litter, the cumulative CO₂ emission was highest in AN treatment, followed by CM, SP, and LP treatment, with a value of 1033.96 ± 48.18 mg-C kg soil⁻¹, 1016.07 ± 29.82 mg-C kg soil⁻¹, 949.13 ± 55.10 mg-C kg soil⁻¹, and 733.47 ± 30.70 mg-C kg soil⁻¹, respectively. In the litter mixture, the cumulative CO₂ emission was highest in SP+CM+LP+AN treatment and lowest in SP+LP treatment, with values of 1246.86 ± 29.21 mg-C kg soil⁻¹ and 804.56 ± 22.01 mg-C kg soil⁻¹.

Alpine steppe soil was also the “source” of N₂O, and similarly, the addition of litter to microcosms can significantly enhance N₂O emissions (Fig. 1 b1–b3). The cumulative N₂O emissions were 29.59 ± 1.57 μg-N kg soil⁻¹ in control treatment. In monospecific litter, soil with SP treatment had the highest cumulative N₂O emission, with a value of 85.6 ± 6.88 μg-N kg soil⁻¹, followed by AN, CM, and LP treatment, with values of 76.92 ± 3.07 μg-N kg soil⁻¹, 59.15 ± 1.09 μg-N kg soil⁻¹, and 44.88 ± 3.95 μg-N kg soil⁻¹. In litter mixture, the cumulative N₂O emission was highest in CM+LP+AN treatment and lowest in SP+LP+AN treatment, with values of 82.27 ± 1.79 μg-N kg soil⁻¹ and 57.66 ± 4.64 μg-N kg soil⁻¹, respectively.

Alpine steppe soil with and without litter treatments were the “sink” for CH₄ (Fig. 1 c1–c3). After the 61-day incubation, the cumulative CH₄ uptake was 64.76 ± 3.77 μg-C kg soil⁻¹ in control treatment. Litter amending either decreased or had no significant effect on soil CH₄ absorption. In monospecific litter, AN treatments can significantly decrease CH₄ uptake, with the value of 29.49 ± 0.73 μg-C kg soil⁻¹. SP, CM, and LP treatment had no significant effect on soil CH₄ uptake, with values of 73.76 ± 7.08 μg-C kg soil⁻¹, 65.79 ± 3.02 μg-C kg soil⁻¹, and 67.39 ± 2.54 μg-C kg soil⁻¹, respectively. In litter mixture, the cumulative CH₄ uptake was lowest in SP+AN treatment, with a value of 35.59 ± 3.33 μg-C kg soil⁻¹.

Testing non-additive effects of litter diversity. Significant SpInt term was recorded for CO₂ and N₂O, indicating that litter mixing had non-additive effects on cumulative CO₂ and N₂O emission (Table 1). The effects were time dependent, as significant interactions between SpInt and incubation time were detected (see Supplementary Table S1). Although SpInt term was not significant for CH₄ uptake, a significant interaction between SpInt and incubation time also indicated the non-additive effects of litter mixing for CH₄ uptake (Supplementary Table S1).

For cumulative CO₂, N₂O emission and CH₄ uptake, the replacement of the SpInt term with the Richness term did not identify richness as driving the non-additivity (CO₂: F = 2.231, P = 0.122; N₂O: F = 0.01, P = 0.99; CH₄: F = 0.311, P = 0.735), indicating that the non-additive effects only arose from

