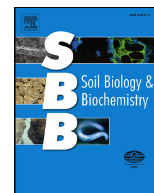
Soil Biology and Biochemistry 125 (2018) 37–43

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

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



A DNA metabarcoding approach to characterize soil arthropod communities

Angela M. Oliverio[a,b], Huijie Gan[c], Kyle Wickings[c], Noah Fierer[a,b,*]

[a] Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO, 80309, United States
[b] Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO, 80309, United States
[c] Department of Entomology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY, 14456, United States

ARTICLE INFO

Keywords:
Soil DNA
Soil arthropods
Morphological identification
Environmental DNA sequencing
CO1 metabarcoding

ABSTRACT

Belowground arthropod communities are diverse and our ability to characterize them remains logistically difficult and time consuming. Molecular metabarcoding techniques are routinely used to assess the diversity of both microbial and some 'macrobial' taxa across a range of environments, but the use of such techniques for characterizing soil arthropod diversity remains limited. Here we used three approaches to profile soil arthropod communities at the family level of resolution across 10 distinct sites via morphological identification, metabarcoding of DNA from the extracted arthropods, and metabarcoding directly from bulk soils. Although the three methods differed to some degree in their ability to detect some individual taxa, we found that all three methods yielded well-correlated site-level estimates of diversity (Spearman's $\rho \geq 0.63$ with $P < 0.05$ for all correlations) and overall arthropod community composition (Mantel $\rho \geq 0.45$ with $P < 0.05$). Of particular note is that DNA extracted directly from bulk soil yielded results comparable to analyses of DNA from extracted arthropods. Thus, DNA metabarcoding of bulk soil will likely be a useful tool for those researchers looking to incorporate multi-domain comparisons or for studies that require rapid assessments of arthropod diversity across a large number of soil samples.

1. Introduction

Soil fauna, including nematodes, annelids, and arthropods (e.g. mites, springtails, centipedes) are key contributors to the functioning of ecosystems with the biomass of soil animals typically representing 40–80% of the total animal biomass found in ecosystems (both aboveground and belowground combined; Fierer et al., 2009). The arthropods are particularly ubiquitous and diverse in soil: a 1 m square plot may host hundreds to thousands of soil arthropod species (Schaefer and Schauermann, 1990). However much of the faunal diversity in soil remains poorly described, making the quantification of faunal diversity and identification of specific taxa difficult (Wall et al., 2005).

Progress in soil arthropod research has been constrained by methodological challenges (André et al., 2002). The morphological identification of soil arthropods is time consuming (Bienert et al., 2012; Querner and Bruckner, 2010) and usually requires significant taxonomic expertise as the diversity is often difficult to characterize due to morphological ambiguity and cryptic diversity (e.g. André et al., 2001; Smith et al., 2008; Yu et al., 2012). Further, there are known biases in the methods commonly used for extraction of arthropod communities (André et al., 2002; Edwards, 1991) and extraction efficiency varies for different taxa across soil types and under different extraction conditions (André et al., 2002; Macfadyen, 1962). These challenges can constrain efforts to characterize soil arthropod communities, particularly when seeking to analyze a larger number of soils in a relatively short period of time or when taxonomic expertise is lacking (Querner and Bruckner, 2010). For example, rapid surveys or environmental assessments, such as the biomonitoring of pests or invasive species; or large-scale studies investigating how soil arthropod communities are distributed in time and space and how they respond to global change factors are often difficult to execute due to these methodological challenges.

