Feeding behaviour of epi-anecic earthworm species and their impacts on soil microbial communities

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ABSTRACT

Earthworms contribute to numerous ecosystem services provided by soils. Most of the studies focusing on the contributions of earthworms on leaf litter decomposition were conducted by comparing distinct ecological categories (epigeic, epi-anecic, anecic strict and endogeic), whereas their specific contributions within a given ecological category remains largely unknown. In this context, the aim of this study was to determine the contribution of four epi-anecic earthworm species (*Lumbricus rubellus, Lumbricus festivus, Lumbricus centralis* and *Lumbricus terrestris*) to the leaf litter decomposition of three plant species (*Lolium perenne, Holcus lanatus* and *Corylus avellana*) with contrasted litter traits located at both the soil surface and at a depth of 10 cm. Fungal and bacterial communities inhabiting epi-anecic earthworm burrows were also assessed using T-RFLP analysis. Epi-anecic earthworms improved the leaf litter mass loss solely at the soil surface, while leaf litter mass loss was mainly due to microbial activity at 10 cm deep. Leaf litter mass loss was positively correlated to the initial biomass of the epi-anecic earthworms and the intensity of this relationship was dependent on litter type. Interestingly, *L. festivus* seemed to have a higher contribution to surface leaf litter mass loss that was linked to a stimulation of the fungal communities in its burrows. Fungal communities were thus impacted by both the litter type and the epi-anecic earthworm identity whereas soil bacterial diversity and richness were stimulated in the earthworm burrows whatever the epi-anecic earthworm species considered. Overall, epi-anecic earthworms contributed to enhance the diversity of the drilospheric microbiota.

1. Introduction

Earthworms constitute the dominant biomass of soil invertebrates (Lee, 1985; Curry, 1994) and are considered as both ecosystem engineers (Jones et al., 1994) and keystone species (Blondel and Aronson, 1995) due to their contribution to physical, chemical and biological modifications of soil properties, thus driving key ecosystem services provided by soils (Blouin et al., 2013). Earthworms are classified into three ecological categories (epigeic, anecic and endogeic species) based on their ecological behaviour, with a continuum between these three categories (Bouché, 1977). Briefly, epigeic earthworms live in and consume surface organic matter, endogeic earthworms burrow horizontal galleries to feed on soil organic matter, and anecic earthworms burrow vertical galleries to feed on a mixture of surface and soil organic matter. Within the anecic earthworms, Jégou et al. (1998, 2000), based on studies from Bouché (1972, 1977), distinguished the strict-anecic from the epi-anecic earthworms: strict-anecic earthworms construct a high density of semi-permanent burrows and have low surface litter incorporation rates whereas the epi-anecic earthworms build permanent burrows into which they incorporate high quantities of fresh litter from the soil surface.

Within the drilosphere, corresponding to the soil influenced by earthworm activities (Lavelle, 1988), this deep litter incorporation into their burrows enhances soil microbial development (Tiunov and Dobrovolskaya, 2002) and mineralization activity (Winding et al., 1997; Brown et al., 2000). Soil microorganisms are indeed responsible, at the fine scale, for soil organic matter decomposition (Coûteaux et al., 1995). Among them, fungi are commonly the main decomposers of fresh plant litter (Chapin et al., 2002) through the production of extracellular enzymes able to decompose complex material with low nutrient concentration such as lignocellulose compounds (Ingold and Hudson, 1993). Meanwhile, bacteria mainly absorb soluble and easily available substrates allowing them to develop quickly in substrate-rich habitats (Chapin et al., 2002).