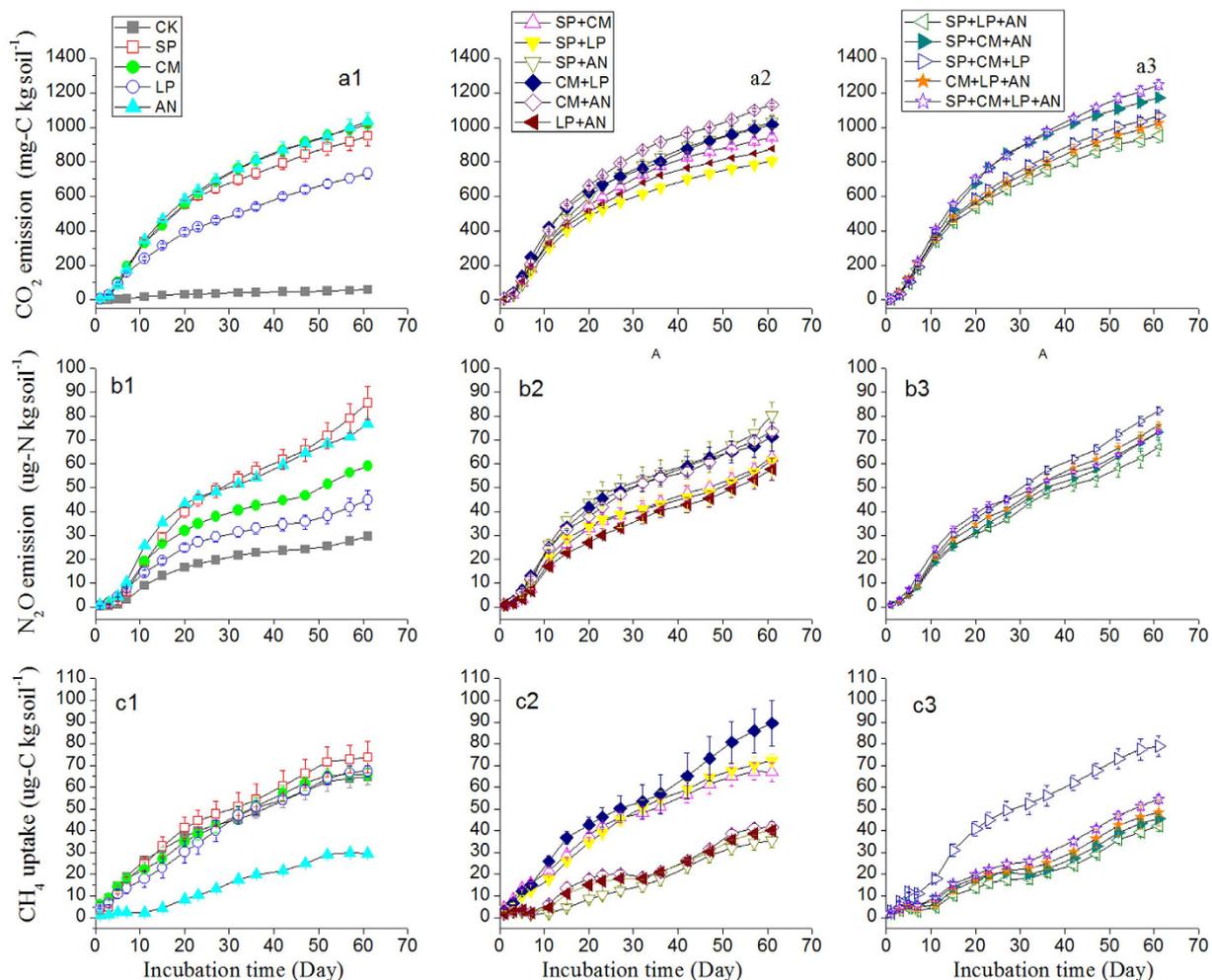


Figure 1. Cumulative CO₂ (a1–a3), N₂O (b1–b3) emissions and CH₄ (c1–c3) uptake from control and litter treatments. CK, control; SP, *S.purpurea*; CM, *C.moorcroftii*; LP, *L.pusillum*; AN, *A.nanschanica*. Error bars represent the standard error (SE, n = 3)

composition. To determine which species contributed to the non-additivity, we compared the observed value for all mixtures involving each species against those that would be expected based on the average of that species in monoculture and the treatment that contained the other species involved, as described by Ball, *et al.*²⁴. Composition effects interacted with time for cumulative CO₂, N₂O emissions and CH₄ uptake (Supplementary Table S1); here we took the cumulative values at the end of the 61-day experiment as the example. As shown in Fig. 2, each species can contribute to non-additive effects. The four species all tended to increase cumulative CO₂ emissions (Fig. 2a); SP tended to decrease N₂O emissions, while the other three species tended to increase emissions (Fig. 2b); the four species tended to decrease CH₄ uptake as shown in Fig. 2c.

Synergistic and antagonistic effects of litter diversity. The observed/expected method showed that non-additive effects were more frequent than additive effects for cumulative CO₂, N₂O emissions and CH₄ uptake through incubation time (Fig. 3). 71.6%, 65.9%, and 65.3% of cases showed non-additive effects for cumulative CO₂, N₂O, and CH₄ values, respectively. Synergistic effects were more frequent in non-additivity for CO₂ and N₂O emissions, with 70.5% and 47.1% of total cases, respectively. Antagonistic effects were more frequent for CH₄ uptake, with 60.3% of the total examined cases.

The strength of the synergistic and antagonistic effect was calculated as *LME* (Supplementary Fig. S1). Stepwise multiple regression showed that *LME* of cumulative CO₂, N₂O, and CH₄ emission/uptake can be positively or negatively effected by litter lignin, N, total phenol, and lignin:N during decomposition (Fig. 4).

