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# Plant species richness leaves a legacy of enhanced root litter-induced decomposition in soil

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ABSTRACT

Increasing plant species richness generally enhances plant biomass production, which may enhance accumulation of carbon (C) in soil. However, the net change in soil C also depends on the effect of plant diversity on C loss through decomposition of organic matter. Plant diversity can affect organic matter decomposition via changes in litter species diversity and composition, and via alteration of abiotic and/or biotic attributes of the soil (soil legacy effect). Previous studies examined the two effects on decomposition rates separately, and do therefore not elucidate the relative importance of the two effects, and their potential interaction. Here we separated the effects of litter mixing and litter identity from the soil legacy effect by conducting a factorial laboratory experiment where two fresh single root litters and their mixture were mixed with soils previously cultivated with single plant species or mixtures of two or four species. We found no evidence for litter-mixing effects. In contrast, root litter-induced $CO_2$ production was greater in soils from high diversity plots than in soils from monocultures, regardless of the type of root litter added. Soil microbial PLFA biomass and composition at the onset of the experiment was unaffected by plant species richness, whereas soil potential nitrogen (N) mineralization rate increased with plant species richness. Our results indicate that the soil legacy effect may be explained by changes in soil N availability. There was no effect of plant species richness on decomposition of a recalcitrant substrate (compost). This suggests that the soil legacy effect predominantly acted on the decomposition of labile organic matter. We thus demonstrated that plant species richness enhances root litter-induced soil respiration via a soil legacy effect but not via a litter-mixing effect. This implies that the positive impacts of species richness on soil C sequestration may be weakened by accelerated organic matter decomposition.



## 1. Introduction

Plant production generally increases with plant species richness, and this cascades to other ecosystem services (Cardinale et al., 2012). Enhanced plant production, resulting in greater litter input, could result in increased soil carbon (C) sequestration. Indeed, three large grassland biodiversity experiments have demonstrated that increases in plant species richness promote C accumulation in soil through enhanced root biomass production (Fornara and Tilman, 2008; Steinbeiss et al., 2008; Cong et al., 2014). In one of the largest biodiversity experiments, root mass loss from litter bags was lower in 16-species plots than in monocultures (Fornara et al., 2009), suggesting that both increased plant production and reduced C loss in high diversity mixtures contribute to the increase in soil C storage. However, in the Wageningen biodiversity study, we found that belowground plant production increased by 60% in eight-species mixtures compared to the average monoculture, while soil C storage after 11 years increased by only 18% (Cong et al., 2014). This discrepancy in effect sizes could potentially be explained by enhanced decomposition of organic matter and hence larger C loss through soil respiration with higher plant species richness. Here, we examine therefore whether plant species richness affects the rate of C loss through respiration upon the addition of organic matter to the soil.

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Plant species richness can potentially affect soil respiration rates upon litter addition through a litter quality effect (Wardle and Lavelle, 1997). Studies using leaf litter mixtures reported non-additive litter-mixing effects (Wardle et al., 1997; Mikola et al., 2002; Handa et al., 2014): mixing litters accelerated or inhibited decomposition compared to expected decomposition from single litters (Gartner and Cardon, 2004; Hättenschwiler et al., 2005). These phenomena are commonly observed when the components of litter mixture vary in resource quality (Wardle et al., 1997), suggesting that mixing litters from different functional groups of plant species could cause a non-additive effect. For example, N released from decomposing N-rich litter may facilitate the decomposition of N-poor litter (Wardle et al., 1997; Harguindeguy et al., 2008; Vos et al., 2013; Handa et al., 2014). On the other hand, when one of the litter components contains inhibitory compounds (e.g. polyphenols), this will inhibit microbial growth and activity of the whole community, consequently impeding decomposition of both litters (Schimel et al., 1998). Little is known of litter-mixing effects on decomposition of root litter despite the fact that root litter represents the main input of organic matter in grassland systems (Gill and Jackson, 2000).

Another potential mechanism through which plant species richness could affect the rate of soil respiration resulting from organic matter decomposition is through a soil legacy effect, i.e. through changes in abiotic and/or biotic attributes of the micro-environment in which decomposition takes place (Hector et al., 2000). Several grassland biodiversity studies have shown contrasting effects of plant species richness on decomposition rate of standard substrates, ranging from positive effects (Hector et al., 2000; Knops et al., 2001; Spehn et al., 2005; Vogel et al., 2013) to no effects on decomposition rates of senesced leaves or cotton strips (Spehn et al., 2000; Scherer-Lorenzen, 2008), or even negative effects on decomposition of senesced root litter (Fornara et al., 2009). These contrasting results can be related to the decomposability of standard materials or caused by differential impacts of diversity-induced effects on soil abiotic factors (such as moisture, nutrients) and biotic factors (e.g. microorganisms). For example, Vogel et al. (2013) attributed the faster decomposition in plots with more species to increased soil water availability. Some studies showed that plant diversity decreases soil nitrogen (N) availability through enhanced N uptake (Tilman et al., 1996; Hooper and Vitousek, 1997; Hooper et al., 2005), which may retard decomposition of organic matter. Interestingly, greater plant diversity has also been found to increase soil N availability in three large grassland biodiversity grassland experiments (Dybzinski et al., 2008; Oelmann et al., 2011; Mueller et al., 2013), which may accelerate decomposition. Moreover, other studies suggest that species richness may enhance organic matter decomposition through increases in microbial biomass, activity, or enzymatic diversity in soil microbial communities (Stephan et al., 2000; Zak et al., 2003; Chung et al., 2007; Eisenhauer et al., 2010, 2011).

