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Dietary switching of collembola in grassland soil food webs

Natalia Ladygina [a,*], Tancredi Caruso [b], Katarina Hedlund [a]

[a] Department of Ecology, Lund University, Sölvegatan 37, SE-223 62 Lund, Sweden
[b] Department of Environmental Sciences, University of Siena, via Mattioli 4, 53100 Siena, Italy

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ABSTRACT

Soil food webs are characterised by complex direct and indirect effects among the organisms. Consumption of microorganisms by soil animals is considered as an important factor that contributes to the stability of communities, though cascading effects within the food web can be difficult to detect. In a greenhouse experiment, an addition of a high number the fungal feeding collembola *Folsomia quadrioculata* was applied to grassland soil food webs in monocultures of three plant species: *Plantago lanceolata* (forb), *Lotus corniculatus* (legume) and *Holcus lanatus* (grass). The abundance of microorganisms, determined as the abundances of phospholipid fatty acids (PLFAs) and the abundances of resident invertebrates, nematodes and collembolans, did not change due to the addition of *F. quadrioculata*. Trophic positions of collembolans were determined by analyses of natural abundances of ^{15}N stable isotopes. The use of food resources by microorganisms and collembolans was determined by ^{13}C analysis of microbial PLFAs and solid samples of collembolans. $\delta^{13}C$ values of the resident collembola *Folsomia fimetaria* were lower in the presence of *F. quadrioculata* than in the control food webs indicating a use of more depleted ^{13}C food resources by *F. fimetaria*. The $\delta^{15}N$ values of *F. fimetaria* did not change at the addition of *F. quadrioculata* thus no change of trophic levels was detected. The switch of *F. fimetaria* to a different food resource could be due to indirect interactions in the food web as the two collembolan species were positioned on different trophic positions, according to different $\delta^{15}N$ values.

1. Introduction

Soil communities have a high diversity of organisms compared to aboveground communities and are highly resilient to disturbance by the complexity of direct and indirect interactions (Wardle et al., 2004). However, the interactions between soil organisms and the processes that maintain high diversity and resilience of soil communities are still largely unknown (Wardle, 2006). Current knowledge of soil food web interactions is partly based on theoretical work that can predict stability or productivity of soil food webs (De Ruiter et al., 1995). The models consider consumption of microorganisms by soil invertebrates as one of the key factors that contribute to food web stability (Neutel et al., 2002, 2007).

Experiments that set out to determine effects of interaction in soil food webs have frequently failed to demonstrate, e.g., how microbial grazers can influence bacterial biomass in soil food webs (Brussaard et al., 1995; Mikola and Setala, 1998a,b). However, failure to detect changes in parts of the food web as, e.g., grazing on microorganisms may be due to indirect interactions that are manifested in other parts of the food web, and not only due to lack of direct responses.

To detect interactions in food webs Yodzis (1988) used so called press perturbation experiments that can determine direct trophic changes but also determine effects of indirect interactions in food webs. A press perturbation acts to population densities to a higher or lower level so that a persistent change or disturbance will influence the resident population or community (Yodzis, 1988). Addition of resources or a higher trophic level, as predator, to soil food webs can both increase microbial biomass and activity (Hedlund and Ohrn, 2000; Baudoin et al., 2003; Lenoir et al., 2007; Paterson et al., 2007). Changes in feeding behaviour or niche partitioning caused by changed resource use have been difficult to detect in natural soil communities as we lack knowledge and have so far not been able to detect the interacting population responses to perturbations on a high resolution.