Discussion

Total soil CO₂ efflux is overwhelmingly the product of respiration by roots (autotrophic respiration) and soil decomposers (heterotrophic respiration) in natural terrestrial ecosystems²⁵. In our incubation

Source	Type I SS	df	Mean Square	F	P
%CO₂					
Block	95760.20	2	47880.10	3.25	0.054
SP	124150.39	1	124150.39	8.44	0.007
CM	1785556.45	1	1785556.45	121.33	0.000
LP	197548.80	1	197548.80	13.42	0.001
AN	1107789.51	1	1107789.51	75.28	0.000
SpInt	741113.89	10	74111.39	5.04	0.000
Error	412057.48	28	14716.34		
%N₂O					
Block	1229.55	2	614.78	3.31	0.051
SP	1106.70	1	1106.70	5.96	0.021
CM	1227.72	1	1227.72	6.61	0.016
LP	1125.35	1	1125.35	6.06	0.020
AN	1433.69	1	1433.69	7.72	0.010
SpInt	14701.47	10	1470.15	7.92	0.000
Error	5198.196	28	185.65		
%CH₄					
Block	513.93	2	256.97	0.80	0.461
SP	1123.90	1	1123.90	3.48	0.072
CM	5969.67	1	5969.67	18.51	0.00
LP	3565.62	1	3565.62	11.05	0.002
AN	82942.20	1	82942.20	257.14	0.00
SpInt	2852.59	10	285.26	0.88	0.559
Error	9031.70	28	322.56		

Table 1. Summary of repeated measures of ANOVA testing for non-additive effects of litter mixing on cumulative CO₂, N₂O, and CH₄ dynamics using Type I SS. SP, *S.purpurea*; CM, *C.moorcroftii*; LP, *L.pusillum*; AN, *A.nanschanica*.

experiment, CO₂ emission of microcosms may have come primarily from the decomposition of soil organic matter and plant litter driven by soil microbes. Alpine steppe soil was the source of CO₂, and significant positive effects on CO₂ emissions could be found when plant litters were added to alpine steppe soil. (Fig. 1a1–a3). The enhanced emissions may be due to the increased soil fertility and supply of energy during litter decomposition, which stimulates significant increases in microbial activity²⁶. It has been reported that litter quality could affect litter decomposition^{27,28} and soil C, N cycling^{15,29}. For example, the higher N content in litter tended to simulate litter decomposition and soil C mineralization^{8,30}. In this study, the significantly positive relationship between litter N content and soil CO₂ emission supported the idea that the different enhancement levels of litter treatments on soil CO₂ emissions may be partly due to the differences of N content in litter combinations (Supplementary Fig. S2).

N₂O is mainly produced in soil by nitrification and denitrification, and it is generally assumed that nitrification is the predominant process in the aerobic alpine steppe soil³¹. In this study, alpine steppe soil was the weak source of N₂O, which also was supported by Wei *et al.*³² and Cai *et al.*³¹. Similarly with CO₂ emission, litter treatments were able to simulate N₂O emissions from alpine steppe soil (Fig. 1b1–b3). The availability of N plays a crucial role in determining limitations on N₂O production³³. Bowman³⁴ documented that higher litter N concentration may have increased decomposition and may be responsible for the increased N turnover and N₂O emission. Thus, compared with the control, the higher N availability in litter-addition soil may explain the enhancement of N₂O in our study. A significant positive relationship between litter N concentration and cumulative N₂O emission can also be detected in alpine steppe soil (Supplementary Fig. S2), which may explain the different simulation effects among litter treatments.

Alpine steppe soil was the sink for CH₄ (Fig. 1c1–c3), in line with the findings of Cai, *et al.*³¹ at the same site. Similar results can also be found in other ecosystems^{35–37}. The uptake of CH₄ may be ascribed to aerobic biological CH₄ oxidation of alpine steppe soil³⁸. Litter treatments could either decrease or have no effect on soil CH₄ uptake (Fig. 1c1–c3). The inhibition of CH₄ uptake in litter treatments was probably due to both the positive effect of nutrition (e.g., N, P) input on CH₄ production and the specific

