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Collembola feeding habits and niche specialization in agricultural grasslands of different composition

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ABSTRACT

The introduction of white clover in grassland is a common practice to improve the quality of the pasture and to limit the use of industrial sources of inorganic fertilizer N inputs. However, little is known about the extent to which the introduction of different crop compositions affects soil quality in general, and soil biota in particular. Recent studies have shown that epedaphic and euedaphic Collembola can have distinct differences in feeding strategy that suggests trophic niche differentiation according to soil habitat. Combining fatty acid (FA) biomarkers with the carbon isotopic ratios technique, we investigated belowground trophic interactions. We compared the FA composition of the three most predominant groups of Collembola (*Isotoma* spp., *Lepidocyrtus cyaneus* and Poduromorpha) and bulk soil in white clover-only, a grass-clover mixture, and grass-only plots. Differences between FA-$\delta^{13}C$ of Collembola groups and the FA-$\delta^{13}C$ signatures of potential food sources (plant material and soil microorganisms) were calculated to explore trophic links in the three cropping combinations. Collembola showed difference in FA compositions between groups. We observed a lower amount of neutral lipid fatty acids (NLFAs) in *Isotoma* spp. compared to *L. cyaneus*. Poduromorpha showed a higher relative percentage of the NLFAs 16:1ω7 and 20:4 compared to *L. cyaneus*, and of 18:3 compared to *Isotoma* spp. The NLFA oleic/linoleic ratio (18:1ω9/18:2ω6,9) ratio was higher in Poduromorpha compared to *L. cyaneus* in all the treatments. Soil FA analysis highlighted a larger fungal/bacterial ratio in grass compared to clover plots, while in Collembola no differences in the FA pattern were detected in relation to the treatments. Nevertheless, results of $\delta^{13}C$ fractionation in Collembola disclosed differences in the food sources used depending on the crop composition. Apparently, *Isotoma* spp. and *L. cyaneus* fed on clover-related diet in clover plots and more on grass-related diet in mixture treatments while Poduromorpha fed more on a plant-related diet, such as leaf litter, in the mixture treatment. Our results suggest differentiation in Collembola feeding strategy and in the diet in relation to food availability and palatability.

1. Introduction

Soil mesofauna promotes degradation processes by grazing on primary decomposers and redistributing carbon through the soil profile. In natural ecosystems, soil faunal activity may contribute directly to the decomposition processes by 5–10%, but may indirectly be responsible for >25% (Seastedt, 1984). Soil mesofauna is a key component of the food web and an important determinant for energy and carbon flows through terrestrial systems, and therefore for soil productivity (Van Eekeren et al., 2009). Grassland management directly and indirectly influences the soil biota and their functions (Bardgett, 2005). In order to develop and optimize sustainable grassland systems, insight is needed into how grassland management influences the soil quality in general, and soil biota in particular (Van Eekeren et al., 2009). Changes in land use and vegetation dynamics of grassland systems may cause considerable decrease or increase of carbon fluxes (Post and Kwon, 2000) and it has been suggested that trophic links and carbon fluxes through the

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food web vary with crop plant and farming system (Haubert et al., 2009). The introduction of white clover in grassland is a common practice to improve the quality of the pasture and to limit the use of nitrogen input. However, little is known about the extent to which the introduction of clover affects soil biota and the functioning of the soil-plant system under grassland (Van Eekeren et al., 2009). The diverse trophic links observed for Collembola to soil microbiota and plant material (Ngosong et al., 2011) support the trophic classification of Collembola as generalist feeders (Bardgett et al., 1993; Rusek, 1998; Johnson et al., 2005). Nevertheless, it has been shown that epedaphic and euedaphic collembolans have distinct differences in feeding strategy suggesting trophic niche differentiation varying with the specific soil habitat (Chahartaghi et al., 2005; Endlweber et al., 2009; Ngosong et al., 2011).

Recent studies have shown that $\delta^{13}C$ natural abundances of individual fatty acids (FAs) of potential prey (diet) and consumers may allow identifying carbon fluxes and trophic links in soil food webs. This technique is particularly helpful in identifying trophic links between microbial grazers (Chahartaghi et al., 2005; Chamberlain et al., 2005; Ruess et al., 2005b; Ngosong et al., 2011). Overall, combining fatty acid biomarkers with their isotopic ratios allows detailed insight into belowground trophic interactions. The fine resolution of compound-specific fatty acid analysis can be a valuable tool to assess effects of agricultural management on soil biota, especially when using relative biomarkers that can be synthesized by consumers or can occur in more than one diet; in the latter case of relative biomarkers, the stable isotope signal is particularly helpful (Ngosong et al., 2011).

In this study, we focused on identifying trophic relations in Collembola and how these relations may change depending on different crop combinations. We compared the fatty acid (FA) composition of Collembola, plants and bulk soil in grass-only, white clover-only and grass-clover mixture. Our objectives were (1) testing the effect of white clover and perennial grass on the collembolan feeding strategy and (2) identifying trophic links between microbes and Collembola in a clover, grass and grass-clover mixture.

We hypothesized that different grassland composition affect trophic relationships by shifting between bacterial versus fungal driven soil food webs. In grass, we hypothesized a food web with a relative dominance of fungi and a consequent shift in the source of energy in this direction.

