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Major mechanisms contributing to the macrofauna-mediated slow down of litter decomposition

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ABSTRACT

To understand why excrements of soil macrofauna often decompose more slowly than leaf litter, we fed *Bibio marci* larvae the litter of tree species differing in litter quality (*Alnus glutinosa*, *Salix caprea*, and *Quercus robur*) and then measured respiration induced by litter and excrements. We also measured respiration induced by the same litter artificially modified to mimic faunal effects; the litter was modified by grinding, grinding with alkalinization to pH = 11, grinding with coating by kaolinite, and grinding with both alkalinization and coating. Decomposition of excrements tended to be slower for willow and was significantly slower for oak and alder than for the corresponding litter. With oak, decomposition was slower for all artificially modified litter than for non-modified litter. The reduction in the decomposition was similar for excrements and for alder and willow litter that was ground, coated, and alkalinized. In alder, a similar reduction was found in ground and alkalinized litter. ^{13}C NMR indicated that gut passage increases aliphatic components and decreases polysaccharides. Pyrolysis indicated that gut passage increases the ratio of guaiacyl to hydroxymethyl derivatives in lignin. Our findings indicate that the decreased decomposition rate of excrements might result from the removal of easily available polysaccharides, the increase in aliphatic components, an increase in the resistant components of lignin, the accumulation of microbial cell walls, and the binding of nitrogen into complexes with aromatic components. Several of these mechanisms are supported or determined by litter alkalinization during gut passage.

1. Introduction

With global warming, soil organic matter is receiving substantial attention as an important part of the global carbon (C) cycle. Soil contains three-times more C than the atmosphere and because of its dynamic nature, soil organic C could serve as either a significant sink or source for atmospheric carbon dioxide (Post et al., 1982). Most of the terrestrial net primary production enters the soil decomposer system as dead organic matter, namely leaf litter and dead roots (Wardle et al., 2004; García-Palacios et al., 2013). Leaf litter decomposition is affected by a complex interplay of climate, litter chemistry, soil properties, and activity of soil organisms (Schmidt et al., 2011).

Among soil organisms, research has more often focused on microorganisms than on fauna; the effect of fauna has been neglected in part because the assimilation efficiency of soil fauna is generally low (Anderson and Ineson, 1984; Lavelle et al., 1997; Kadamannaya and Sridhar, 2009), and consequently, most ingested organic matter is only transformed from litter into excrements. Fauna excrements, however, differ substantially from the original litter, and these differences greatly affect microbial activity and decomposition (Anderson and Ineson, 1984; Lavelle et al., 1997; Wolters, 2000). During faunal feeding or soon after excrements are produced, microbial activity is increased, and the decomposition rate is greater for the new excrements than for the original litter. As soil faunal excrements age, however, the decomposition rate decreases and becomes lower than that of the original litter (Van der Drift and Jansen, 1977; Hassall et al., 1986, 1987; Griffiths et al., 1989; Lavelle and Martin, 1992; Frouz et al., 1999; Frouz and Šimek, 2009; Kaneda et al., 2013; Frouz et al., 2014, 2015). Factors causing the increase of microbial activity in new faunal excrements

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(litter fragmentation, nutrient release due to the effects of the gut on the litter and to the killing of the ingested microflora) have often been studied (Hassall et al., 1986, 1987; Griffiths et al., 1989; Frouz et al., 1999), but the mechanisms causing a reduction in microbial activity in older excrement have been less studied. This phenomenon has received much more attention for earthworms (Lavelle and Martin, 1992; Zhang et al., 2003; Frouz et al., 2014) than for other litter-feeding macrofauna. This is despite the fact that soil macrofauna such as millipedes, isopods, and insect larvae can consume 20–100% of annual litter fall in many ecosystems and especially in temperate, broadleaf forests (Karpachevsky et al., 1968; Szabo, 1974; Tajovský, 1992; García-Palacios et al., 2013).

The aim of this contribution is to explore mechanisms that may reduce the rate of microbial decomposition in older excrements of litter-feeding macroarthropods. To do so, we studied the decomposition (measured as loss of C in CO_2) of litter and of excrements produced from this litter together with artificial litter treatments that mimic some of the modifications in litter caused by passage through the faunal gut.

Bibio marci (Diptera: Bibionidae) was used as an example of a litter-feeding macroarthropod; in addition to dipteran larvae, such macroarthropods also include millipedes and terrestrial isopods. Bibionidae occur worldwide and are especially abundant in tropical and temperate regions, where larvae in litter and soil can achieve high biomasses (Frouz, 1999) and consume all of the annual litter fall (Karpachevsky et al., 1968; Szabo, 1974).