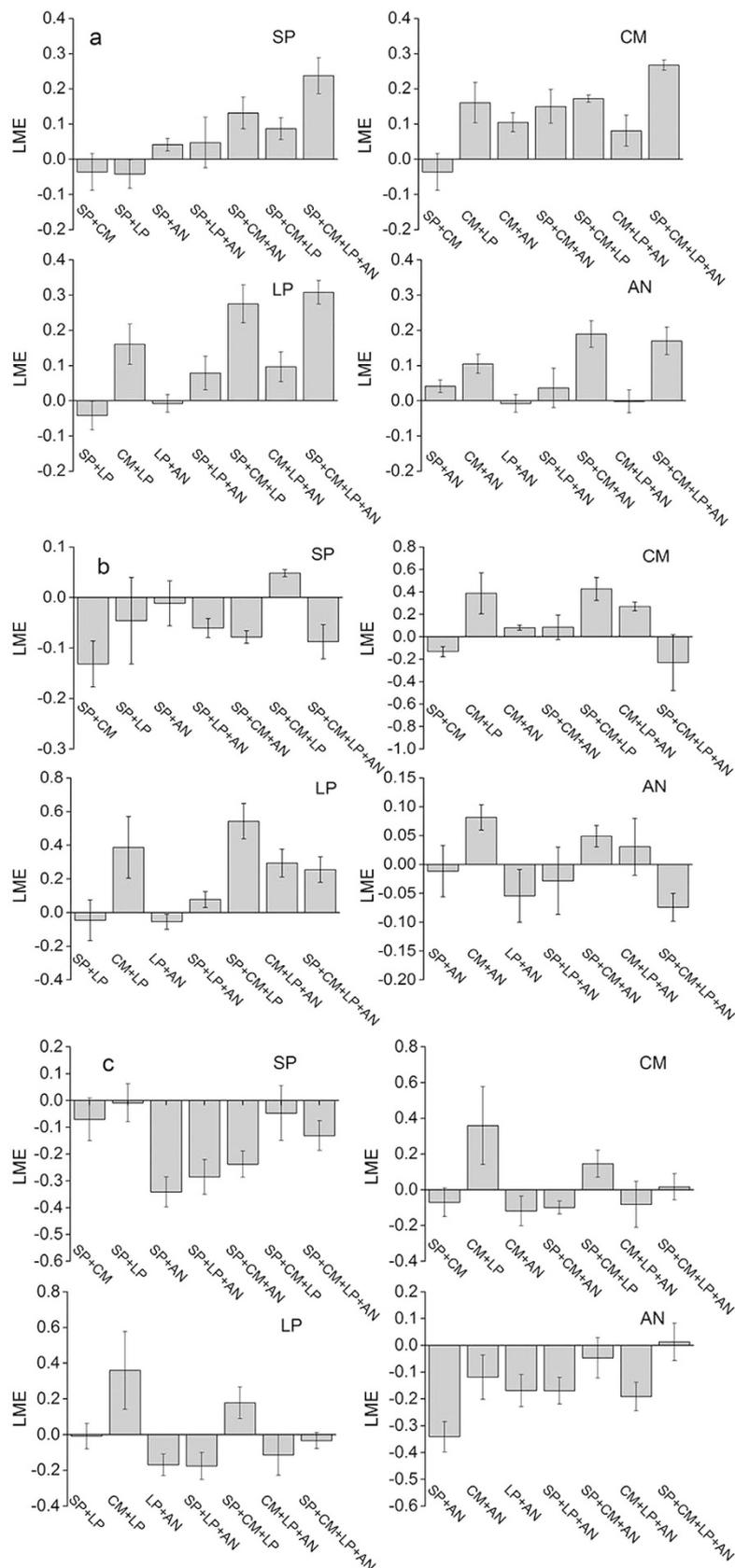


Figure 2. Potential non-additive response of CO₂ (a), N₂O emissions (b), and CH₄ uptake (c) driven by each of the four species used. LME means litter-mixing effect. SP, *S. purpurea*; CM, *C. moorcroftii*; LP, *L. pusillum*; AN, *A. nanschanica*. Error bars represent 95% confidence intervals (CI); Cls that did not cross the x-axis were used to represent the non-additivity.