While metabarcoding techniques have been widely applied to investigate soil microbial communities (e.g. Bates et al., 2013; Delgado-Baquerizo et al., 2018; Tedersoo et al., 2014) the use of these techniques to survey soil arthropod communities remains limited. While previous studies have successfully used metabarcoding techniques to assess the diversity of specific lineages such as springtails (Hogg and Hebert, 2004), nematodes (Griffiths et al., 2006; Read et al., 2006; Waite et al., 2003), and earthworms (Bienert et al., 2012; Porco et al., 2013), relatively few studies have tested the fidelity of metabarcoding of soil arthropods for community-scale analyses. Soil nematode communities were recently profiled across a range of soil types (Griffiths et al., 2018; Treonis et al., 2018), although both studies noted significant discrepancies between the molecular and morphology-based

* Corresponding author. Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO, 80309, United States.
E-mail address: noah.fierer@colorado.edu (N. Fierer).

https://doi.org/10.1016/j.soilbio.2018.06.026
Received 22 March 2018; Received in revised form 25 June 2018; Accepted 27 June 2018
0038-0717/ © 2018 Elsevier Ltd. All rights reserved.

assessments which require further standardization. Although a handful of studies have sequenced regions of the 18S rRNA gene from extracted soil DNA to characterize soil fauna (Wu et al., 2011), the evaluations of these molecular-based methods have found significant differences between the molecular and morphological profiles (Hamilton et al., 2009; Wu et al., 2009). However, DNA metabarcoding has been well-validated for characterizing aboveground arthropod communities (Madden et al., 2016), including arthropods that accumulate in pitfall traps (Ji et al., 2013; Yang et al., 2014).

In this study, we used three approaches to characterize and compare soil arthropod communities at the order and family level: morphological identification from Berlese funnel extraction, metabarcoding of DNA from extracted arthropods, and metabarcoding of DNA from bulk soil. We chose to identify invertebrates to the order and family levels because these taxonomic levels represent a compromise between taxonomic detail and known ecological function and are commonly used for bioindicator and other soil ecological studies (Gan and Wickings, 2017; Gergócs and Hufnagel, 2009; Gulvik, 2007; Ruf, 1998; Socarrás, 2013; Wickings and Grandy, 2013). Further, many soil arthropods can only be classified with confidence to the family level of resolution using readily available taxonomic keys and minimal slide preparation for morphological identification.

We tested the efficacy of the three approaches across a range of soils from ten sites that varied with respect to vegetation type and management intensity. Our objectives were twofold. First, we wanted to compare a metabarcoding approach (high-throughput sequencing of a portion of the mitochondrial CO1 gene, a 'barcode' commonly used for characterization of arthropod diversity, Madden et al., 2016) versus a more traditional, morphology-based approach for quantifying the structure of soil arthropod communities. Second, we wanted to determine whether the metabarcoding approach can be used with bulk soil DNA, bypassing the need to first extract arthropods from soil using standard approaches (e.g. Berlese funnels) as this method is still time intensive and efforts to extract arthropods from soil can introduce significant biases. We addressed these objectives by comparing how the three approaches differed with respect to their estimates of standard ecological metrics: detection of specific arthropod families, community richness, and overall arthropod community composition.

2. Materials and methods

2.1. Site description and soil sampling

We collected surface (0–5 cm) soil samples from ten sites near Ithaca, New York. The ten sites sampled were diverse, spanning a range of vegetation types (grass, forest, crops) and soil management intensities (from sites with no management, including designated natural areas and state forests, to heavily managed sites, including apple orchards and golf courses). For detailed site information, see Supplementary Table 1.

At each site, we randomly selected four 1 m by 1 m plots at least 100 m apart. Four pairs of soil cores of 5 cm × 5 cm were collected from each plot after removing plant litter from the soil surface. Four soil cores from each plot (one core from each pair) were bulked, resulting in four composited soil samples per site for extraction of soil arthropods using Berlese funnels and subsequent morphological and DNA-based identification of the extracted arthropods. The remaining four soil cores from the same plot were combined for bulk soil DNA extraction. As such, a total of eight composited soil samples were collected from each site for comparative identification by morphology and CO1 metabarcoding: yielding a total of 80 soil samples collected for the study. All soil samples were transferred to the lab in coolers, with the soil samples collected for DNA extraction immediately frozen and stored at −20 °C prior to extraction. Importantly, we note that our goal was not to exhaustively census all arthropod taxa found in each of the 10 sites, rather we used these four composited samples per site to broadly survey the dominant arthropod communities from diverse habitats and evaluate different methodologies for characterizing these communities.