Today, the analyses of stable isotopes of both soil animals and microorganisms can be used to address resource use and trophic positions of organisms in the soil food web (Ponsard and Arditi, 2000; Albers et al., 2006; Dijkstra et al., 2006). Naturally occurring C and N stable isotope ratios reflect the feeding history of the organism as stable isotopes participate in metabolic reactions at different rates, which lead to enrichment of the heavier isotope (Fry, 2006). The accumulation of the heavy stable isotope of carbon is lower than that of nitrogen and values of ^{13}C are often routed to the foraging animal and thus provide information on the isotope ratio of the food source (Ruess et al., 2005). The stable isotope of

* Corresponding author. Tel.: +46 46 2224450; fax: +46 46 224716.
E-mail addresses: natalia.ladygina@ekol.lu.se (N. Ladygina), tancredicaruso@unisi.it (T. Caruso), katarina.hedlund@ekol.lu.se (K. Hedlund).

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nitrogen can give information on the trophic positions of the organisms as it is accumulated in the organism (Minagawa and Wada, 1984; Ponsard and Arditi, 2000).

In the present study, we performed a press perturbation experiment by adding a microbial-feeding collembolan species to a natural soil food web and examined whether soil microorganisms and microbial-feeding invertebrates already present in the soil responded directly or indirectly to the perturbation. Collembolans are known to occupy different trophic positions depending on habitat and availability of resources (Chahartaghi et al., 2005). Consequently, interactions between collembolan species are expected to lead to changes in the community composition and possibly also changes in trophic positions of other invertebrates and microorganisms in the soil food web. In a mesocosm experiment, high densities of the microbial-feeding collembola *Folsomia quadrioculata* (Tullberg) were added to natural soil communities from a grassland. Three common grassland plant species, *Plantago lanceolata* L. (forb), *Lotus corniculatus* L. (legume) and *Holcus lanatus* L. (grass), were used in the mesocosms in order to test whether the food web responses would be general to different groups of plants. The abundance of collembolans and nematodes were determined to detect changes in population numbers at the press perturbation.

Microbial community composition and biomass were determined using the abundance of phospholipid fatty acids (PLFAs) (Zelles, 1999). Natural abundances of $\delta^{13}C$ and $\delta^{15}N$ of collembola and $\delta^{13}C$ of PLFAs of microorganisms were determined to detect shifts in the use of food resources by microorganisms and collembolans (i.e. changes in $\delta^{13}C$ values) and to identify the trophic positions of collembolans (i.e. changes in $\delta^{15}N$ values).

2. Material and methods

2.1. Experimental design

Soil was collected from a seminatural grassland in South Eastern part of the Netherlands (Van der Putten et al., 2000); the site was taken out of agricultural production (of maize) in 1995. The soil was a sandy loam and had on average 21.3 ± 0.5 g/kg of carbon, 1.3 ± 0.2 g/kg of nitrogen, 0.3 ± 0.02 g/kg of phosphorus, and a pH of 6.3 (Hedlund et al., 2003). Plant monocultures were established with three grassland species *P. lanceolata* (forb), *L. corniculatus* (legume) and *H. lanatus* (grass) that represented common species of the Dutch grassland site (Leps et al., 2001). Seeds were germinated in Petri dishes on a filter paper during one week and three seedlings were planted in 0.5 l pots (diameter 9 cm and height 9.2 cm) with 500 g of soil. The seeds were obtained from Appels Wilde Samen GmbH (Darmstadt, Germany). The pots were kept in a greenhouse with a natural photoperiod for June–July in Southern Sweden and average temperatures 22 °C at day and 18 °C at night. The plants were watered and each pot was adjusted to equal soil water content (20%) by weighing. A total of 30 pots were arranged according to a randomized block design containing five replicates of each plant species (plant factor) and the two treatments, addition of collembolans and control. The collembolan *F. quadrioculata* was sampled from a grassland soil in Lund, South Sweden, and 200 individuals were added to each of the 15 pots, three weeks after the seedlings had been planted.