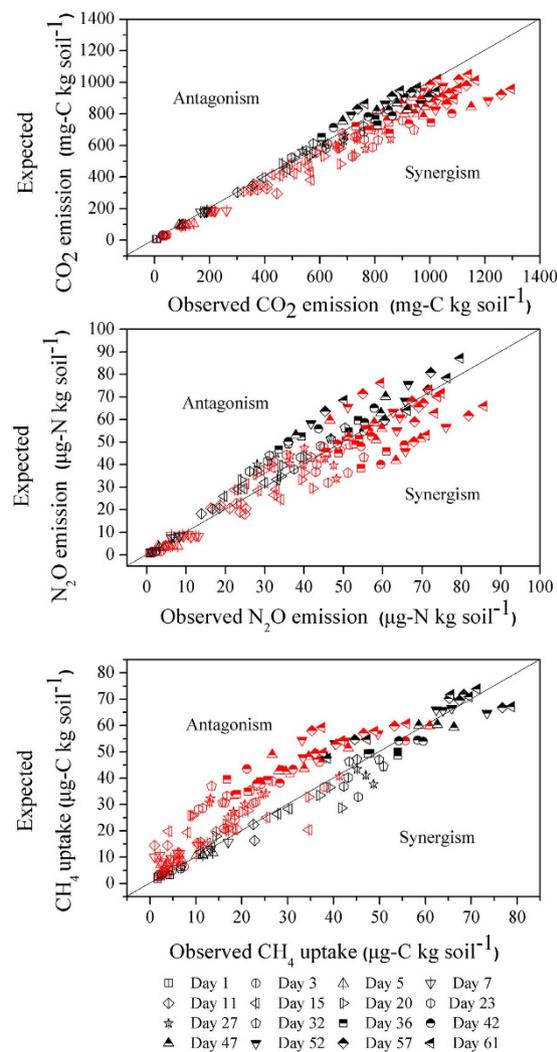


Figure 3. Observed vs expected values of cumulative CO₂ (a), N₂O (b) emissions, and CH₄ uptake (c) in the litter mixture. Red symbols are indicative of statistically significant non-additive effects, and blank symbols imply additive effects.

components (e.g., condensed tannin) released from litter during decomposition, which can suppress the activity of methanotroph³⁹.

Significant species identity effects and non-additive litter diversity effects on cumulative CO₂, N₂O emission and CH₄ uptake were detected in alpine steppe soil of Northern Tibet, and the diversity effects were determined to be due to species composition rather than species richness (Fig. 2). The results suggested that four species were not functionally substitutable in this alpine steppe ecosystem. However, given that we observed pervasive non-additive effects on GHG dynamics, changes in GHG emissions or uptake caused by species loss cannot be statistically predictable based on knowledge of the main effects of each species.

In order to specifically evaluate the synergism and antagonism of non-additivity, a traditional observed/expected method was adopted based on prior works⁴⁰. Previous studies using this method mainly focused on the effect of litter mixing on decomposition (including mass loss and nutrient release), and prevalent non-additive interactions were reported, with a majority of synergistic effects^{2,9}. A review of various studies, conducted by Gartner and Cardon⁹, showed that approximately 30%, 50%, and 20% of reported cases on litter mass loss were additive, synergistic, and antagonistic, respectively. As for GHG dynamics during litter decomposition, Meier and Bowman⁴¹ reported that litter mixture effects on cumulative CO₂ emissions from alpine soil were largely additive in a moist meadow ecosystem in Rock Mountain; Jiang, *et al.*¹⁶ found both additive and non-additive antagonistic effects of litter mixture on CO₂ emissions in an alpine meadow on the Tibetan Plateau. However, little information could be found on GHGs, especially pertaining to N₂O and CH₄ dynamics, with regard to soil during litter-mixing decomposition in alpine steppe ecosystems. Based on the 4-species incubation experiment, we found that litter species diversity created strong synergistic or antagonistic effects on cumulative GHG dynamics

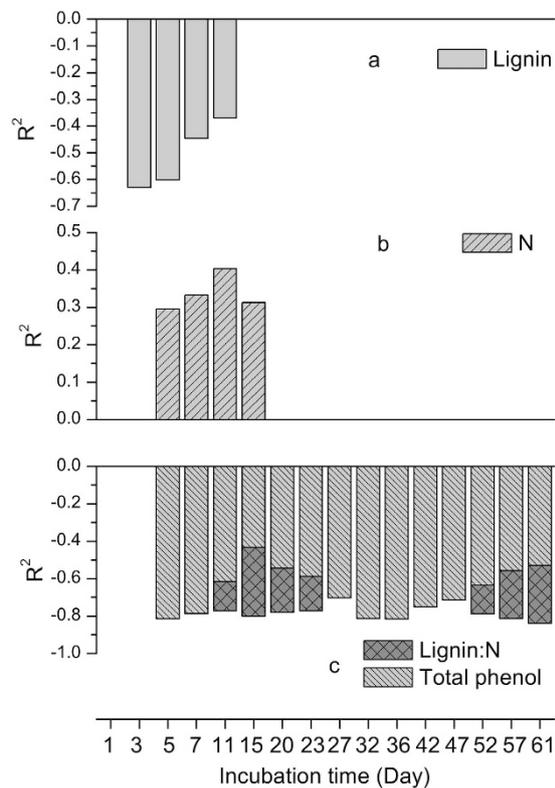


Figure 4. Effects of the litter chemical structure on the litter-mixing effect for cumulative CO₂ (a), N₂O (b) emissions, and CH₄ (c) uptake at different incubation times. Stepwise multiple regression analyses were used to exclude autocorrelation among litter chemistries. All R² values were significant at $P < 0.05$.