In temperate grasslands, epi-anecic earthworms are subjected to leaf litter of various quality depending on the plant species composition. For example, *Lumbricus terrestris* preferentially selects leaves with high nitrogen content (Shipitalo et al., 1988). This feeding preference induces

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strong variations in litter feeding rates by epi-anecic earthworms according to leaf litter traits (Wright, 1972; Edwards and Lofty, 1977; Lee, 1985; Hendriksen, 1990; Šlapokas and Granhall, 1991). In addition, leaf litter can be located at different positions in the soil profile according to agricultural practices. Leaf litter is naturally deposited at the soil surface but, considering some specific agricultural practices, leaf litter can also be incorporated deeper in the soil through temporary grasslands ploughing. Compared to surface leaf litter, this buried leaf litter might thus alter epi-anecic earthworms' contribution to leaf litter decomposition.

The literature on epi-anecic earthworms is biased towards *L. terrestris* (Needham, 1957; Satchell and Lowe, 1967; Curry and Bolger, 1984; Shipitalo et al., 1988; Tiunov and Dobrovolskaya, 2002; Andriuzzi et al., 2016) due to its widespread distribution (Bouché, 1972) and breeding facility (Daniel, 1991; Butt et al., 1994; Daniel et al., 1996). However, the epi-anecic ecological category includes several earthworm species (Diaz Cosin et al., 1992; Decaëns et al., 2008; Cluzeau et al., 2012) which might exhibit distinct feeding preferences and specific interactions with soil microorganisms. For example, the fresh biomass of epi-anecic earthworms can vary from 0.75 g for *Lumbricus festivus* up to 15 g for *L. terrestris* (Bouché, 1972), suggesting different metabolic needs depending on the species identity. Such high differences in earthworm biomass could lead to different quantity and/or quality of leaf litter transported in the burrows and thus to distinct impact on soil microorganisms. It has already been demonstrated that earthworm species from different ecological categories affected soil microbial abundance and activity differently with cascading effects on microbial transformation of labile carbon (Sheehan et al., 2008; Chang et al., 2016). However, to our knowledge, no previous study has been conducted in order to decipher the impact of different earthworm species on microbial communities within a given ecological category.

The purpose of this study was to determine whether, within the epi-anecic ecological category, different earthworm species specifically interact with leaf litter and microbial communities inhabiting their burrows leading to distinct rates of leaf litter mass loss. We conducted a laboratory mesocosm experiment and determined the contribution of four epi-anecic earthworm species (*Lumbricus rubellus, L. festivus, Lumbricus centralis* and *L. terrestris*) to the leaf litter mass loss of three distinct plant species, two grass (*Lolium perenne, Holcus lanatus*) and a tree species (*Corylus avellana*), located at both the soil surface and at a depth of 10 cm after 10 and 20 days of incubation. At the end of the experiment, the community structure of the bacteria and fungi inhabiting the epi-anecic earthworms' burrows were analyzed and compared using Terminal Restriction Fragment Length Polymorphism (T-RFLP). First, we hypothesized that leaf litter mass loss increases according to the initial biomass of the epi-anecic earthworms. Second, we hypothesized a higher epi-anecic earthworms' contribution to leaf litter mass loss at the soil surface compared to a 10 cm depth. Finally, we expected that both the litter type and the epi-anecic earthworm identity control microbial community structure in earthworm burrows.

2. Materials and methods

2.1. Material collection

Soil (deep to 5–20 cm) and epi-anecic earthworms were collected in a temporary grassland near Trans-La-Forêt, France (48°50′ N, −1°58′ W) in the Long Term Ecological Research (LTER) site "Zone Atelier Armorique". The climate of the region is oceanic with a mean annual temperature of 11.7 °C, a mean annual rainfall of 815.0 mm and a mean annual relative humidity of 80.9% (mean values over the period 2010–2016, data from Météo France). Soil collected was hand sieved at 4 mm, homogenized, and a soil sample was sent to the central analytical laboratory of INRA (SAS, Arras, France) for texture, organic matter, C:N ratio and pH measurements. The soil was identified as a brown soil with 48.2% sand, 37.5% silt and 14.3% clay, characterized by 2.9% of organic matter, a C:N ratio of 9.7 and a pH of 6.4. The soil was also pre-incubated for one week at 12 °C under a 12 h:12 h light: dark regime with a water content adjusted to 31% w/w by addition of deionized water prior to the experiment.