2.2. Sampling

Eleven weeks after planting the seedlings, the soil of each pot was collected and separated according to the different types of analyses. For PLFA analysis 10 g of soil was stored at −20 °C until extraction (see below). Collembolans were extracted alive from 250 g of soil by a modified Macfadyen (1961) extractor. All collembolans were counted and identified to species or genus level, specimens were preserved in 70% ethanol before identification (Table S1), while those of the most abundant resident species (*Folsomia fimetaria*) were transferred to tin capsules and dried at 60 °C for 48 h. Thus, 15 tin capsules of *F. quadrioculata*, corresponding to five replicates for each plant species, and 30 tin capsules of *F. fimetaria*, including the addition and the control treatments, were used for stable isotopes analysis (see below). Each sample consisted of 1–120 specimens and was stored at −20 °C until analysis. Nematodes were extracted from the soil with a Baermann (1917) funnel technique by heating at 37 °C for 24 h. The number of collected nematodes was lower than the detection limit of the stable isotope analysis and was thus excluded from the analyses.

2.3. Lipid analysis and fatty acid signatures

Phospholipid fatty acids (PLFAs) were extracted from 3 g of fresh soil according to Frostegård et al. (1993). The lipids were separated into neutral lipids, glycolipids, and phospholipids on prepacked silica columns (100 mg of sorbent mass; Varian Medical Systems, Palo Alto, CA, USA) by eluation with 1.5 ml of chloroform, 6 ml of acetone, and 1.5 ml of methanol, respectively. The fatty acid residues in the neutral lipids and phospholipids were transformed into free fatty acid methyl esters and analyzed by gas chromatography (Hewlett–Packard, Palo Alto, CA, USA) using a 30 m × 0.25 mm fused silica capillary column (HP-5) with H_2 as the carrier gas (for details, see Hedlund, 2002). The fatty acids were identified from their retention times relative to that of a standard. A total of 27 PLFAs were identified and used as markers for microbial community composition analysis. The following PLFAs were used as indicators of bacterial biomass (Frostegård and Bååth, 1996): 15:0, i15:0, a15:0, i16:0, 16:1ω9, i17:0, a17:0, cy17:0, 18:1ω7, and cy19:0. Biomass of saprophytic fungi was indicated by the PLFA marker 18:2ω6,9 (Frostegård and Bååth, 1996), and arbuscular mycorrhizal (AM) by the fungal marker NLFA 16:1ω5 (Hedlund, 2002). Actinomycete biomass was indicated by the PLFA marker 10Me18:0 (Kroppenstedt, 1985). The nomenclature of the PLFAs follows that used by Tunlid and White (1992).

2.4. Stable isotope analyses

2.4.1. PLFAs

Due to a higher detection limit in the GC-Isotope Ratio Mass Spectrometer (IRMS) system some of the fatty acids identified in GC analysis were not detected. Thus, in the ^{13}C analysis i15:0, a15:0, i16:0, cy17:0, 18:1ω7, and cy19:0 PLFAs were used as bacterial markers while NLFA 16:1ω5 and PLFAs 18:2ω6,9 and 10Me18:0 were used as markers of AM fungi, saprophytic fungi and actinomycetes, respectively. The $^{13}C/^{12}C$ ratios of PLFAs were measured by GC–(CF)-IRMS using a Hewlett–Packard (HP) 6890 GC (Palo Alto, CA, USA) interfaced with a PDZ Europa Scientific Instruments (Crewe, UK) 20–20 Stable Isotope Analyzer. The samples were automatically injected with a CTC Combi PAL (Zwingen, Switzerland) at splitless mode and helium was used as the carrier gas. The GC was equipped with a 30 m × 0.25 mm fused silica capillary column (HP-5). Temperature programming was 80 °C for 1 min; 20 °C/min; 160 °C, followed by 3 °C/min to 270 °C and kept isothermal 10 min. After the eluation of the solvent peak, all compounds were diverted to a reaction furnace (oxidized copper wire at 860 °C). Combusted gases were dried in a Nafion filter and directed into the IRMS. The compounds were calibrated against reference CO_2 gas that was introduced directly to the ion source at the beginning and at the end of each chromatographic run.

2.4.2. $\delta^{15}N$ and ^{13}C analyses of solid samples

Samples of collembolans were sealed in ultra-clean tin capsules and combusted in an Automatic Nitrogen and Carbon Analyzer with