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The biochemical transformation of oak (*Quercus robur*) leaf litter consumed by the pill millipede (*Glomeris marginata*)

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Abstract

Soil macrofauna play an essential role in the initial comminution and degradation of organic matter entering the soil environment and yet the chemical effects of digestion on leaf litter are poorly understood at the molecular level. This study was undertaken to assess the selective chemical transformations that saprophagous soil invertebrates mediate in consumed leaf litter. A number of pill millipedes (*Glomeris marginata*) were fed oak leaves (*Quercus robur*) after which the biomolecular compositions (lipids and macromolecular components) of the leaves and millipede faeces were compared using a series of wet chemical techniques and subsequent analysis by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). It was found that the concentrations of short chain ($<C_{20}$) *n*-alkanoic acids, sterols and triacylglycerols reduced dramatically in the millipede faeces relative to the leaf litter. Hydrolysable carbohydrates and proteins both decreased in concentration in the faeces, whereas similar yields of phenolic components were observed for the cupric oxidation products of lignin, although the oxygenated functionalities were affected by passage through the millipede gut, yielding a more highly condensed state for lignin. This shows that the chemical composition of fresh organic matter entering the soil is directly controlled by invertebrates feeding upon the leaf litter and as such that they are key contributors to the early stages of diagenesis in terrestrial soils.

Keywords: Litter decomposition; *Quercus robur*; *Glomeris marginata*; Faeces; Lipids; Biopolymers; Soil; Carbohydrate; Protein; Lignin

1. Introduction

Soil organic matter (SOM) is currently estimated to comprise a carbon reservoir of ca. 1760 Pg (O'Neill, 1993). Whilst this constitutes less than 0.01% of the global carbon budget (over 99% of carbon is stored in the ocean and marine sediments), it represents 24% of the terrestrial carbon pool. The relatively high turnover of carbon in soils means that any changes in assimilation and mineralisation fluxes for this dynamic system will have important ramifications for the global carbon cycle as a whole. Current concerns over climate change have conferred an increased impetus to research concerned with factors affecting the biological immobilisation of organic matter and the feedback effect that rising CO_2 concentrations are having on the soil fauna that mediate this process (e.g. Verhoef and Brussard, 1990; O'Neill, 1994; Yeates et al., 1997). Whilst there has been much work concerning the role that microorganisms play in this process (e.g. Cheng and Johnson, 1998; Cardon et al., 2001; Cheng, 1999) surprisingly little attention has focussed on the role of meso- and macrofauna operating within the soil environment. Indeed, for the majority of soils the importance of these larger organisms in the pre-processing and comminution of deadfall cannot be overstated. For example, in the top F layer of a northern temperate forest soil up to 90% of the residual organic matter will exist in the form of arthropod faecal material (Bocock, 1963; Nicholson et al., 1966). The exact changes in the biochemical composition of litter resulting from digestion by arthropods of fresh organic matter entering the soil are mostly unknown and as such represent a significant gap in our understanding of the initial stages of diagenesis in terrestrial soils.

Of the larger animals present in soil ecosystems, earthworms (Annelida, Oligochaeta) have been most widely investigated and contribute to the biodegradation of natural residues primarily through the physical alteration of the structure of plant tissue and the soil matrix enhancing microbial activity (Coleman and Crossley, 1996). This paper focuses on the role of the pill millipede (Diplopoda, *Glomeris*

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marginata) in the decomposition of leaf litter. *Glomeris* are important detritivores that inhabit the litter layer of forests, particularly those overlying calcerous soil, and are found in densities of 2.5–7.5 individuals per m^2 although they have been recorded in densities of up to 24 per m^2 (Thiele, 1956). *Glomeris* ingest leaf litter, assimilating 0.3–7%w/w of the ingested material in the process (Byzov et al., 1996) and the resultant faecal material is an important food source for other saprophagous animals such as earthworms (Scheu and Wolters, 1991). *Glomeris* are important in the comminution of litter since they increase the surface area available for microbial growth and their associated decomposition processes during gut passage and in faecal pellets (Striganova, 1971). The presence of *Glomeris* in soil broadens microbial diversity whilst indirectly stimulating nitrogen mineralisation and mobilisation of cations such as potassium and sodium (Anderson et al., 1983; Visser, 1985). Furthermore, faecal pellet formation results in an increase in availability of nutrients such as nitrogen and phosphorous to microorganisms. However, the uptake of these nutrients is dependent on the availability of labile organic matter in the fragmented litter (Maraun and Scheu, 1996).

