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Does introduction of clover in an agricultural grassland affect the food base and functional diversity of Collembola?

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Introduction of legumes (i.e. white clover) in agricultural grasslands is a common practice to improve yields, but how this affects soil fauna populations, particularly mesofauna, is still poorly understood. We investigated taxonomical and functional differences of Collembola communities between plots with either perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.) or a mixture of both in a Danish agricultural grassland 6 and 14 months after establishing the leys (September and May, respectively). Diet preferences were investigated via stable isotope analyses (SIA) of carbon (^{13}C) and nitrogen (^{15}N). Collembolan abundance data were used to analyse morphological and ecological traits of the collected taxa and calculate functional diversity indices. Our stable isotope results show that root-derived resources made larger contributions to epedaphic and hemiedaphic species in the white clover than ryegrass plots. Changes in taxa specific density and traits distribution as a response to the C:N ratio of plant material, suggest that plant material quality was the main factor affecting the collembolan community, especially when comparing the two sampling occasions. Functional richness decreased under conditions of low quality material. In contrast to our hypothesis, population densities did not increase under mixture treatment and functional richness decreased. Our results suggest that habitat changes, via different plant composition, can affect some functional groups, having in turn effects on the functional diversity of the community.

1. Introduction

Collembola, or springtails, are a key group of microarthropods within the soil food web that feed on soil microbiota with derived effects on soil nutrients dynamics (Rusek, 1998). Collembola form feeding guilds of microbial feeders (Bardgett, 1998; Rusek, 1998) or generalist feeders foraging on microbiota, plant roots and nutrient rich detrital matter (Hopkin, 1997). Our understanding of collembolan feeding habits at the species or functional group level is steadily increasing (Chamberlain et al., 2006; Ngosong et al., 2011; Potapov et al., 2016a; Ruess et al., 2007; Sechi et al., 2014), but several gaps still exist. It is known that Collembola are closely associated with the rhizosphere food web being nutritionally supported by root-derived resources (Endlweber et al., 2009; Larsen et al., 2007; Sabais et al., 2012 Larsen et al., 2016b), but the path through which they obtain these resources is not yet clear. While euedaphic and hemiedaphic species have been shown to incorporate recent photosynthate carbon (C) from crops (Larsen et al., 2007; Ostle et al., 2007), Potapov et al. (2016b) recently demonstrated that only a fraction of these plant derived resources derives from direct foraging on roots. Hence, Collembola are influenced by plant-related changes that affect availability, quality and palatability of microbial derived food sources. Introduction of legumes in agricultural grasslands – a practice for reducing the dependence on amended nitrogen (N) fertilizers – can constitute a change in the soil habitat, possibly having consequences for the soil biota. Some studies have been carried out testing the effects of plant diversity (Sabais et al., 2011; Salamon et al., 2004) and

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presence of legumes on soil fauna (Birkhofer et al., 2011; Larsen et al., 2016a; Mulder et al., 2002; Schon et al., 2011; van Eekeren et al., 2009), but the effects of these crop types on the community composition of Collembola are complex and have not yet been resolved (Eisenhauer et al., 2011; Kooistra, 1964; Salamon et al., 2004; Sechi et al., 2014).

In Denmark more than 500.000 ha (20%) of the agricultural land is grassland and 60% of these grasslands are in rotation with cultivation every 2–4 years (Pedersen and Pedersen, 2013). These crop rotations represent a crucial stage in building up soil fertility for the next crops. While increased plant diversity in production grasslands increases organic matter and microbial biomass, legumes are crucial for increasing soil N, especially for soils poor in nutrients (Küchenmeister et al., 2012; Nyfeler et al., 2011). Perennial ryegrass and white clover, commonly used in combination in production grasslands, have distinct characteristics. Ryegrass has a much denser root system, which in turn positively affects the microbial biomass (van Eekeren et al., 2009), while white clover may have a negative effect on fungal biomass, suppressed by N in root exudates or litter (de Vries et al., 2006). Therefore, ryegrass generally increases microbial biomass and fungal:bacteria ratio (see also Sechi et al., 2014), more than white clover, which instead tends to support a more bacterial-dominated food channel. Taken together, these distinct characteristics are likely to affect the food base for Collembola. In a study that took place in the same experimental field site as ours, Sechi et al. (2014) found that Collembola living in ryegrass plots obtained most of their C from fungal-rather than from plant-derived sources, and vice versa for Collembola living in white clover plots. The authors also found in mixed ryegrass-clover plots that Collembola obtained relatively more C from grass-than clover-derived resources (Sechi et al., 2014).

