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The role of *Armadillidium vulgare* (Isopoda: Oniscidea) in litter decomposition and soil organic matter stabilization

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ABSTRACT

We studied the effects of the terrestrial isopod *Armadillidium vulgare* on organic matter decomposition and stabilization in a long-term (65-week) laboratory experiment. We quantified the microbial activity in leaf litter (*Acer pseudoplatanus*) which did not come into contact with isopods, in *A. vulgare* feces produced from the same litter, and in unconsumed leftover of this litter. Freshly fallen leaf litter and up to 3 day old feces and leftover of litter were used. All materials were air dried immediately after collection and rewetted 1 day before use. Simultaneously, we measured how microbial activity in litter and feces are affected by fluctuations in humidity and temperature and by the addition of easily decomposed substances (starch and glucose).

Microbial respiration was lower in feces than in litter or unconsumed leaf fragments. At the same time, moisture and temperature fluctuations and addition of glucose or starch increased respiration much more in litter than in feces. The results indicate that the processing of litter by *A. vulgare* reduces microbial respiration and reduces the sensitivity of microbial respiration to environmental fluctuations. ^{13}C NMR spectra from feces indicated preferential loss of polysaccharide-carbon and accumulation of lignin with some modification to the aromatic-carbon. TMAH-Py-GC MS showed that lignin content was higher in feces than in litter and that lignin quality differed between the two substrates. Guaiacyl units were depleted in the feces, which indicated breakdown of guaiacyl associated with gut passage. As a conclusion, the results suggest that this common isopod greatly affects leaf litter decomposition. Decomposition of isopod feces in a long-term experiment is lower than litter decomposition which may support stabilization of organic matter in soil. This is caused mainly due to higher content of aromatic carbon in feces, which may cause its considerable resistance to bacterial degradation.

1. Introduction

Plant litter decomposition is one of the main processes in material cycling and energy transformation in terrestrial ecosystems (Mellillo et al., 1982). While climate and litter chemistry greatly affect litter decomposition at the global and regional scales (Mellillo et al., 1982; Aber et al., 1991; Austin and Vitousek, 2000; Vanderbilt et al., 2008), soil organisms greatly affect plant decomposition and nutrient release at the local scale (Aerts, 1997; Frouz et al., 2007).

Decomposition of plant litter can be substantially affected by the interactions of soil microflora and fauna (Scheu et al., 2002). Whereas the soil microflora play a primary role in the chemical transformation and mineralization of soil organic matter, soil fauna contribute to litter decomposition by digesting substrates, increasing substrate surface area through fragmentation, and enhancing microbial activity (Coleman et al., 2004; Wolters, 2000).

The effects of earthworms on the distribution of organic matter in the soil profile and on decomposition and carbon sequestration have been well studied (Hendriksen, 1997; Lavelle et al., 1997; Zhang et al., 2003; Frouz et al., 2007), but less is known about effects of other macrofauna including terrestrial isopods. Isopods mainly inhabit the litter layer; by fragmenting leaf litter (Zimmer et al., 2005), they facilitate litter decomposition and nutrient cycling (Hassall et al., 1987; Zimmer and Topp, 1999; Xin et al., 2012). As a consequence, terrestrial isopods indirectly affect the activity and community composition of the soil microflora (Hanlon and Anderson, 1980; Lavelle et al., 1997). The isopod *Armadillidium vulgare* (Latreille) is a common and abundant member of the saprophagous soil macrofauna in Europe but has recently become invasive in other parts of the world (Hassall et al., 1987; Frouz et al., 2008). *A. vulgare* may reach field densities as high as 10,000

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individuals per m^2 (Frouz et al., 2004) and may consume significant amounts of litter thus producing large quantities of fecal pellets (Zimmer, 2002).

The vast majority of studies on the effects of soil macrofauna on plant litter decomposition have focused on short-term effects occurring over days or several weeks; as noted, these studies generally indicate that soil macrofauna enhance decomposition. Researchers have paid less attention to the long-term and overall effects of soil macrofauna on the stabilization and accumulation of soil organic matter (Wolters, 2000). An increasing number of studies, however, show that, in the long-term, soil fauna decrease microbial respiration and consequent carbon loss (Lavelle and Martin, 1992; Frouz and Šimek, 2009).

In the current study, we investigated the overall effect of *A. vulgare* on litter respiration in a long-term (65 week) laboratory experiment. As there were no other mechanisms by which organic matter may have left the experimental system (e.g. leaching), we may use cumulative amount of C loss by respiration and C loss from the decomposing material. We compared carbon loss by microbial respiration and chemistry of litter, feces, and unconsumed residuals of litter (leaf fragments that have not been ingested). Although most previous studies were done under constant conditions, the natural environment is seldom constant and we therefore determined how microbial respiration in litter and in feces responded to moisture and temperature fluctuations. Previous studies reported that soil fauna removes easy accessible carbon from litter namely saccharides and polysaccharides (Hopkins et al., 1999). A previous study showed that feces addition to the soil causes lower priming effect than litter addition (Kaneda et al., 2013). Priming effect is when the addition of available carbon increases the rate of decomposition of existing organic matter (Fontaine et al., 2004). Finally, we used solid-state 13C nuclear magnetic resonance (NMR) spectroscopy and TMAH-Py-GC MS to elucidate how changes in decomposability caused by *A. vulgare* feeding correspond to changes in organic matter chemistry in general and to changes in the content and composition of aromatic compounds in particular.