While previous studies have examined the two potential pathways (i.e. litter-mixing effect and soil legacy effect) independently, it is essential to disentangle the two pathways and assess their relative impacts and potential interactive effects on organic matter decomposition within one study. Recent studies have indicated that litter decomposes faster in soil collected from an area dominated by the plant species from which the litter was derived compared to soil dominated by another plant species (irrespective of the plant litter inherent chemical composition), the so called 'home-field advantage' (HFA, Ayres et al., 2006, 2009b). It is hypothesized that HFA results from specialization of soil microbial communities in decomposing the litters they are most exposed to (Strickland et al., 2009; Ayres et al., 2009a). Most studies on HFA focus on forest ecosystems and find variable effect sizes ranging from −8% to 28% (with mean value of 8%). But only a few studies have investigated HFA in grassland systems and none could detect any HFA (Hunt et al., 1988; Strickland et al., 2009; Osanai et al., 2012). Possibly the differences in litter quality of grass species are not large enough to lead to specialization of soil microbial communities, thus inducing smaller or no HFA in grasslands than forest systems (Vivanco and Austin, 2008; Ayres et al., 2009a).

Here, using a factorial laboratory experiment with soil and root litter collected from an 11-year grassland biodiversity experiment without legumes, we attempt to disentangle the diversity-induced litter-mixing and soil legacy effects on C loss through soil respiration upon the addition of organic matter and assess whether both plant-diversity mediated factors interact. We hypothesize that (1) plant species richness influences organic matter decomposition when root litter is added to soils through both litter-mixing and soil legacy effects, and (2) there is no significant interaction between the two effects. We divide a potential soil legacy effect into abiotic (i.e. soil N availability) and biotic factors (i.e. soil microbial community biomass and fungal/bacterial ratios). In addition we amended soils with compost, a substrate more recalcitrant than root litter, to test whether any soil legacy effect depends on the decomposability of the substrate.

## 2. Materials and methods

### 2.1. Field sampling

We collected samples from the Wageningen biodiversity experiment, in which a positive plant diversity–productivity relationship was shown before (Van Ruijven and Berendse, 2005, 2009). The Wageningen biodiversity experiment is described in detail in Van Ruijven and Berendse (2003). Therefore, a brief description is given here: The experiment was established on a former arable field in Wageningen, the Netherlands in early spring 2000 and consisted of 102 plots of 1 $m^2$. The original topsoil was removed to a depth of 45 cm and replaced by a mixture of 'black' soil and pure sand (1:3). Other characteristics of the soil were measured in each plot in 2001 (0–10 cm), including pH (7.26 ± 0.04), organic matter content (1.31% ± 0.02) and soil C/N (12.8 ± 0.1). The species pool consisted of four C3 grasses (*Agrostis capillaris* L., *Anthoxanthum odoratum* L., *Festuca rubra* L., and *Holcus lanatus* L.) and four forbs (*Centaurea jacea* L., *Leucanthemum vulgare* Lamk., *Plantago lanceolata* L., and *Rumex acetosa* L.). In each plot, 144 seedlings were planted following a substitutive design. The experimental plots were distributed over six blocks. Each block contained seventeen treatments: all eight monocultures, four mixtures of two species, four mixtures of four species and one eight-species mixture. Species composition of the two- and four-species mixtures was selected randomly without replacement from the species pool. Aboveground biomass was removed by clipping plants at 2.5 cm above the soil surface in late August annually.

For the purpose of this experiment, we used four blocks and sampled a subset of the plots varying in abundance of *A. odoratum* and/or *C. jacea* (Table 1). We selected *A. odoratum* and *C. jacea* based on three criteria: (1) the two species are from different functional groups (grass vs forb) (2) the two species had sufficient fresh roots at the sampling time to conduct the decomposition experiment and (3) the two species were dominant in the previous experimental years (Van Ruijven and Berendse, 2009). To examine the effect of these species on root litter decomposition in monocultures and species mixtures, we took soil samples from monocultures of *A. odoratum* (Ao; $n = 4$) and *C. jacea* (Cj; $n = 4$), a mixture of the two species (Ao + Cj; $n = 1$) and three mixtures of four species: *A. odoratum* plus three other species excluding *C. jacea* (Ao + 3;