2.2. Soil extractions and identification by morphology

We compared arthropod community composition and diversity across the ten sites using three methods: morphological identification of funnel-extracted arthropods, CO1 gene sequencing of funnel-extracted arthropods, and CO1 sequencing of DNA extracted directly from bulk soil samples. We refer to these three methods in brief as 'morph ID', 'arthropod DNA', and 'bulk soil DNA', respectively. To extract arthropods for morphological identification, bulked soil from each plot was placed on Berlese funnels for heat extraction, with the temperature directly above the soil sample starting at 30 °C and increased in 5 °C increments daily reaching a final temperature of 50 °C. Organisms that passed through the funnels were collected in 95% ethanol in plastic cups which were placed directly underneath the funnels. Arthropods were counted under a dissecting microscope at 50× magnification and identified to family level. Slide mounting and examination under a compound microscope at 200x magnification was necessary for accurate identification of some organisms. The following keys were used: Krantz and Walter (2009) for mites; Christiansen and Janssens (2010) for springtails; and Triplehorn and Johnson (2005) for insects; Zhang (1998) for myriadpods; Buddle (2010) for pseudoscorpions; and Kaston (1978) for Araneae. Around 1–2% of the individuals collected were not identifiable due to damage in the key structure of the specimens or small body size (< 0.1 mm). After sorting, the specimens were stored in 95% ethanol before DNA extraction, which was conducted within 4 months of sample collection.

2.3. Identification by CO1 barcodes

To characterize the soil arthropod communities via barcode sequencing, we first extracted DNA from bulk soils. We homogenized 10 g of soil with liquid N_2 and extracted DNA from 0.2 g duplicate subsamples from each of the 40 samples as per Oliverio et al. (2017), with an additional subsample extracted from 10 of the soil samples (one from each site), yielding 90 soil DNA extractions in total. We also pooled all of the preserved arthropods (including any soil falling through the Berlese funnel during extraction) that were used for the morphological identifications by plot (n = 40) and then extracted DNA using the PowerSoil DNA extraction kit (MoBio Laboratories Inc.). We ran multiple extraction blanks to check for potential contamination.

We then amplified a portion of the CO1 gene with arthropod-specific primers as per Madden et al., 2016. Briefly, we PCR amplified a ~158 bp region of the mitochondrial cytochrome *c* oxidase subunit I gene with arthropod-specific primers (Zeale et al., 2011). The primers were modified to permit multiplex sequencing on the Illumina MiSeq platform with the appropriate Illumina adapters included on both primers with the reverse primers also having an error-correcting 12-base pair barcode unique to each sample to permit multiplexed sequencing (Hamady et al., 2008). Duplicate PCR reactions were conducted on all extracted DNA samples along with multiple 'no template' negative controls per plate to check for contamination. Amplicon concentrations were standardized using SequalPrep Normalization plate kits (Invitrogen) and then sequenced on the Illumina MiSeq platform at the University of Colorado Next Generation Sequencing Facility with the 2 × 150 base pair (bp) paired-end chemistry.

We generated approximately 8 million sequence reads across 170 samples including 80 from extracted invertebrates and 90 from bulk soil. Sequences were demultiplexed with the custom Python script 'prep_fastq_for_uparse_paired.py' (Leff, 2016) and then sequences were merged, quality filtered, and clustered into phylotypes with UPARSE (Edgar, 2013). We merged forward and reverse reads, retaining 3.7 million reads (47%). Next, reads were discarded if the paired end reads did not have a minimum overlap of at least 16 bp or if the merged reads

Download English Version:

https://daneshyari.com/en/article/8362416

Download Persian Version:

https://daneshyari.com/article/8362416

Daneshyari.com