



# Chemical diversity and incubation time affect non-additive responses of soil carbon and nitrogen cycling to litter mixtures from an alpine steppe soil



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## ABSTRACT

The mechanisms of litter-mixing effects on soil carbon (C) and nitrogen (N) cycling in alpine ecosystems remain inconclusive. In this study, we employed a four-month litter-mixing experiment to examine the relationship between litter chemical diversity, incubation time and litter-mixing effects on soil C and N fluxes from an alpine steppe ecosystem in Northern Tibet. Litter mixtures caused predominantly non-additive effects on soil C and N fluxes, with more synergistic effects for carbon dioxide (CO<sub>2</sub>)/nitrous oxide (N<sub>2</sub>O) emissions while more antagonistic effects for soluble organic C (SOC), total inorganic N (TIN), microbial biomass C (MBC) and urease activity (UA). Litter-mixture would largely increase the fluxes of CO<sub>2</sub>, N<sub>2</sub>O emission and SON, while decrease SOC, TIN, MBC and UA concentration. We calculated six chemical diversity indices, and found litter chemical diversity correlated with the strength of litter-mixing effect on soil C and N, but the indices we chose may be influenced our understanding of the relationship. Our results also showed that models including the chemical diversity indices and incubation time generally gave better explanation on variations of litter-mixing effects. This work demonstrated a general relationship between litter chemical diversity and non-additive responses of soil C and N cycling, and suggested that incubation time is an important factor in understanding the litter-mixing effects.

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## 1. Introduction

Plant litter decomposition is a fundamentally important ecosystem process that control carbon (C) and nitrogen (N) cycling, plant growth as well as community structure in many terrestrial ecosystems (Hättenschwiler et al., 2005). Previous studies on single litter decomposition have improved the understanding of factors that influence decomposition rate, including climate (Berg et al., 2000; Zhang et al., 2008a), geographical variables (Aerts, 1997; Silver and Miya, 2001), vegetation and litter types (Prescott et al.,

2000), decomposer community structure (Graca, 2001; Gao et al., 2015), and litter chemistry (Valachovic et al., 2004; Zhang et al., 2008b). However, terrestrial ecosystems usually consist of more than one plant species, and the decomposition process in mixed litters may be quite different from that of single species.

Litter mixture may have additive or non-additive effects on decomposition processes (Gartner and Cardon, 2004; Hättenschwiler et al., 2005). The additive effect means the decomposition of a litter species is not affected by the other species in the mixture, thus, the decomposition processes of the litter mixture, such as decomposition rate, can be predicted from the monospecific decomposition processes of the component litter species. The non-additive effect, however, means there are interactions among the constituted species, which will accelerate or decrease the decomposition processes in litter mixture comparing

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with the expectation based on the monospecific decomposition of constituted species (Gogo et al., 2016). Previous studies showed that litter mixtures may show more non-additivity (synergism or antagonism) than additivity in their decomposition rates (Gartner and Cardon, 2004; Hättenschwiler et al., 2005; Duan et al., 2013). The non-additive effects from litter mixtures indicated that summing the individual species cannot explain the decomposition rate of multiple plant species (Kagata and Ohgushi, 2012), and thus, the decomposition processes of single litter species may not adequately represent unmanaged ecosystems where litters decompose in mixture (Hättenschwiler et al., 2005). What is more, the widely existed non-additivity in litter mixture may also increase the uncertainties in C and nutrient flux prediction in the global decomposition models, where the input data on litter decomposition are derived from single species studies (Adair et al., 2008; Makkonen et al., 2013).

Although there are a growing number of studies on mixed litter decomposition in past decades, the mechanisms that underlie the litter-mixing effects are still questioned (Gartner and Cardon, 2004; Meier and Bowman, 2008; Frainer et al., 2015). A few hypothesized mechanisms include nutrient (e.g., N) transfer among litter types, the influences of specific compounds like secondary metabolites, complementary resource use, and the improvement of micro-environmental conditions (Hättenschwiler et al., 2005; Lummer et al., 2012; Liu et al., 2016). Despite a variety of mechanisms, the common source of the non-additive effects comes from the chemical or structural differences in mixed litters (Tardif and Shipley, 2015). What's more, species "richness" and "composition" are often used as the index of "diversity" in previous studies, but their influences on decomposition processes are also intimately related to the chemical traits of litters included in the mixture (Ball et al., 2008; Jiang et al., 2013). Therefore, litter chemistry is considered to be an effective way to examine litter-mixing effects (Epps et al., 2007; Lecerf et al., 2011). Litter chemical traits, such as lignin, total phenol, soluble sugar and nutrients (e.g., N, P, K, Fe, etc.), have been reported in affecting litter decomposition (Madritch and Hunter, 2004; Mungai and Motavalli, 2006; Bonanomi et al., 2010; Meier and Bowman, 2010). These chemical components released from plant litter may affect the production and activity of microbes, which ultimately influence the decomposition process positively or negatively (Sinsabaugh et al., 2002). Following the recent studies on mixed litter decomposition, litter-mixing effects might be due to chemical traits diversity (i.e., the dissimilarity of chemical traits) among the constituent litter species (Epps et al., 2007; Lecerf et al., 2011). However, some other studies reported the opposite (Frainer et al., 2015). Experimental evidence for this hypothesis is inconclusive, and the role of chemical diversity in generating litter-mixing effects remains questioned. Furthermore, results of previous studies which adopted the notion of "chemical diversity" should be interpreted with caution, as most of these studies only used a single chemical diversity index to examine the litter-mixing effects (Lecerf et al., 2011). The selection of chemical diversity index is not trivial, as the metrics we choose may greatly influence the evaluation of relationships between biodiversity and ecosystem processes (Petchev and Gaston, 2006; Frainer et al., 2015).