2. Materials and methods

2.1. Field site

The experimental plots were located in the dairy crop rotation experiment of Aarhus University at Foulum (9°34′ E, 56°29′ N), with mean annual rainfall of 770 mm and mean annual temperature of 7.7 °C. Since 1987 the site has had intensive dairy farming with grassland-arable crop rotations (Eriksen et al., 1999, 2004). The soil is classified as a typical hapludult with 6.4% clay, 8.5% silt, 44% fine sand, 39% coarse sand and 1.6% carbon. The experimental design involved three treatments: grass-only (G), clover-only (C) and a mixture of clover and grass (CG) in plots of 3 × 18 m in a randomized block design with four replicates. Each plot was sown following spring ploughing in 2011 with either ryegrass (*Lolium perenne* L., 28 kg ha^{-1}), white clover (*Trifolium repens* L., 6 kg ha^{-1}), or with a mixture of the two (4 kg ha^{-1} white clover and 24 kg ha^{-1} ryegrass).

2.2. Sampling

The soil sampling took place in the last week of September 2011. All the samples were collected in the centre of the plots, at least one metre from the border between plots, to avoid border effects. In order to analyse fatty acid composition of soil microorganisms, three soil cores (5 cm diam. × 6 cm depth) were taken in each plot and immediately divided into layers of 3 cm thickness. The samples were stored at −18 °C in plastic bags until fatty acid extraction. Leaves were randomly collected from four plants in each plot of the experimental field and stored at 5 °C. The day after they were washed with demineralized water, frozen, freeze dried and crumbled with scissors. Samples were stored at −18 °C until further analyses.

Collembola were extracted from two soil blocks of 20 × 20 cm (12 cm depth) per plot using a modified Berlese method by heating the soil with 60 W light bulbs. The three most dominant Collembola taxa, i.e. *Isotoma* spp. (hemi-epedaphic; represented by *Isotoma anglicana* and *Isotoma viridis*), *Lepidocyrtus cyaneus* (hemi-epedaphic) and Poduromorpha (hemiedaphic; mostly *Brachystomella parvula* – Brachystomellidae – and *Ceratophysella succinea* – Hypogastruidae–), were collected in trays with plaster of Paris (plaster mixed with activated charcoal, w:w = 8:1). Euedaphic species were not found in sufficient numbers to be included in the study. The three groups of Collembola were hand-picked daily from the plaster and frozen at −18 °C in glass tubes. The extraction and collection of Collembola was conducted during a period of three days to achieve enough biomass for FA extraction. Afterwards they were grouped in tin capsules, freeze dried for 24 h, weighed and stored at −18 °C until FA extraction and isotopic analyses.

2.3. Fatty acid extraction

A sufficient amount of soil (2.0–2.5 g fw), leaves (2–3 mg dw) and Collembola (0.3–0.5 mg dw) were transferred to glass test tubes and extracted with a one-phase mixture of chloroform, methanol and buffer (1:2:0.8 v/v/v) as described by Bayley et al. (2001). The lipid extracts were dissolved in 100 μl chloroform and fractionated on pre-packed columns with 100 mg silic acid (Bond Elut Extraction Cartridges, Varian US). Neutral lipids were eluted with 1.5 ml chloroform, and lipids of intermediate polarity were eluted with 6 ml acetone (but discarded). Finally, polar lipids (mainly phospholipids) were eluted with 1.5 ml methanol. Neutral lipid fatty acids (NLFAs) and phospholipid fatty acids (PLFAs) were methylated to fatty acid methyl esters (FAMEs) as previously described (Nielsen and Petersen, 2000). A Shimadzu GCMS-QP2010 with autosampler was used to perform the analysis of NLFA and PLFA composition based on external standards (Supelco 37 component FAME mix and Bacterial Fatty Acid methyl esters CP mix, Supelco, Bellefonte, PA, USA). The gas chromatographer was equipped with a Supelco Omegawax 320 capillary column (length 30 m, inner diameter 0.32 mm, film thickness of 0.25 μm; Sigma Aldrich, Stockholm, Sweden). The injection volume was 1.0 μl and the autosampler was operating in split mode with a split ratio of 10. Helium was used as carrier gas with a total flow of 22.7 ml min^{-1} and a column flow at 1.79 ml min^{-1}. The injection temperature was maintained at 220 °C and the oven was programmed as follows: initial temperature of 50 °C was held for 2 min, then increased to 240 °C with a rate of 20 °C min^{-1} and held for 5 min. The mass spectrometer was operated in electron ionization mode at 70 eV. The ion source temperature was 250 °C and the interface temperature 240 °C. Identification of individual fatty acids was based on the comparison of the obtained mass spectra to known FAME standards. The area of identified peaks was calculated using GCMS Solution software and the molar ratio between the different fatty acids calculated. Fatty acids were designated as *X*:*Y*ω*Z*, where *X* is the number of carbon atoms, *Y* the number of double bonds, and *Z* indicates the position of the first double bond from the methyl end of the molecule. We avoided indicating double bonds' positions when it was not possible to detect them with sufficient certainty.