from alpine steppe soil. Synergistic effects were more frequent than antagonistic effects in non-additivity for CO₂, N₂O emission, while antagonistic effects were predominant for CH₄ uptake (Fig. 3). These results indicated that the higher litter diversity might significantly enhance CO₂ and N₂O emission but inhibit CH₄ uptake in this alpine steppe ecosystem in Northern Tibet.

Although the results showed that species identity and composition with litter mixture is a strong influence on non-additive GHG response to diversity, the mechanisms by which litter diversity affects these soil ecological processes remain unclear¹⁵. Traditionally, studies investigating the effects of species diversity on ecosystem function have measured species richness as a surrogate for species diversity, and positive relationships between species richness and above-ground ecosystem processes, such as primary productivity, have been well documented^{3,4}. However, species richness usually failed at predicting diversity effects on below-ground processes, such as soil C and N dynamics^{15,16}. In this study, we also found that the non-additive responses of soil GHGs to litter diversity were not caused by species richness. Soil C and N dynamics are driven by microbial activities, which should respond to the amount and type of substrates available within litter mixtures, but species-rich mixtures may be functionally redundant compared with mixtures containing fewer species if litter mixtures are composed of chemically similar species¹⁵. This may explain why litter species richness often correlates poorly to soil processes, and, on the other hand, indicates that litter chemical structure may be the key underlying the composition effects on GHG dynamics.

Stepwise multiple regression analyses showed that litter chemical traits such as lignin, N, lignin:N, and total phenol had significant effects on response of CO₂, N₂O emissions, and CH₄ uptake to litter diversity during incubation (Fig. 4). Lignin is commonly viewed as a microbe inhibitor⁴², and the negative relationship between LME and lignin in this study indicated that lignin may decrease the strength of synergistic response to litter mixture for CO₂. For the nutrient-limited alpine steppe ecosystem, plants with relatively high N contents may alleviate N limitation by increasing decomposition rates, and consequently affect soil C and N dynamics². In this study, the positive relationship between LME and litter N content indicated that N tended to enhance the synergistic effects in litter mixture for N₂O. Production of phenolic compounds is particularly significant if plants are growing in nutrient-poor conditions, short growing seasons, or are under other types of environmental stress⁴³. Phenolic compounds, especially condensed tannins, have been shown to affect C and N mineralization with regard to complex proteins and possibly other N-containing compounds, to induce toxicity to microbes, and to inhibit enzyme activities in the soil⁴⁴. In our study, LME for CH₄ were mainly negative values (antagonistic effects); the negative relationship between LME and total phenol suggested that a higher litter phenolic content could

Bulk density (g cm ⁻³)	pH	SOC(g/kg)	TN (g/kg)	TIN (mg/kg)
1.59 (0.12)	8.13 (0.02)	7.90 (0.07)	0.82 (0.01)	7.75 (0.23)

Table 2. Characteristics of the alpine steppe soil. Values are means with standard errors given in brackets (n = 3). BD, bulk density; SOC, soil organic C; TN, total N; TIN, total inorganic carbon.

enhance the antagonistic effects. These results indicated that the direction and strength of non-additivity may be affected by the chemical structure of the litter, which was also supported by Meier and Bowman⁴¹.

In addition, the relationship between *LME* and litter chemical traits appear to have been time dependent (Fig. 4). This result indicates that incubation time is an important design consideration if the goal is to capture the full range of the relationship between chemical traits and the litter diversity response. For example, the effects of litter N content and *LME* on N₂O emissions cannot be detected if the cumulative value of 61-day incubation is used, which may weaken our understanding of the role of chemical traits in litter diversity effects. The pattern of the relationship between *LME* and chemical traits may be ascribed to the dynamic changes in the content of litter chemistries during decomposition. More research is needed in the future to explore the relationship between *LME* on GHGs and incubation time.

