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Species-specific effects of earthworms on microbial communities and the fate of litter-derived carbon

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ABSTRACT

Soil respiration is frequently measured as a surrogate for biological activities and is important in soil carbon cycling. The heterotrophic component of soil respiration is primarily driven by microbial decomposition of leaf litter and soil organic matter, and is partially controlled by resource availability. In North American temperate deciduous forests, invasive European and Asian earthworms are known to variously affect soil properties and resource availability through their feeding, burrowing, and casting behaviors, and may affect different components of soil respiration through modulating the microbial communities. By tracing litter-derived C from ^{13}C and ^{15}N double-enriched leaf litter into soil and CO_2 efflux in a mesocosm experiment, we tested the hypothesis that earthworms inhibit litter C-derived soil respiration by reducing resource availability and microbial biomass, and further examined how species-specific effects of earthworms on soil respiration are mediated by soil microbial community. We showed that while earthworms generally had no effect on total soil respiration, the interaction between *Octolasion lacteum* and *Lumbricus rubellus* had a significant negative non-additive effect, presumably through affecting anaerobic microsites in the soil. Moreover, litter C-derived soil respiration was reduced by the Asian *Amynthas hilgendorfi*, the European *L. rubellus*, and the North American native species *Eisenoides lonnbergi*, but not by the European species *O. lacteum*. Phospholipid fatty acid (PLFA) analysis and structural equation modeling indicated that while soil bacteria and fungi abundances were affected by earthworm species identities, the observed reduction of litter C-derived soil respiration could not be fully explained by changes in microbial biomass. We attributed these effects to earthworm-induced aggregate formation, reduction of microbial transformation of labile carbon, and antimicrobial peptide activities, and concluded that the mechanisms through which the four earthworm species affect the fate of litter-derived C and its mineralization are species-specific.

1. Introduction

The heterotrophic component of soil respiration is primarily the result of decomposition and is driven by microbial activity. Soil macrofauna, such as earthworms, influence soil microbial communities and activities through complex direct and indirect processes. A recent meta-analysis concluded that the presence of earthworms increases soil respiration by an average of 33% (Lubbers et al., 2013), but the effect is transient and relatively short-term, usually observable only during short-term manipulation experiments or at the leading edge of the earthworm invasion front (Eisenhauer et al., 2011; Xia et al., 2011; Crumsey et al., 2013). The long-term field study by Fisk et al. (2004) supports this conclusion, showing no differences in soil respiration between plots with and without earthworms. However, these conclusions do not imply that the underlying processes contributing to soil respiration and their relative importance remain unchanged under earthworm invasion.

The overall effects of earthworms on soil microbial communities and activities could be positive, negative or neutral. In general, earthworms can indirectly affect soil microbes through changing resource availability by consuming leaf litter and soil organic matter (SOM), by casting, and by vertical translocation of C and N in the soil (Eisenhauer et al., 2007). Vertical mixing of leaf litter by litter-feeding species tends to increase microbial biomass in the soil as a result of the combination of the release of labile substrates and translocation of readily mineralizable C, while soil-feeding species

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tend to decrease microbial biomass in the soil due to their ingestion of recalcitrant organic matter (McLean et al., 2006). The drilosphere, which consists of earthworm burrows, casts and middens, is rich in soluble C and earthworm mucus when fresh and is generally considered a hotspot of microbial activities (Aira et al., 2009; Stromberger et al., 2012). Bacteria are also known to be an important part of earthworm nutrition (Larsen et al., 2016). Earthworms may selectively feed on bacteria or bacteria-colonized patches (Jayasinghe and Parkinson, 2009; Zirbes et al., 2011), potentially leading to reduced bacteria abundance. Their burrowing activities can cause disruption of fungal hyphae (Butenschoen et al., 2007), leading to the reduction of fungi. Some of these behaviors and the associated impacts can be species-specific.

While the effects of earthworms on resource availability and soil microbes have been widely studied (Butenschoen et al., 2007; Jayasinghe and Parkinson, 2009; Eisenhauer et al., 2011; Dempsey et al., 2011, 2013; Sackett et al., 2013), less is known about how the different processes involved mediate the effects of earthworms on soil respiration and its different components. The earthworm-induced short-term increase in soil respiration, followed by gradual decrease back towards the baseline (e.g. Xia et al., 2011; Crumsey et al., 2013), is consistent with the theoretical responses of soil microbes following a C pulse event, and can be readily explained by the enzyme-driven theoretical models of microbial C and N limitation (Schimel and Weintraub, 2003; Waring et al., 2013). Earthworms are also known to change the fate of litter-derived C through a combination of their feeding, burrowing, and casting behaviors (Fahey et al., 2013a; Chang et al., 2016). Therefore, the contribution of litter-derived C to soil respiration may still be altered even under situations where total soil respiration is not affected. Recently Snyder et al. (2009) suggested that soil macrofauna could reduce soil respiration derived from leaf litter. This new phenomenon has only been observed in two cases, one with the millipede *Pseudopolydesmus erasus* native to the USA and the other with the Asian invasive earthworm *Amynthas corticis* (Snyder et al., 2009). It is unclear whether this phenomenon is common across different species with different feeding and burrowing behaviors, and the mechanisms leading to the decrease have never been investigated.

