Soil Biology and Biochemistry 116 (2018) 245–252

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Seasonal and annual variation in fungal communities associated with epigeic springtails (Collembola spp.) in boreal forests

Sten Anslan[a,*], Mohammad Bahram[a,b], Leho Tedersoo[c]

[a] Institute of Ecology and Earth Sciences, University of Tartu, 14A Ravila, 50411 Tartu, Estonia
[b] Department of Organismal Biology, Evolutionary Biology Centre, Uppsala University, SE 75236 Uppsala, Sweden
[c] Natural History Museum, University of Tartu, 14A Ravila, 50411 Tartu, Estonia

ARTICLE INFO

Keywords:
Collembola
High-throughput sequencing
Fungal community
Arthropod-microbial associations
Seasonality
Temporal changes

ABSTRACT

Soil fauna mediate nutrient cycling through engineering physical properties and altering microbial communities in soil. Collembola is one of the most abundant groups of soil fauna, which regulates microbial communities by consumption and dispersal. The spatial structure of associations between Collembola and soil microbes have been described in several studies, but temporal variation of these associations remains unclear. Using high-throughput sequencing, we studied the fungal communities on Collembola (*Entomobrya nivalis*, *Orchesella flavescens*, *Pogonognathellus longicornis*) body surface, gut and their immediate habitat (topsoil samples) in four seasons across three years. The soil samples were characterized by fairly uniform relative abundance of saprotrophic and mycorrhizal fungi, whereas collembolans were associated mostly with saprotrophs. The structure of fungal communities from all substrate types exhibited comparable patterns of temporal distance decay of similarity. Unlike in soil, fungal richness and composition in Collembola body and gut samples exhibited seasonal and annual variation, with a significant interaction term, indicating low predictability. These results reflect spatial and temporal plasticity of the fungal communities associated with epigeic Collembola, indicating the high adaptability of collembolans to available conditions. We found that the Collembola associations with fungi (including diet) did not vary among the studied epigeic Collembola species. The detected high diversity of fungi associated with Collembola suggests that dispersal by arthropod vectors may represent a powerful alternative to aerial dispersal of fungal propagules.

1. Introduction

Soil microbes play a fundamental role in terrestrial ecosystem processes such as decomposition and nutrient cycling, acting as important regulators of plant productivity (van der Heijden et al., 2008). These microbial effects are often manipulated by soil fauna, especially microbivores such as soil arthropods. Collectively, soil microbial and faunal communities and their interactions mediate a variety of processes including soil formation, microclimate regulation as well as control and spread of diseases (Friberg et al., 2005; Lavelle et al., 2006; Crowther et al., 2015). Therefore, soil faunal and microbial communities make a strong contribution to soil health that promotes soil fertility and hence ecosystem productivity and economy (Lal, 2016).

Springtails (Collembola) are one of the most abundant and ubiquitous group of soil mesofauna. Through their interactions with microbial communities, primarily through direct predator-prey interactions, Collembola may affect the dynamics of nutrient cycling (McGonigle, 1995). In particular, fungi are one of the main sources of collembolans' diet. Based on isotopic signatures, it has been suggested that Collembola species prefer saprotrophic (SAP) fungi (Potapov and Tiunov, 2016). However, as Collembola species occupy various microhabitats and have distinct trophic niches (Ferlian et al., 2015; Potapov et al., 2016; Malcicka et al., 2017), they may exhibit species-specific impact on soil microbial communities (Tordoff et al., 2008; Fujii et al., 2016). Besides active feeding, Collembola may affect the structure of microbial communities through active dispersal by carrying spores and hyphal fragments on the body surfaces or stomach. Especially for fungi forming corticioid fruit-bodies among litter, soil fauna may be key agents for spore release and dispersal (Lilleskov and Bruns, 2005). Unlike wind, soil arthropods and rodents disperse spores into direct contact with roots, which may be especially beneficial for mycorrhizal fungi and perhaps root pathogens (Friberg et al., 2005; Frank et al., 2009).

