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# Impact of vegetation community on litter decomposition: Evidence from a reciprocal transplant study with $^{13}C$ labeled plant litter

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ABSTRACT

Litter decomposition has been highlighted as one of the crucial biochemical carbon sequestration processes in grassland ecosystems. Vegetation changes of grasslands are associated with an alteration of litter quality and decomposer communities. How they affect litter decomposition and associated carbon flows at the plant-soil interface, is not well understood. We designed a reciprocal transplant litter experiment by incubating $^{13}C$-labeled litter in selected patches. The primary aim of this study was to (1) determine the relative effects of litter quality and soil microbial community on litter decomposition rates; (2) estimate the associated carbon incorporated into different groups of the soil microbial community; and (3) verify if the dominant litter decomposed faster at "home" sites, *i.e.* home-field advantage (HFA) effect. Our data indicated that the interaction between litter type and decomposition site significantly affected litter decomposition. Cellulose contributed the most C during decomposition, and fungal phospholipid fatty acids incorporated the highest level of $^{13}C$ from labeled litter. There was a higher decomposition rate of cellulose and more $^{13}C$ incorporation in fungi, when each type of litter was incubated in its dominated sites. HFA index presented positive values that illustrates that the dominant litter had advantage in mediating decomposition processes. Gram positive (GP) bacteria and actinomycetes, they were found to be closely associated with the C priming of native-SOM. From this perspective of HFA, our results help understanding mechanisms of leaf litter–soil feedbacks in grassland, including the return of carbon (C) to the soil through litter decomposition and fraction in functional microbial groups. Practically, the results also imply that a higher biodiversity of grassland will affect the carbon cycle and formation of soil organic matter in ecosystem. Once such litter-soil feedbacks process is considered, vegetation management in grassland will contribute to carbon cycle, formation of soil organic matter in ecosystem.



## 1. Introduction

Grasslands, which cover 40% of the earth land surface, possess about 20% of the soil organic carbon (SOC) stocks globally (Schuman et al., 2002). Annual litter fall and its decomposition process play important roles in the formation and turnover of soil organic matter in grassland ecosystems (Liski et al., 2002). Previous studies have built models for litter decomposition that aid the prediction of energy and matter flux in the decomposition process and can help develop better strategies for grassland management (Berg and McClaugherty, 2008). Climate, litter quality and related decomposer communities have traditionally been considered as the main drivers regulating the rate of the litter decomposition (Bradford et al., 2015). Biogeochemical models that describe litter decomposition have been extensively calibrated and validated based on these controlling factors. Recent studies have illustrated that the interactions between litter quality and soil decomposers may have both positive and negative effects on litter decomposition (Ayres et al., 2009; St John et al., 2011; Perez et al., 2013). However, few details are available regarding how such interactions affect decomposition and C flow.

Plant species traits, which determine litter quality, are thought to be the predominant control on litter decomposition rates within biomes worldwide (Cornwell et al., 2008). Different species vary greatly in their rate of decomposition due to their chemical composition. Cleveland et al. (2014) noted that the decomposition of a species' litter is consistently correlated with that species'

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carbon strategy. The more accessible C compounds (non-structural carbohydrates, phenolic) and less recalcitrant C (condensed tannins, lignin) can promote litter decomposition (Hättenschwiler and Jørgensen, 2010).

Soil microorganisms play a central role in litter decomposition and partitioning C between $CO_2$ evolution and the sequestration of C into semi-permanent pools in soils. Litter decomposition is greatly influenced by the activities of soil decomposers. Bacteria and fungi comprise more than 90% of the soil microbial biomass and are the main agents in decomposition (Rinnan and Baath, 2009). Considerable progress has been made in understanding how the factors affect the rate of litter decomposition and formation of soil organic matter (Stevenson, 1994). The role of microbial functional groups during C decomposition and sequestration in soils is based on the methods such as activity measurements and biomass. Communities with relatively high proportions of fungi have advantages over communities dominated by bacteria because fungi are able to extend hyphae into the soil to extract nutrients and water (Holland and Coleman, 1987), whereas bacteria are limited in movement. In addition, fungi are major forces in decomposition and formation of soil organic matter (SOM), because they have a wide range of extracellular enzymes that can degrade recalcitrant materials.

Stable isotope probing (SIP), combined with isotopic labeling techniques and microbial biomarkers, has been extensively used technique to elucidate the pathway for the translocation of focus elements in particular substrates (Cowie et al., 2010). It has been demonstrated that phospholipid stable isotopic probing could provide more details on soil biogeochemical cycles (Bai et al., 2016), in which microbial consumption and incorporation of $^{13}$C-labeled substrate processes are involved (Boschker et al., 1998; Watzinger, 2015). An understanding of the interactions between litter quality and decomposer is particularly important for revealing the biological driving power of the decomposition process.