Food preference experiments have been conducted for *Glomeris* feeding on *Quercus ilex* litter. David and Gillon (2002) reported a preference for decomposed leaf material over freshly fallen litter. It has been reported that for the leaf litter to be palatable to *Glomeris,* colonistion of the litter by microorganisms must occur to soften the leaves (Hassall and Rushton, 1984) and accelerate the loss and degradation of phenolic feeding inhibitors (Edwards, 1974). Mean weight consumption rates of 14 g dry weight of leaf litter per gram of millipede per year have been reported (David and Gillon, 2002), however, laboratory experiments with the millipede *Harpaphe haydeniana* reported daily consumption of conifer litter of between 10 and 20% of millipede fresh biomass that could equate to 36% of total annual litter fall (Carcamo et al., 2000). Soil saprophagous invertebrates (millipedes, woodlice and Diptera larvae) consume between 20 and 100% of plant litter input in a year with consumption being highest during spring (David and Gillon, 2002). However, despite the obvious importance of this pathway for the flux of terrestrial carbon, very little is understood of its effect on the biochemical composition and the associated biogeochemical ramifications.

A variety of non-destructive chemical techniques have been used to analyse the chemical changes that occur when leaf litter is consumed by saprophagous invertebrates. Gillon and David (2001) have used near infrared spectroscopy (NIRS) in conjunction with calibration equations to study *Glomeris* consuming *Q. ilex* leaf litter while ^{13}C nuclear magnetic resonance (NMR) has been used to study other saprophagous soil invertebrates such as termites and earthworms and the transformations they effect on organic matter (Guggenberger et al., 1996; Hopkins et al., 1998). The advantages of using non-destructive techniques lies with the ease of analysis and speed at which samples can be processed, although obtaining quantitative data is non-trivial with ^{13}C NMR analysis being semi-quantitative at best (Preston, 1996). However, these techniques do yield an overview of the changes in chemical composition when used in conjunction with other techniques. NIRS studies concluded that the faeces contained significantly higher concentrations of lignin, but contained less non-structural compounds and nitrogen (Gillon and David, 2001). A significant disadvantage of such approaches is that important changes in composition at the molecular level may be undetectable.

Previous studies have all reported that decomposability is not necessarily greater in millipede faeces than in leaf litter (e.g. Scheu and Wolters, 1991; Maraun and Scheu, 1996; Gillon and David, 2001). *Glomeris* digest a proportion of the cellulose and hemicellulose ingested and readily assimilate compounds such as amino acids (e.g. Bignell, 1989; Scheu and Wolters, 1991). Although *Glomeris* have been extensively studied, the chemical transformations occurring due to the digestive process are poorly understood. Changes in microbial biomass, respiration and nutrient status of beech (*Fagus sylvatica*) leaf litter processed by *Glomeris* have been studied. Using C/N ratios and ergosterol as a fungal indicator, the investigation concluded that *Glomeris* reduced fungal biomass in beech leaf litter more than the bacterial biomass (Maraun and Scheu, 1996). There has been no molecular characterisation of the biochemical transformation of leaf litter consumed by *Glomeris* hence this study was initiated to test the hypothesis that the pre-processing of leaf litter by this arthropod has specific defined effects on organic matter at the molecular level that are likely to affect the subsequent stages of biodegradation of organic matter in soil.

2. Experimental

Pill millipedes and oak leaf litter were collected from a woodland that was formerly associated with the Centre of Ecology and Hydrology Merlewood, Cumbria, UK (Grid reference 341,000, 479,600) and cultured in a single food source microcosm. Briefly, oak leaf litter was placed in a pre-furnaced glass jar and a number of pill millipedes added. The jar was placed in the dark, in a temperature-controlled room held at 20 °C. The millipede faeces were removed from the jar and analysed according to the methods described below.

2.1. Sample preparation and solvent extraction

All samples were crushed with a pestle and mortar facilitated by the addition of liquid nitrogen and subsequently passed through a 2 mm and a 75 μm sieve. Hexadecan-2-ol, nonadecane, heptadecanoic acid, 5β-pregnan-3α-ol and hexadecyloctadecanoate were added as internal standards. Samples were extracted ultrasonically at room temperature (5×) using dichloromethane (DCM):acetone (9:1 v/v); total volume 50 ml. The extracts were combined to form a total lipid extract (TLE) and solvent was removed by evaporation under reduced pressure.