Fresh leaf litter of *Lolium perenne* and *Holcus lanatus* was collected from non-permanent grasslands close to the earthworm and soil sampling location. Freshly abscised leaves of *Corylus avellana* were collected from trees close to the Biological Station of Paimpont, France (48°01 N, −2°17 W). The three litter types were thereafter air-dried at room temperature and stored until the beginning of the experiment. *L. perenne* and *H. lanatus* leaves were cut into sections of approximately 7 cm length while *C. avellana* leaves were used intact. *H. lanatus* and *L. perenne* are two grass species typical of temporary grasslands, whereas *C. avellana* is a shrub frequently encountered in hedges surrounding these grasslands.

Four epi-anecic earthworm species were studied (Bouché, 1972, 1977): *L. rubellus rubellus* (Hoffmeister, 1843; hereafter referred to as LR), *L. festivus* (Savigny, 1826; hereafter referred to as LF), *L. centralis* (Bouché, 1972; hereafter referred to as LC) and *L. terrestris,* (Linné, 1758; hereafter referred to as LT). Epi-anecic earthworms were hand collected a week before the experiment, placed in a sample of the hand-sieved soil and fed with litter composed of a mixture of air-dried leaves of the three plant species than the ones used in the experiment.

2.2. Initial litter characteristics

Carbon (C) and nitrogen (N) concentrations were determined by thermal combustion using a Vario Pyro cube CNS analyzer (Elementar France SARL, Lyon, France). Lignin, cellulose, hemicellulose and water soluble compound (WSC) concentrations were determined according to the Van Soest extraction protocol (Van Soest and Wine, 1967) using a fiber analyzer (Fibersac 24; Ankom, Macedon, NJ, USA). Phenolic concentrations were measured colorimetrically using the method described in Santonja et al. (2015) with gallic acid as a standard. To determine the water holding capacity (WHC), intact leaf litter samples were soaked in distilled water for 24 h, drained and had mass determined. The dry mass was determined after drying samples at 60 °C for 48 h. WHC was calculated as (moist mass / dry mass) × 100% (Santonja et al., 2015). Specific leaf area (SLA) was determined by using the Image J software (https://imagej.nih.gov/ij/, MA, USA). SLA was calculated as the ratio between leaf area and leaf dry mass. Initial litter traits were determined from four samples of each of the three litter types (except for WHC and SLA for which n = 10).

With the exception of cellulose, all of the initial litter characteristics varied between the three litter types (Table 1). Nitrogen and WSC concentrations decreased according to the gradient *L. perenne* > *H. lanatus* > *C. avellana*, whereas WHC and SLA followed the gradient *H. lanatus* > *L. perenne* > *C. avellana* (Table 1). Carbon, lignin and phenolic concentrations, in addition to C:N and lignin:N ratios, were higher in *C. avellana* litter than in *H. lanatus* and *L. perenne* (Table 1).

2.3. Experimental setup

A 5 × 3 factorial design was performed with 4 replicates and for two incubation times (10 and 20 days): with or without (control accounting for the litter mass loss due to microbial decomposition or leaching) one of the four epi-anecic earthworm species; with one of the three litter types placed at both the soil surface and at 10 cm deep. For each litter type, 30 litterbags (11 × 9.5 cm; 1.2 cm mesh size) were prepared and filled with 2 g of air-dried leaves re-humidified just before the experiment. In parallel, 2 g of leaves were dried at 72 °C for 48 h to determine the initial litter dry mass. Each mesocosm (PVC cylinder, 30 cm high, 10 cm diameter sealed at the base) was filled with 4.9 kg of soil in two steps. First, half of the soil was placed in the mesocosm and compacted to a bulk density of 1.3 g cm^{-3} and a litterbag was placed