



# Non-additive effects vary with the number of component residues and their mixing proportions during residue mixture decomposition: A microcosm study

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## ABSTRACT

Poplar leaf litter and crop residues (leaves and stems) of two main crops (soybean and maize) collected from semiarid agroforestry systems of Northeast China were used in our microcosm study. The aims were to examine whether non-additive effects (synergistic or antagonistic) between poplar leaf litter and crop residues exist during decomposition and to identify the influence of residue mixing proportion on the incidence of non-additive effects of residue mixture for the same plant residues. We determined residue decomposition rate by measuring mass loss and N release. Synergistic effects between poplar leaf litter and crop residues were more common than additive effects in terms of mass loss and N release. Moreover, the interactive effects between tree leaf litter and crop residues on decomposition varied with the number of component residues and their mixing proportion. Three-residue mixtures produced synergistic effects on mass loss and N release, although two-residue mixtures showed an additive effect in some cases. In addition, as compared with equal proportion, mixing residues with unequal proportion increased the incidence of non-additive effects during decomposition of residue mixture. These findings highlight that residue decomposition dynamics in ecosystems should be assessed on the basis of plant residue mixtures and their mixing proportions, which may help us better understand nutrient dynamics and guide our decisions on nutrient management.

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## 1. Introduction

Plant residue decomposition can regulate soil organic matter buildup, improve soil nutrient availability and sustain plant productivity in terrestrial ecosystems (Aerts, 1997; Cadisch and Giller, 1997; Gartner and Cardon, 2004; Hauser et al., 2005). Considerable effort has been invested in documenting the patterns of plant residue decomposition and nutrient release. It has been suggested that residue decomposition rate and nutrient release are regulated by climate (Aerts, 1997; Coûteaux et al., 1995), residue quality (Aerts, 1997; Dossa et al., 2009) and soil biota (Hättenschwiler et al., 2005). However, most of the previous studies have focused on the decomposition rate and nutrient dynamics of single plant residues (Berg and McLaugherty, 2008). Indeed, in natural or managed ecosystems, plant residues from more than one species usually mix and decompose together. Plant residue mixtures frequently produce non-additive effects on decomposition dynamics (Gartner and Cardon, 2004; Gessner et al., 2010; Hättenschwiler et al., 2005). Moreover, during decomposition of litter mixtures, non-additive effects may vary with the difference in residue quality of component species

(Hoorens et al., 2002; Wardle et al., 1997), residue mixing proportion (Bonanomi et al., 2010; Swan et al., 2009), environmental conditions (Gessner et al., 2010) and decomposer community composition and diversity (Gessner et al., 2010; Hättenschwiler and Gasser, 2005). Therefore, residue decomposition dynamics in ecosystems cannot be accurately assessed on the basis of single plant residues, and further work is required to determine the residue mixture effects.

Agroforestry systems contain a mixture of plant species such as trees and crops that have different growth forms and can produce plant residues with different chemical composition. Moreover, residues of trees and crops do not decompose alone, and usually become mixed and decompose simultaneously. In tropical agroforestry systems, Sakala et al. (2000) found that mixing maize (*Zea mays*) with pigeonpea (*Cajanus cajan*) residues had much greater N immobilization than the values predicted from the mineralization pattern of the individual components. However, there is little information about the interactive effect of mixed residues of trees and crops on decomposition in temperate agroforestry systems (Zeng et al., 2010). A better knowledge about the interactions of mixing tree and crop residues on decomposition dynamics and nutrient release is essential for improving soil nutrient availability and sustaining plant productivity in temperate agroforestry systems.

In Northeast China, poplar (*Populus* spp.) has been widely planted in agroforestry systems. Many smallholder farmers usually intercrop poplars with soybean (*Glycine max*) and maize, two of the main

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crops in Northeast China. In the present study, we reported the effect of mixing poplar leaf litters and crop residues (stems and leaves) on mass loss and N release under laboratory conditions. Our previous studies found that mixing poplar leaf litter and crop residues had an additive effect on microbial respiration during 49 days of incubation periods (Zeng et al., 2010). Considering that microbial respiration and mass loss are fundamental aspects of residue decomposition (Taylor and Parkinson, 1988), we have presumed that residue mixtures may show a shift in pathways of decay from microbial respiration to mass loss. In order to examine our presumption, we determined plant residue decomposition rate by measuring mass loss and N release in the present study.

The aims of our study were (1) to examine whether non-additive effects exist in the decomposition process of residue mixtures. We hypothesized (hypothesis one) that mixing poplar leaf litters and crop residues would have non-additive effects on mass loss and N release; and (2) to identify the influence of residue mixing proportion on the incidence of non-additive effects of residue mixture for the same plant residues. We hypothesized (hypothesis two) that residue mixing proportion would change the incidence of non-additive effects during decomposition of residue mixtures.

## 2. Materials and methods

### 2.1. Material collection and preparation

Soil and plant residues used for the microcosm study were collected from poplar-based agroforestry systems in Taipingzhuang Town (41°47'N and 119°15'E, 632 m above sea level), Jianping County, Liaoning Province, Northeast China. The climate of the study site belongs to the semiarid monsoon of a moderate temperate zone. Mean annual temperature is 6.5 °C, precipitation is 467 mm (more than 60% falling between June and August), and frost-free period is 148 days. The soil type in the study site is a silty soil, and is classified as Entisols of suborder Fluvent in the US Soil Taxonomy. The agroforestry systems were established in April 2005. The trees were planted with nursery-grown seedlings at a spacing of 3 m × 4 m. The tree rows allowed six parallel rows of crops to be planted 75 cm apart on both sides of a tree row, at a within row spacing of 50 cm. Soybean and maize were cropped annually and solely within the tree rows. The crop residues were crushed to a small size using a machine and returned to the systems after harvest.

