The influence of agricultural tillage practices on soil biodiversity: Soil metagenomic methods, arthropod community

Jeff Strohm, Robert Hanner, Richard J Heck

Abstract

This protocol provides the sampling and molecular biology lab methods used to prepare arthropod amplicons for MiSeq sequencing.

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Protocol

Sample collection

Step 1.
Bulk soil samples were collected to a depth of 15cm using a trowel and placed in 2L plastic bags.

Soil processing

Step 2.
Samples were placed in BioQuip Collapsible Berlese funnels with the only modification being an addition of a two-ply layer of cheese cloth to the metal grate.

REAGENTS
Collapsible Berlese Funnel 2832 by BioQuip

Soil processing

Step 3.
60W light bulbs were used to dry the soil samples until they felt completely desiccated (1 week).

Soil processing

Step 4.
Emerging arthropods were collected in 70% ethanol.

Soil processing

Step 5.
After fixing, 100% ethanol was added to increase the concentration to 95% and the samples were stored at -20 ºC. Since only eight Berlese funnels were available, half of the bulk soil samples were stored at 4 ºC for 1 week.

Specimen sorting

Step 6.
Arthropods were visually sorted under a dissecting microscope and identified to the taxonomic levels of class or order

Specimen sorting

Step 7.
Larger arthropods such as Coleoptera or Diplopoda were sub-sampled by removing either a leg or a few body segments. For smaller Arthropods such as mites and Collembola, entire organisms were used.

Specimen sorting

Step 8.
All organisms from a sample were placed together in a 1.5mL Eppendorf tube in 95% ethanol and stored at -20 ºC until DNA extraction.

Arthropod DNA Extraction

Step 9.
Ethanol was removed from all tubes using a heated SpeedVac centrifugal evaporator.

Arthropod DNA Extraction

Step 10.
The glass fiber insect DNA extraction protocol developed for the Canadian Center for DNA Barcoding was used (supplementary methods)

PCR inhibitor removal

Step 11.
The MO BIO PowerMax Soil DNA isolation kit was chosen as it contains two inhibitor precipitation reagents and was used for the microbial soil samples from the study

REAGENTS

PowerMax® Soil DNA Isolation Kit 12988-10 by Mobio

PCR inhibitor removal

Step 12.
All reagent volumes were scaled down by a factor of 0.03 so the entire volume of DNA extract could be purified using Epoch Biolab spin columns and all centrifuge spin times and speeds were adopted from the bulk Arthropod DNA extraction protocols previously outlined

PCR inhibitor removal

Step 13.
All samples were eluted in 60µL of solution C6 (10mM Tris-HCL).

PCR

Step 14.
The high fidelity polymerase KAPA HiFi HotStart in the ReadyMix format was used.

REAGENTS

HotStart ReadyMix (KAPA HiFi PCR kit) KK2601 by Kapa Biosystems

PCR

Step 15.
Annealing temperature gradient experiments were used to select 52 ºC, 55 ºC and 58 ºC as "low, medium and high" annealing temperatures

PCR

Step 16.
Reaction set-up and cycling conditions were identical to those used for microbial PCR with aside from the annealing temperatures and with 0.75µL primer cocktail (0.6µM) being used. As with microbial PCR, a negative control was set-up to monitor contamination.

PCR Purification

Step 17.

Aline PCRClean DX was used to purify all amplicons, a ratio of 0.55 (beads):1 (sample) was used.

REAGENTS

PCRClean DX C-1003-5 by Aline Biosciences

PCR Purification

Step 18.

An Invitrogen E-gel was used to confirm purification success.

PCR Purification

Step 19.

Amplicons were stored at 4 ºC for index PCR the following day.

Index PCR

Step 20.

A master mix tube was prepared for each index primer. Half the total volume of master mix was added to each reaction well from either master mix tube containing the forward or reverse index primer

Index PCR

Step 21.

For PCR setup and cycling conditions see supplementary methods

Purification

Step 22.

Aline PCRClean DX was used for purification, but this time, at the full concentration of 1.8X (beads): 1(sample).

REAGENTS

PCRClean DX C-1003-5 by Aline Biosciences

Purification

Step 23.

Amplicons were stored at 4 ºC to be quantified and normalized the following day.

Quantification and Normalization

Step 24.

An Invitrogen Q-Bit Fluorometer was used to measure all amplicon concentrations

Quantification and Normalization

Step 25.

All samples were normalized to 4nmol with the addition of 10mM Tris-HCL (pH 7.6).

Quantification and Normalization

Step 26.

Amplicons were then pooled and submitted to the Advanced Analysis Center Genomics Facility at The University of Guelph for Illumina MiSeq sequencing using the 250bp paired end read chemistry.

Sequencing

Step 27.

Amplicons were loaded into the MiSeq as a 6pM denatured library which was 15% PhiX.