Knowledge about effects of litter mixture on litter decomposition is derived mostly from experiments carried out in temperate ecosystems, and most of these studies focused on mass loss and nutrient release (Makkonen et al., 2013). Previous studies demonstrated that soil ecological processes, including soil C and N cycling, are closely related to litter decomposition (Vargas et al., 2006; Meier and Bowman, 2008; Jiang et al., 2013). The understanding of litter-mixing effects on soil C and N processes is necessary for studying the relationship between biodiversity and ecosystem

functioning (Lorena et al., 2005). However, very few investigators have addressed effects of litter mixture on these soil processes, especially for the alpine ecosystems. What's more, the few existing results of soil processes' responses to litter mixture only came from very short-time experiments (e.g., six weeks incubation experiment in Meier and Bowman (2010), 38 days experiment in Jiang et al. (2013)), and none of these works considered the influence of incubation time (i.e., the length of experiment). In our previous study, we demonstrated that litter chemical components play an important role in affecting soil C and N dynamics using a 61 days' result of litter-mixing experiment (Chen et al., 2015). Now, in this study, we used a longer result of experiment (four months) to explore the effect of litter chemical traits diversity and incubation time on non-additive response of soil C and N processes, including soil CO<sub>2</sub>/N<sub>2</sub>O fluxes, soluble organic C (SOC), total inorganic N (TIN), soluble organic N (SON) as well as microbial characteristics including microbial biomass C (MBC) and urease activity (UA), from an alpine steppe ecosystem in Northern Tibet. The aims of our work were to test the following hypotheses: (1) Non-additive effects (synergistic and antagonistic effects) on soil C and N fluxes occur frequently during litter mixture decomposition, and (2) the non-additive effects were affected by litter chemical diversity and incubation time.

## 2. Material and methods

### 2.1. Experiment design

Alpine steppe plants and soil samples were collected from a site at the Xainza Alpine Steppe and Wetland Ecosystem Observation Station (N 30°57', E 88°42', 4675 m a.s.l.) in Northern Tibet. Detailed information about our study site could be found in Lu et al. (2012) and Chen et al. (2015). Litters of four abundant alpine steppe species: *Stipa purpurea* Griseb. var. *arenosa* Tzvel (SP), and *Carex moorcroftii* Falc. ex Boott (CM), *Artemisia nanschanica* Krasch. (AN) and *Leontopodium pusillum* (Beauv.) Hand.-Mazz. (LP) were collected in September 2013 from an alpine steppe community at Xainza station. We pooled litters of each species into a single sample, and air-dried all litter samples in a well-ventilated room in Xainza station for about one month. The air-dried litters were chopped into lengths of 1 cm in order to minimize "size effect", and then stored in paper bags at room temperature until the beginning of incubation experiment. The alpine soil we studied is a Cryic Aridisols according to the Chinese soil taxonomy, with pH of 8.72, organic C of 7.9 g kg<sup>-1</sup>, total N of 0.82 g kg<sup>-1</sup> and bulk density of 1.52 g cm<sup>-3</sup>, and comprised 91% sand, 7% silt, and 2% clay at top 10 cm depth soil. In early June 2014, soil at the depth of 0–10 cm was collected from seven random locations in the community after snow-melt. All soil samples were mixed thoroughly, air dried, crushed, passed through a 2-mm sieve, and visible roots were removed. Soil samples were then transported to the laboratory and stored in sealed containers at 4 °C before incubation experiment.

We employed a litter-mixing experiment under laboratory condition to investigate plant litter-mixing effects on soil carbon and nitrogen cycling. In this experiment, we incubated 50 g (dry-weight basis) alpine steppe soil with 0.6 g (dry-weight basis) litter in monoculture or combination. The experiment involved 16 treatments: each of four species (SP, CM, LP, AN), the combination of two (SPCM, SPLP, SPAN, CMLP, CMAN, LPAN), three (SPLPAN, SPCMAN, SPCMLP, CMLPAN) and four species (SPCMLPAN) (see Table S1), and a control treatment (CK) with un-amended soil. Litter combination, with the total weight of 0.6 g, contained an equal mass of each species. Before litter amendment, we placed 50 g (dry-weight basis) alpine steppe soil in 250-ml triangular flasks and pre-incubated them at 13.6 °C and 30% water holding capacity (WHC)

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