In October 2009, soils were randomly sampled from 0 to 15 cm layer in poplar-based agroforestry systems, and thoroughly mixed to form a composite sample. After removing roots, macrofauna and debris, the soil was sieved (2 mm) and stored at 4 °C for laboratory incubation experiment. The soil had the following properties: a pH in a 1:2.5 (weight:volume) water suspension of 8.3; organic C of 5.25 g kg<sup>-1</sup>; total N of 0.46 g kg<sup>-1</sup>.

Soybean leaves, soybean stems, maize leaves, maize stems and naturally-senesced poplar (*Populus euramericana* cv. 'N3016') leaves were collected from agroforestry systems. Crop residues were collected during harvest. Poplar leaves were collected immediately after leaf fall in October 2009. Meanwhile, plant residue mass was sampled in four randomly selected points in agroforestry systems by using a squared frame (1 m × 1 m), then oven-dried to a constant mass at approximately 65 °C for 48 h, and weighed. Plant residues were mixed carefully, oven dried at 65 °C until constant weight and divided into two subsamples. The first group of subsample was used for the incubation experiment. In order to minimize the effect of residue size on decomposition, the plant residue was cut into pieces of approximately 1 cm long (crop stems) or 1 cm<sup>2</sup> (tree and crop leaves). The second group of subsample was milled (<0.25 mm) for chemical analysis. Organic C concentration was determined using the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>-H<sub>2</sub>SO<sub>4</sub> wet oxidation method of Walkley and Black (Nelson and Sommers, 1996). Total N concentration was analyzed by the Kjeldahl method

**Table 1**  
Plant residue mass in agroforestry systems and their initial chemical composition.

Residue types	Residue mass	Total C	Total N	C/N ratio
	Mg hm <sup>-2</sup>	mg g <sup>-1</sup>	mg g <sup>-1</sup>	
PL	2.60	424.9	7.84	54.1
GL	1.11	375.7	13.15	29.1
GS	1.29	390.4	6.39	61.9
ZL	1.30	389.3	8.36	45.5
ZS	1.73	416.5	4.73	89.1

PL, poplar leaves; GL, soybean leaves; GS, soybean stems; ZL, maize leaves; ZS, maize stems.

(Bremner, 1996) using a continuous-flow autoanalyzer (AutoAnalyzer III, Bran+Luebbe GmbH, Germany). The mass and initial chemical composition of plant residues in agroforestry systems are presented in Table 1.

### 2.2. Experimental design and residue decomposition measurements

In our incubation experiment, 17 treatments were installed: five single plant residues (poplar leaves, PL; soybean leaves, GL; soybean stems, GS; maize leaves, ZL; maize stems, ZS) and 12 residue mixtures. We divided 12 residue mixtures into two groups: one group where the residues were mixed with an equal proportion (50PL + 50GL, 50PL + 50GS, 50PL + 50ZL, 50PL + 50ZS, 33PL + 33GL + 33GS and 33PL + 33ZL + 33ZS), and another group where the residues were mixed with the actual proportion based on the residue mass survey (70PL + 30GL, 67PL + 33GS, 67PL + 33ZL, 60PL + 40ZS, 52PL + 22GL + 26GS and 46PL + 23ZL + 31ZS). For example, 70PL + 30GL treatment represented that, in the residue mixture, PL accounted for 70% residue mass, and GL accounted for 30% residue mass. Each treatment was replicated five times.

Plant residue decomposition was determined using a modified laboratory microcosm method similar to that described by Ganjgunte et al. (2005) and Vargas et al. (2006). We prepared 255 microcosms by filling plastic (polyvinyl chloride) cylinders (11 cm in diameter, 15 cm tall) with field-moist soils (approximately 100 g dry weight). One point five grams of plant residues were placed on the soil surfaces, and separated from the soils by 1-mm nylon mesh. Each microcosm was adjusted to 60% water-holding capacity with distilled water, and covered with a perforated adherent film to reduce humidity losses while allowing gaseous exchange. Meanwhile, total weight of the microcosm was recorded. The microcosms were incubated for 84 days at 25 ± 1 °C. Throughout the incubation, soil moisture content was maintained at 60% water-holding capacity by weighing the flask every 3–5 days and adjusted by adding distilled water when necessary. We acknowledge that this microcosm study does not fully reflect plant residue decomposition dynamics in the field. However, our approach does offer a means to examine the effects of plant residue numbers and their mixing proportions on non-additive effects during the early stage of residue mixture decomposition under controlled conditions. After 14, 42 and 84 days of incubation, a total of 85 microcosms, five replicates of 17 treatments, were harvested, respectively. Litters were collected by lifting the nylon mesh to avoid any loss of decomposing litter materials. Litters were cleaned from soils attached to them, dried at approximately 65 °C to constant mass and weighed. After that, the remaining litter was milled (<0.25 mm) for measurement of total N concentration. Total N concentration was analyzed by the method as described above. Residue mass remaining was calculated as the ratio of the final residue mass to initial residue mass, and expressed as a percentage of initial residue mass. Residue N remaining was calculated by multiplying final residue mass by the total N concentration of final residue, and expressed as a percentage of initial N content. For the single plant residues, residue mass remaining was fitted with a negative exponential

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