Although several studies have addressed the spatial structure of Collembola-fungal associations (e.g. Visser et al., 1987; Greenslade et al., 2002), only a few have examined the temporal aspect of these interactions. Seasonal fluctuations in the fungal particle consumption

* Corresponding author.
E-mail address: sten.anslan@gmail.com (S. Anslan).

http://dx.doi.org/10.1016/j.soilbio.2017.10.021
Received 25 August 2017; Received in revised form 18 October 2017; Accepted 21 October 2017

by epigeic collembolans have been already noted by Anderson and Healey (1972). Using stable isotope analysis, Potapov et al. (2014) also demonstrated the seasonal variation in collembolans' diet. In contrast, more recent studies demonstrated that the diet of Collembola is independent from the seasonality-driven fungal resources and structure of microbial communities in bulk litter (Ferlian et al., 2015; Anslan et al., 2016). All these studies, however, have been performed within a single year, which disallows to distinguish seasonality from random temporal change.

Here we used high-throughput sequencing to determine fungal communities on the body surface and in gut contents of epigeic collembolans in four seasons across three years. To be able to understand any shifts in preferences for diet, we studied the local fungal communities by analyzing soil samples in each collection period. We hypothesized that 1) the richness and structure of fungal communities on the body surface reflects uniform seasonal changes across years; and 2) the Collembola-associated fungal community composition differs among Collembola species. We predicted that the fungal diet of Collembola consists of mainly saprotrophic rather than mycorrhizal fungi and that it is independent of seasonal and annual variation.

2. Methods

2.1. Sampling

The study sites included two Norway spruce (*Picea abies* (L.) H. Karst) dominated forests in Estonia (Ilmatsalu [IL] and Kardla [KA] sites as described in Anslan et al., 2016). Specimens of epigeic collembolans, *Entomobrya nivalis* Linnaeus 1758, *Orchesella flavescens* Bourlet 1839 and *Pogonognathellus longicornis* Tullberg 1871 were collected during three vegetation periods from May to September 2013–2015 from circular 2500 m^2 plots (collection dates are specified in Table S1). These species are abundant on the forest floor of mixed and coniferous forests in North Europe throughout the vegetation period. The relatively large size of these collembolans (*E. nivalis*: up to 2 mm, *O. flavescens*: up to 5 mm, *P. longicornis*: up to 6 mm) allowed the individual specimen approach for separate analysis of body surface and gut content. At least four specimens of each Collembola species were collected during each sampling period with a single-animal aspirator. Specimens were instantly treated with chloroform to prevent their excretion of gut contents. To characterize the local fungal communities, a total of 22 composite soil samples were collected and processed (following Tedersoo et al., 2014) at both sites during each collection period (except for June of 2013; Table S1). These composite samples consisted of 40 subsamples and covered the 5 cm of topsoil that includes degraded litter, humus and uppermost mineral soil (A horizon).

2.2. Molecular analyses

Collembolans were prepared for molecular analysis on the day of collection. The body surface (particles associated with appendages, such as legs, antennae and some body hairs or scales) and gut content samples were prepared in a biosafety cabinet with laminar flow as described in Anslan et al. (2016). Soil samples were air-dried and homogenized, followed by DNA extraction from 2 g of fine soil powder using Power-Soil®DNA Isolation Kit (MoBio, Carlsbad, CA, USA) as instructed by the manufacturer. PCR and Illumina MiSeq (2 × 300 bp) sequencing were performed as described in Anslan et al. (2016) using the forward primer gITS7 (Ihrmark et al., 2012; excludes animals) and reverse primer ITS4ngs (Tedersoo et al., 2014), which was tagged with one of the 10–11 base molecular identifiers (MIDs; Table S2). Sequencing libraries were prepared separately for body, gut and soil samples with positive and negative controls throughout the experiment. The quantity and purity of DNA extracts ranged between 10.3 and 25.8 ng/μl and 1.8–1.9 (UV absorption ratios of 260/230 and 260/280 nm), respectively.