In temperate deciduous forests in eastern North America, European earthworm invasion into habitats with low native earthworm abundance or habitats previously devoid of earthworms has led to growing concerns from researchers and land managers. Through feeding, burrowing and casting behaviors, invasive European earthworms negatively affect the understory vegetation, reducing the leaf litter and organic layers and mixing the organic matter into the mineral soil (Hale et al., 2005, 2006; Nuzzo et al., 2009; Dempsey et al., 2011; Dobson and Blossey, 2015). Earthworm activities also lead to major changes in soil properties and biogeochemistry, including altered water retention capacity, pH, and soil C and N distribution and availability, as well as increased bulk density, aggregate formation, incorporation of organic matter, and CO_2 and N_2O efflux (Bohlen et al., 2004a; Hale et al., 2005, 2008; Eisenhauer et al., 2007; Szlavecz et al., 2011; Lubbers et al., 2013; Ma et al., 2013; Dobson and Blossey, 2015; Lyttle et al., 2015).

In recent years, a group of Asian earthworms, *Amynthas*, has been widely reported invading forests already inhabited by European species, leading to a "second wave of invasion" where the soil ecosystem, already modified by European species, is going through another transition. Two of the invading species, *Amynthas agrestis* and *Amynthas hilgendorfi*, are of special concern due to their high abundance and biomass (Callaham et al., 2003; Görres and Melnichuk, 2012; Greiner et al., 2012), ability to spread with facilitation from human activities (Belliturk et al., 2015), and potential to completely displace other earthworm species (Greiner et al., 2012; Chang et al., 2016). While recent studies have shown that the effect of *Amynthas* on soil C may be similar to that of the European species (Snyder et al., 2011, 2013; Greiner et al., 2012), our understanding on the mechanisms through which different *Amynthas* and European earthworm species affect soil C dynamics is still limited. One contrasting difference between the two *Amynthas* and most common European species in eastern North America is the annual life cycle of the two *Amynthas* species. This characteristic is accompanied by dietary flexibility, voracious foraging, fast growth, and dramatic seasonal biomass fluctuation (Callaham et al., 2003; Greiner et al., 2012; Chang et al., 2016), and may have distinct impacts on soil C biogeochemistry.

Redistribution of organic matter by earthworms through vertical mixing alters resource availability to soil microbes and therefore affects C sequestration and mineralization. Earthworms in different functional groups have distinct feeding and burrowing behaviors, and, in theory, can have their own characteristic soil mixing and C translocation patterns distinguishable from other functional groups. The most commonly used functional classification categorizes earthworms into three groups: 'epigeic species' are litter feeders and leaf litter/soil surface dwellers; 'endogeic species' are soil feeders and live predominantly in the soil; 'anecic species' are litter feeders that live in permanent vertical burrows extending deep into the soil (Bouché, 1977). A fourth group, 'epi-endogeic species', is sometimes separated from the true epigeic earthworms for species living in the litter-soil interface but also burrowing into the surface mineral soil. Since all species in a functional group are assumed to exhibit functional equivalency (Blondel, 2003), the three or four-category classification has been widely used in recent research focusing on belowground processes (Bohlen et al., 2004b; Crumsey et al., 2013). However, several studies have demonstrated that using functional groups alone failed to reasonably explain and predict patterns of feeding, vertical mixing, and C translocation in a wide range of earthworm species (Neilson et al., 2000; Zicsi et al., 2011; Chang et al., 2016), stressing the importance of explicitly considering species identities and species-specific effects when studying earthworm impacts on soil C and N biogeochemistry.

Leaf litter with a unique ^{13}C signature, artificially altered through either enrichment or depletion, allows us to trace litter-derived C into soil and CO_2, and to partition soil respiration into litter- and soil-derived components. In this study, we conducted a lab mesocosm experiment using ^{13}C and ^{15}N double-enriched leaf litter to address the complex processes mediating the earthworm effects on soil respiration and to evaluate how soil respiration is affected by four earthworm species with distinct behaviors. We hypothesized that (1) earthworms have an overall negative effect on litter C-derived soil respiration, (2) epi-endogeic earthworms negatively affect litter C-derived soil respiration by reducing leaf litter, (3) endogeic earthworms reduce litter C-derived soil respiration by reducing substrate availability and microbial biomass in the soil, and (4) the interspecific interactions have either additive, or positive non-additive effects on both litter C and soil C-derived soil respiration.

2. Methods

2.1. Experimental design

A laboratory experiment with ecologically different earthworm species and species combinations was conducted using ^{13}C and ^{15}N double-enriched leaf litter as a food resource (Chang et al., 2016). Four species of earthworms were selected based on their origin and functional groups: *Amynthas hilgendorfi*, an epi-endogeic species of Asian origin, *Lumbricus rubellus*, an epi-endogeic species from Europe, *Octolasion lacteum*, an endogeic species from Europe, and