Methods

Study site. The Northern Tibet region, located in the interior of the Tibetan Plateau, spans an area of approximately 0.39 million km² with a mean altitude of more than 4500 m a.s.l. It is the headwater of many high mountain lakes and important rivers in China and other Asian countries, including the Yangtze River, Nu (the Salween River), and Lancang (the Mekong River). Alpine grassland is the dominant ecosystem in this region, covering approximately 94.4% of the total area⁴⁵.

Soil and litter used in this study were collected from an alpine steppe at the Xainza Alpine Steppe and Wetland Ecosystem Observation Station (N 30°57', E 88°42', 4,675 m a.s.l.) located in Northern Tibet. The average annual air temperature and precipitation at this location is 0 °C and 300 mm, respectively. There is no absolutely frost-free season, and the frosty period lasts up to 279 days. Vegetation in our study area is dominated by *Stipa purpurea* Griseb. var. *arenosa* Tzvel. and *Carex moorcroftii* Falc.ex Boott. The soil is mostly equivalent to Cryic Aridisols. Several of the soil characteristics are presented in Table 2. A detailed description of the study site can be found in Lu *et al.*⁴⁶ and Cai *et al.*³¹.

Litter and soil sampling. In September 2013, we harvested senescent standing and recently fallen leaves of four abundant alpine steppe species: *S. purpurea* (SP), *C. moorcroftii* (CM), *Artemisia capillaris* Thunb. (AC), and *Leontopodium pusillum* (Beauv.) Hand.-Mazz. (LP) from a permanent plot at the Xainza station. The litter samples were air-dried in a well-ventilated laboratory of the Xainza station for approximately one month. We then chopped the air-dried litter into 1-cm long pieces and stored them in paper bags at room temperature prior to experimental use. Alpine steppe soil was collected from seven random locations at a depth of 0–10 cm in the plot after snow-melt in early June 2014. All soil samples were mixed thoroughly; the visible roots and stones were removed; and the samples were air dried, crushed, passed through a 2-mm sieve, and then transported to the laboratory, stored in sealed containers at 4 °C prior to the incubation experiment.

Aerobic incubation experiment. We placed 50.0 g (dry-weight basis) of alpine steppe soil in 250-ml triangular flasks and pre-incubated the soil at 13.6 °C for one week by adjusting soil moisture to 30% water holding capacity (WHC). The incubation temperature and moisture were adapted according to mean growing season soil temperature and moisture levels⁴⁷. After pre-incubation, the soil was amended with 0.6 g of litter (dry-weight basis). There were 15 litter treatments and one control treatment (three replicates per treatment) in our incubation experiment. The litter treatments included monocultures from each of four species (SP, CM, LP, and AN) and the combination of two (SP+CM, SP+LP, SP+AN, CM+LP, CM+AN, LP+AN), three (SP+LP+AN, SP+CM+AN, SP+CM+LP, CM+LP+AN) and four species (SP+CM+LP+AN). The litter combination contained an equal mass of each species. All microcosms were incubated at 13.6 °C in a growth chamber for 61 days. Soil moisture was maintained at 30% WHC by watering the soil along the flask borders every three days as needed.

The emission rates of CO₂, CH₄, and N₂O were measured every two days for the first week, and then every three or four days throughout the remainder of the experiment. Before gas sampling, the flasks were sealed with airtight butyl rubber stoppers perforated by centered polyvinyl chloride tubes, after which the headspace air in the flasks was thoroughly flushed with ambient air for 9 min at a rate of 1200 ml min⁻¹. After two hours of incubation, 6 ml of the headspace gas of the bottle were sampled with an airtight syringe in order to measure CO₂, CH₄, and N₂O concentrations. Concentrations of CO₂, CH₄, and N₂O were measured by means of a gas chromatograph (Agilent 7890A, Santa Clara, CA) equipped with a flame ionization detector for CO₂ and CH₄ analysis, and an electron capture detector for N₂O analysis.

Species	SP	CM	LP	AN
C (%)	41.29(0.05) ^c	38.94(0.17) ^b	26.09(0.12) ^a	39.61(0.16) ^b
N (%)	1.13(0.01) ^b	1.17(0.03) ^b	0.76(0.03) ^a	1.27(0.02) ^c
Lignin (%)	10.43(0.08) ^c	7.06(0.11) ^b	5.60(0.30) ^a	6.83(0.12) ^b
Cellulose (%)	15.58(0.32) ^b	18.66(0.029) ^c	11.08(0.10) ^a	10.93(0.21) ^a
Total phenol (g/kg)	10.5(0.31) ^b	11.13(0.08) ^b	7.95(0.02) ^a	19.42(0.22) ^c

Table 3. Characteristics of the plant litter used in this study. Values are means with standard errors given in brackets ($n = 3$). SP, *S.purpurea*; CM, *C.moorcroftii*; LP, *L.pusillum*; AN, *A.nanschanica*. Values in a row with different superscripted letters indicate that significant differences exist among litter species at $P < 0.05$.