2.3. Bioinformatics

Bioinformatics for sequencing data were performed using PipeCraft analysis platform (Anslan et al., 2017). Raw paired-end Illumina data were merged and quality-filtered using vsearch (v1.11.1; Rognes et al., 2016) with expected errors = 1 and minimum overlap = 10. After assembling and quality filtering, the data were inspected with FastQC (Andrews, 2010) to confirm the inclusion of only high quality reads (quality scores across all bases > 30). Sequences were allocated to samples based on MIDs using mothur (v1.36.1; Schloss et al., 2009). Potential chimeras were detected and removed using vsearch *de novo* and reference-based (UNITE v7.0 reference dataset; Abarenkov et al., 2010) chimera filtering algorithms. For clustering purposes, reads were processed using ITS Extractor (v1.0.11; Bengtsson-Palme et al., 2013) to obtain full-length fungal ITS2 without flanking gene fragments. Before the clustering step, ITS2 reads were subjected to error-correction (denoising) using UNOISE2 (Edgar, 2016). Denoised sequences were assigned to operational taxonomic units (OTUs) using 97% similarity threshold. OTUs were generated using multilevel clustering with first CD-HIT (Fu et al., 2012) and then SCATA (scata.mykopat.slu.se; usearch clustering engine). Representative sequences for taxonomy annotation were chosen using mothur abundance method. Blastn searches (Camacho et al., 2009) were performed for the representative sequence of each non-singleton OTU against both INSD and UNITE database sequences. OTUs were further checked and filtered based on blastn search values as well as positive and negative controls to remove contaminants, non-fungal OTUs, potential artefacts and tag-switching errors (Nguyen et al., 2015; Schnell et al., 2015). OTUs with 75, 80, 85, 90, 95 and 97% similarity were considered to represent phylum, class, order, family, genus and species level, respectively (Tedersoo et al., 2014). Based on taxonomic assignments, fungal OTUs were parsed into trophic guilds according to FUNGuild (Nguyen et al., 2016). For the statistical analysis we included clusters that contained at least 10 sequences across the whole data set. Sequencing data sets have been deposited in the Sequence Read Archive (SRA) under submission SUB2903651 (BioProject: PRJNA399829).

2.4. Statistical analysis

To determine whether the dissimilarity of fungal community composition (FCC) was related to temporal distance, we performed separate Mantel tests (with 4999 permutations) for both sites as implemented in vegan package (Oksanen et al., 2015) of R v.3.2.2 (R Core R-Core-Team, 2015). For this purpose, samples from body surface and guts were pooled by sampling time to match soil data that comprised a single composite sample per sampling period in both sites (Table S1). The Hellinger-transformed OTU matrix and sampling dates were converted into Bray-Curtis dissimilarity and Euclidean distance matrices, respectively. Mantel test was also used for analysis of dissimilarity correlations between FCC in body, gut and soil samples. To determine the temporal autocorrelation of fungal richness data, we calculated the autocorrelation coefficient using Moran's I test as implemented in the ape package of R (Paradis et al., 2004).

The effect of season (fixed factor with levels May, June, August and September), year (fixed factor with levels 2013, 2014, 2015), Collembola species (fixed factor with levels *E. nivalis*, *O. flavescens*, *P. longicornis*) and site (random factor with levels IL and KA) on log-transformed fungal OTU richness were tested using factorial ANCOVA (Type III SS) followed by Tukey HSD tests. To minimize the effect of differential sequencing depth across samples, the number of sequences (log-transformed) per sample was included in the analysis as a covariate. To detect the differences in FCC, we performed PERMANOVA analysis (Anderson, 2005) on Bray-Curtis similarity matrix based on Hellinger transformed data. Number of sequences (log-transformed) per sample and temporal distance (days) were accounted as covariates in the PERMANOVA analysis with 4999 permutations (Type I SS). As