Inappropriate grassland management, such as overgrazing has led to serious degradation in typical steppes of northern China (Barger et al., 2004). In this area *Leymus chinensis*, *Stipa krylovii* as well as *Artemisia frigida* are native species. Meanwhile, the plant communities dominated by the three species represent vegetation conditions from better to worse condition (Li, 1994). Specifically, it results in deterioration (barren, drought) when plant communities change from *L. chinensis* (LC) patch to *A. frigida* (AF) patch. Litter and soils in such grasslands are therefore the ideal means to test the interactions of litter types and decomposers, as well as other associated issues. Furthermore, reciprocal litter transplant experiment design we used is helpful to estimate the interaction during litter decomposition.

Plant community composition alters in grassland due to disturbances by activities of human being or climate change. This field-based study aimed to provide insights into the impact of vegetation community on litter decomposition. We hypothesized that (1) both litter quality and soil microbial community will affect litter decomposition rates; we expect that (2) carbon incorporated different groups of the soil microbial community; and finally we expect that (3) dominant litter decomposed faster at “home” sites (home-field advantage).

## 2. Materials and methods

### 2.1. Study site and experimental setup

The study was performed in the Duolun Restoration Ecology Station of the Institute of Botany of the Chinese Academy of Sciences (42°02′N, 116°17′E), Inner Mongolian, China. The study area was located at a semi-arid steppe with a mean annual precipitation of 383 mm and a mean annual temperature of 2.1 °C. The study area has a long history of grazing and haymaking for animal production. Soil types were classified as Haplic Calcisols according to FAO classification. The plant community was dominated by *L. chinensis*, *S. krylovii*, *A. frigida*, *Cleistogenes squarrosa* and *Agropyron cristatum* (Yang et al., 2014).

The plot was fenced off in 2003 for ecological research. We chose 3 types of patches for the experiment according to the community dominance. Study sites dominated by *L. chinesis (lc)* were recognized as *L. chinesis* sites (*LC*). Similarly, the community dominated by *S. krilovii* (*sk*) and *A. frigida* (*af*) were denominated as *S. krilovii* sites (*SK*) and *A. frigida* sites (*AF*), respectively. Prior to initiating the experiment, soil cores (6 cm diameter; 10 cm in depth) were randomly taken from each plot to estimate physical and chemical properties, including pH, bulk density, and soil texture.

### 2.2. Labeling of plant litter

The plants were labeled in July at the time when RGR (Relative Growth Rate) of the plant is photosynthetically most active. Three days prior to the labeling, a collar of 50 cm × 100 cm in size was installed to the depth of 5 cm. The collar possessed a groove on aboveground part to connect the chambers so they had an air-tightly seal when labeling. For $^{13}CO_2$ labeling of the plants, a 250 L (50 cm × 50 cm × 100 cm) polyethylene plastic chamber was set on the collar over the plants. Labeled $CO_2$ was added in pulses through the chemical reaction between $H_2SO_4$ and 13C-$NaHCO_3$ (99 atom % $^{13}$C; Cambridge Isotope Laboratories, Inc. USA). Plants within the chamber were labeled by $^{13}$C throughout photosynthesis. Litters of labeled *S. krylovii*, *A. frigida* and *L. chinensis* were thoroughly mixed and cut into 5 mm pieces to ensure uniformity.

### 2.3. Experimental design

We used a reciprocal transplant design (i.e., all litter types crossed with all sites) with 3 types of plant litter and 3 sites in our study. The experiments were arranged in a complete randomized design with 5 replicates. A total of 60 litter samples were used for the entire study ([3 litter species+1blank] × 3sites × 5 replications per site = 60).

In each plot, 4 PVC collars (20 cm diameter) were inserted into the soil to 2 cm depth in the selected patches. In October 2014, the reciprocal litter transplant experiment was performed. Surface soils in the traps, apart from the blank ones were incubated with 5 g litter, which was a higher amount of plant litter than has been previously investigated in prior experiments. No litter was applied in the equivalent controls. This constituted an attempt to minimize other potential non-microbial community effects on litter decomposition and isotopic fraction. To avoid fresh litter from falling into the collars, we placed a coarse high density PVC nylon net (mesh size: 1 mm × 1 mm) on all collar as soon as we clipped the entire canopy.

### 2.4. Sample analysis

#### 2.4.1. Soil sampling and measurements

After a full year of decomposition, the remaining litters in the collar were collected. Soils samples were taken from the upper layer (0–2 cm) of each treatment in September 2015, and were crumbled and sieved through 2 mm mesh sieves to removed plant material and stones. One portion of the samples was air-dried at room temperature for 2 weeks for chemical analysis, while the other was kept on ice in the field, transported to the laboratory and stored in the −80 °C refrigerator for PLFA analysis.