Our study investigated the influence of ryegrass, white clover and a mixture of both crops on the diversity of a collembolan community. We used a Danish agricultural grassland as setting for the experiment, and we studied the community in two different seasons, autumn and spring. In addition to measuring taxonomic diversity, we characterized the collembolan community via a trait-based approach. The use of traits, which first became popular in plant ecology and later transferred to animal ecology, consists in characterizing a community based on morphological, physiological or phenological traits which impact species fitness via their effects on growth, reproduction and survival (functional traits) (Violle et al., 2007). We investigated the response of the community to the different environmental conditions, by selecting a set of functional response traits. Response traits are a certain type of functional traits able to capture the different characteristics of an organism by determining its response to environmental pressures, and are considered to reflect variation in environmental conditions (Lavorel et al., 1997, 2013; Mulder et al., 2012). Functional traits can be used to calculate the functional diversity of a given community, which has been defined by Díaz and Cabido (2001) as "the value and range of functional traits of the organisms present in a given ecosystem". As demonstrated in recent ecological research with Collembola (e.g. Makkonen et al., 2011; Martins da Silva et al., 2016; Salmon and Ponge, 2012; Salmon et al., 2014; Santorufo et al., 2015), the study of functional traits distribution allows to identify the response of the community to environmental changes (e.g. land use, soil properties, temperature). Thus, we used a traits-based approach to disentangle the influence of different plant crops on the functioning of the collembolan community. Trophic interactions in the community and C and N pathways were also studied by analysing natural abundances of C and N stable isotopes (^{13}C and ^{15}N) in Collembola, soil and plants, for the purpose of identifying Collembola possible food sources. Both isotope species are ideally suited for studying dietary contributions from clover and ryegrass to Collembola, because the two crops have significantly different ^{13}C and ^{15}N values (Larsen et al., 2016a).

We hypothesized that (i) Collembola would be trophically associated with the respective crops, i.e. the isotope values of Collembola would resemble those of either white clover or ryegrass, where (ii) we expect to find more bacterial feeders, such as e.g. *Brachystomella parvula* (Schaeffer, 1896) (Adams and Salmon, 1972), in the former and more fungivorous species, such as e.g. *Lepidocyrtus cyaneus* Tullberg, 1871 (Berg et al., 2004; Sechi et al., 2014), in the latter. We also hypothesized that (iii) Collembola abundance and functional richness would be greatest in plots with mixed crops because mixtures of grasses and legumes have been shown to improve soil structure and fertility compared to soils with monocultures (e.g. Nyfeler et al., 2011). Finally, because in the late season (September) senescent plants provide a pulse of easily decomposable C to support microbial growth, difference in belowground nutrients and exudates inputs will be more evident than in the early season (May) (Bardgett et al., 2005). Therefore, we hypothesized that (iv) differences in collembolan population densities/traits between ryegrass and white clover would be greater in September than in May.

2. Methods

2.1. Experimental setting and sampling

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.

In spring 2011, we established leys with ryegrass (*Lolium perenne* L., 28 kg ha^{-1}), white clover (*Trifolium repens* L., 6 kg ha^{-1}) or a mixture of the two species (4 kg ha^{-1} white clover and 24 kg ha^{-1} ryegrass) after ploughing. Each crop was established in 4 separate plots (each 3 × 18 m) according to a randomized block design, where each block in the field comprised the three different treatments (crops) randomly positioned next to each other, resulting in a total of 12 plots. We tested the effect of the treatments on the collembolan community taxonomic composition, traits distribution and functional diversity at two sampling occasions in different seasons (September 2011; May 2012), when we sampled soil, plant material and collembolans. In spring we also used the collected materials to analyse natural abundances of C and N stable isotopes (^{13}C and ^{15}N), as described below.

Soil core samples (Ø 6 cm; depth 5 cm) were taken from each plot (one soil core per plot in September and three soil cores per plot in May) and used to extract Collembola by a modified MacFayden high gradient extractor (MacFadyen, 1961). Sampling was more extensive in May than September because the sampling campaign in September 2011 was running parallel to other work-demanding project activities at the same location (Sechi et al., 2014; Larsen et al., 2016a). This sampling design allowed us to carry out a more thorough comparison of the effect of crop type on the community composition of Collembola for 14 rather than 6 months after establishing the leys. Specimens were collected in benzoic acid and later transferred to glycerol for counting and long-term storage. Those samples were used for identification and counting. Specimens were identified at species level when possible, or else they were identified at higher taxonomic levels, using the keys of Fjellberg (1998,2007).