Laboratory chemistry analysis. Litter C and N content were determined using the VarioMAX CN element analyzer (Macro Elemental Analyzer System GmbH, Hanau, Germany). Total phenol was assessed via the Folin–Ciocalteu method⁴⁸, and the amount of lignin was determined by means of the acid-detergent digestion technique⁴⁹. Cellulose was determined using the Updegraf method⁵⁰. The chemical traits of litter measured are given in Table 3.

Analysis of litter diversity effects. The effects of litter diversity on cumulative GHG values were analyzed by means of two different approaches. First, following Ball *et al.*²⁴ and Bonanomi *et al.*²¹, an analysis of variance (ANOVA) using Type I sums of squares (SS) was adopted to test for additive and non-additive effects of litter diversity. It should be noted that our approach differed significantly from that of Ball *et al.*²⁴ and Bonanomi *et al.*²¹ due to the repeated measures experiment that we employed in this study, and to the fact that time was treated as the within-subject factor in repeated measures ANOVA. Replicates (three levels) and presence/absence (two levels) of each of the four species were added sequentially to the model as predictive factors. A species interaction term (SpInt) was then added to test for non-additivity. The SpInt term had 11 levels, each representing one of the multi-species combinations. A significant SpInt term (and/or its interaction with time) indicates a significant non-additive interaction among species, due to richness and/or composition. The significant SpInt was then replaced by a Richness term (three levels) to explore the source of non-additivity. If the Richness term (and/or its interaction with time) is not significant, the significant SpInt term must arise through non-additive composition effects. If the Richness term is significant, a Composition term, with 11 possible levels, can be included in the model, while retaining the Richness term, to evaluate whether both non-additive richness and composition effects manifest. A significant species presence/absence term indicates significant effects of that species on cumulative GHG emissions or uptake, while a non-significant species term suggests that the species is functionally replaceable by one of the other species used^{21,24}.

Secondly, a traditional observed/expected method was used to specifically evaluate the synergism and antagonism of non-additivity. We calculated litter-mixing effects (*LME*) via the following equation⁴⁰:

$$LME = \left(\frac{\text{OBS value}}{\text{EXP value}} \right) - 1 \quad (1)$$

where the *OBS* value refers to the measured value of cumulative CO₂, N₂O, and CH₄ emissions/absorption. The *EXP* value was calculated by averaging the results of the respective monoculture experiments using the following equation¹⁶:

$$\text{EXP value} = \sum_{i=1}^S R_i / S \quad (2)$$

where R_i denotes the cumulative GHG value when species i was added alone, and S refers to the total number of species in the litter mixture. Significant differences between *LME* and zero indicate that non-additive effects occur. Stronger synergistic effects would result in a greater positive departure from zero, and stronger antagonistic effects would give rise to a greater negative departure from zero.

Data analysis and statistics. The cumulative emission of CO₂, CH₄, and N₂O was calculated using the following equation (Cai *et al.*, 2013):

$$\text{Cumulative } CO_2 \text{ (or } CH_4, N_2O) \text{ emission} = \sum_{i=1}^n (F_i + F_{i+1}) / 2 \times (t_{i+1} - t_i) \times 24 \quad (3)$$

where F is the emission rate of CO₂, CH₄, or N₂O; i describes the i th measurement; $(t_{i+1} - t_i)$ refers to the days between two adjacent measurements; and n identifies the total number of measurements.

One-way ANOVA followed by Duncan's multiple comparisons was employed to test the differences in litter chemistries. For each mixture, we tested whether the *LME* differed significantly from zero using

one sample *t*-tests. We estimated the influence of initial litter chemical structures (including C, N, lignin, cellulose, total phenol, C:N, Lignin:N, Total phenol:N) on GHG emissions using stepwise multiple regression analyses. All statistical analyses were conducted using SPSS 17.0 (IBM, Chicago, IL, USA) with a significance level of $P < 0.05$.

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Author Contributions

C.Y.C. and L.X.Y. designed the research; C.Y.C., Y.Y. and L.X.Y. conducted the research; C.Y.C, S.J. and X.F.T. analyzed the data and wrote the main manuscript text; W.X.D and C.G.W reviewed the manuscript.

Additional Information

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