The artificial treatments used in our study were litter fragmentation, litter alkalinization, coating by clay, and their combinations. Litter fragmentation is assumed to be the major reason for the initial increase in microbial respiration in excrements (Hassall et al., 1986, 1987; Griffiths et al., 1989; Frouz et al., 1999); therefore, we explored its long-term effect. Alkalinization was studied because many saprophagous arthropods including *B. marci* have a highly alkaline section of their gut (Johnson and Felton, 1996; Brune, 1998; Graça and Bärlocher, 1999; Frouz et al., 2002). Finally, clay coating has been suggested as one reason for the stabilization of soil organic matter processed by earthworms (Zhang et al., 2003; Frouz et al., 2014).

2. Materials and methods

2.1. Materials

A litter bag experiment was conducted in microcosms (bottles) with litter of three tree species, excrements generated from that litter by a soil arthropod, and litter modified to mimic specific changes in litter during passage through the soil arthropod gut. Litter of alder (*Alnus glutinosa*), willow (*Salix caprea*), and oak (*Quercus robur*) was collected at post-mining sites near Sokolov (50°14′ N, 12°39′E). Litter of these species was chosen because of contrasting C:N ratios (Table 1). Fresh litter that had not contacted soil was used. Part of the litter was air-dried; the rest was kept moist, was stored at 4 °C, and was used to feed larvae of the soil arthropod *B. marci*, which were collected in an alder (*A. glutinosa*)-dominated forest near České Budějovice, Czech Republic (48°59′N, 14°25′E), in October 2009. At the same time, samples of the fermentation layer were taken from each site where the litter was collected; the fermentation layer was kept at the original moisture and was stored at 4 °C.

To obtain excrements, larvae were maintained in a plastic container (10 × 20 × 5 cm) at 15 °C with 95–100% relative humidity (RH) and in the dark. About 150 larvae were placed in each of the six containers (3 litter types × 2 replicates) and fed with either alder, willow, or oak litter. To harvest excrements, the contents of each container were passed through a 1-mm sieve every other day. After excrements were separated, larvae were removed from the litter and returned to the container along with new litter. After they were collected by sieving, excrements were spread in a thin layer and air-dried. Excrements derived from the same kind of litter were pooled and stored in a dry, dark place. The first two collections were not used to ensure that the excrements were not contaminated by previously consumed litter.

Besides litter and excrements, the following four artificial treatments were used in the experiment: ground litter (G); ground and alkalinized litter (GA); ground and coated litter (GC); and ground, coated, and alkalinized litter (GCA). These treatments were designed to mimic some of the following effects that fauna may have on litter: fragmentation; alkalinization due to gut passage; and coating by clay particles to mimic the situation when larvae ingest soil attached to litter surface. Ground litter was prepared by processing the dry litter in an electric blender and then passing the fragments through a 0.2-mm sieve. Ground litter was also used to prepare the other modified-litter treatments. For treatment G, the ground litter was not further treated. For treatment GA, the litter bags were placed in water alkalinized to pH 11 by addition of NaOH; after 8 h, the litter bags were washed in distilled water until the water had a neutral reaction, and then the litter bags and litter within were air-dried. For treatment GC, 0.1 g of kaolinite was mixed with the 0.5 g of ground litter before it was sealed in the litter bag. For treatment GCA, the ground litter was alkalinized before it was coated and sealed in litter bags. Excrements and all litter treatments including litter that was not modified were also placed in 2 × 2-cm litter bags (42-μm openings), with 0.5 g (dry weight) of litter or excrements per litter bag.

Before the onset of the litter bag experiment, all litter bags prepared as above were rewetted by placing them for 24 h on sand saturated with a filtered soil suspension. The filtered soil suspension, which was made with water and fermentation layer soil (1:100, soil:water), provided autochthonous microorganisms that may have been lost during litter bag preparation. The litter bags were also sprayed with the suspension every 8 h during the 24 period.

Table 1
CN ration of litter, treated litter, and excrements prepared from the same litter (alder, oak, or willow) before and after 54 weeks of decomposition. G = ground litter, GC = ground and coated litter, GA = ground and alkalinized litter, GCA = ground, coated, and alkalinized litter. Values are means (with SD below), statistically homogenous groups of CN values are marked by the same letter (one-way ANOVA, LSD, $p > 0.05$).

Litter		Excrements		GA	G	GC	GA	GCA
Before	After	Before	After	Before	After	After	After	After
Alder (*Alnus glutinosa*)								
14.6a (0.6)	14.7a (1.0)	15.4a (0.1)	17.4a (0.3)	14.6a (0.1)	15.7a (0.0)	15.5e (0.2)	16.8 (1.6)	16.0a (0.3)
Willow (*Salix caprea*)								
29.4b (2.6)	36.2c (2.7)	14.5a (0.1)	23.9b (1.4)	28.5b (1.1)	28.8b (0.5)	28.9b (1.7)	29.2b (1.3)	29.7b (0.2)
Oak (*Quercus robur*)								
58.0d (3.2)	59.0d (5.5)	17.3a (0.1)	23.0b (0.9)	58.8b (5.9)	59.7d (2.4)	70.4e (0.1)	60.9d (0.8)	63.4de (2.5)

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