With this in mind we decided to test following hypotheses:

1. In a long-term experiment microbial respiration of materials affected by isopod feeding, both feces and unconsumed residues of litter are lower compared to litter unaffected by isopod.
2. As a result of fluctuations of moisture and temperature and of the addition of easily decomposed substances, litter respiration increases more than respiration of isopod feces.
3. Litter is more sensitive to a priming effect than feces.
4. Changes in feces correspond with changes in litter chemistry, namely because feces are enriched by aromatic components more resistant to bacterial decomposition.

2. Materials and methods

2.1. A. vulgare and leaf litter collection

Leaf litter (*A. pseudoplatanus*) was collected from the soil surface at the same site at the time of natural leaf fall (November 2009); the litter was carefully separated by hand from other components such as small branches and woody debris, and then air-dried and stored in paper bags in a dark, dry location.

The terrestrial isopod *A. vulgare* (Latreille) was collected in a deciduous woodland dominated by *Acer pseudoplatanus* on Petřín hill in Prague in May 2010. About 100 individuals of *A. vulgare* were supplied with rewetted leaf litter (*A. pseudoplatanus*) for 2 months. Every third day, the feces and leftover of litter that remained were collected and new rewetted litter was provided. Collected material was separated on feces and leftover of unconsumed litter fragments, immediately air-dried, and stored in paper bags in a dark, dry location. In this way we produced three types of materials litter, which were not yet offered as food for isopods, feces and unconsumed litter fragments. This unconsumed litter differed from original litter as more veins remain in it whereas area between veins was consumed. Rewetting of all material was done 1 day before material was used in experiments (Rouifed et al., 2010), by placing them in to nylon mesh bag 0.02 mm mesh size and submersion these bags in water for 12 h. Litter used in feeding experiment was removed from the bag before used as a food for isopods.

2.2. Respiration experiment

Collected materials (litter, feces, and unconsumed leaf fragments) were divided into two parts; the first part was stored in paper bags for chemical analyses of initial materials and the second part was used for a long-term respiration experiment in bottles. For this experiment, both unconsumed leaf fragments and litter were cut into small pieces (1 cm^2) and placed in nylon litter bags (2 cm × 2 cm, mesh size 0.02 mm); about 0.1 g was placed in each bag. Similar litter bags were used for feces (0.1 g per bag). The litter bags were placed in 250-ml glass bottles (one bag per bottle) with 40 g of fine sand on the bottom. The sand contained no organic matter and was moistened in a way that there was no visible water level in the sand but there was constant capillary fridge from the sand. All three substrates (litter, unconsumed leaf fragments, and feces) in litterbags were rewetted with sterile water to 70% moisture content. Distilled water was added bi-weekly to the sand in each bottle (1 ml per bottle) to maintain constant moisture.

Some litter bags were maintained at a constant temperature (21 °C) and with constant moisture (by addition of distilled water as noted in the previous paragraph). Other litter bags with litter or feces were treated in one of four ways. One group was dried and rewetted once per week; these litter bags were removed from the bottles, air dried for 24 h, then returned to the bottles and rewetted with sterilized water. A second group was frozen and thawed once per week; these litter bags were removed from the bottles, placed at −18 °C for 24 h, and then returned to the bottles at 21 °C. A third and fourth group were treated with glucose or starch; a 2-ml solution containing 0.2 μg of glucose or starch was added to each bottle each week.

For measurement of microbial respiration, bottles were sealed for 6 days every week and supplied with 3 ml of 0.5 M NaOH in a small beaker. The CO_2 produced in the bottle was trapped in the NaOH, and the quantity trapped was determined by titration with 0.05 M HCl after 2 ml of $BaCl_2$ was added (Page, 1982). This was repeated for 65 weeks, at which time the respiration experiment was ended. In all cases five replicates were measured.

2.3. Substrate analyses

Total carbon and nitrogen in the three materials at the start and at the end of the respiration experiment were measured with a CN analyzer (The Elemental Analyzer 1108, Carlo Erba Instruments).

Litter and feces collected at the beginning and end of the respiration experiment were subjected to 13C CP/MAS NMR and TMAH-Py-GC MS. NMR spectra were measured with a Bruker Avance 500 WB/US NMR spectrometer (Karlsruhe, Germany, 2003) in a 4-mm ZrO_2 rotor. Magic angle spinning (MAS) speed was 9 kHz in all cases, with a notation frequency of $B_1(^1H)$ and $B_1(^{13}C)$ fields for cross-polarization $\omega_1/2\pi = 62.5$ kHz. Repetition delay and number of scans was 4 s and 1024, respectively. TPPM (two-pulse phase-modulated) decoupling was applied during evolution and both detection periods. The phase modulation angle was 15°, and the flip-pulse length was 4.8–4.9 μs. The applied notation frequency of the $B_1(^1H)$ field was $\omega_1/2\pi = 89.3$ kHz. The ^{13}C scale was