Report STSM (Cost Action ES1406)

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STSM title: DNA metabarcoding and morphological identification of main soil fauna groups

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STSM dates: from 10.04-20.04.2017

Summary:

Working at this eleven-day research stay was separated into three main parts:

1. collecting of soil samples and extraction of soil fauna
2. morphological assessment and group sorting of soil fauna
3. DNA extraction from soil and soil fauna samples and PCRs of general and specific taxonomical genes

At the first day we took soil samples from four study sites (forest) near Othmarsingen/ Switzerland. From each site, ten soil cores and 1 litter sample were collected for the extraction of earthworms, microarthropods and also for DNA analysis. Soil cores and litter sample were brought to the Research Institute for Organic Agriculture (FiBL), Frick/Switzerland, where the soil fauna was extracted by a MacFadyen-installation for seven days. The earthworms were washed and kept in alcohol for a later on taxonomic determination.
In addition, we developed and tested a self-constructed Berlese funnel in the lab at the WSL Birmensdorf/Switzerland to extract microarthropods from some soil samples. Specifically, we tested different temperatures, mesh sizes and extraction times. These extracted soil organisms and also the extracted soil fauna from the FiBL were investigated with a binocular in the lab.
After extraction of the soil fauna we identified different soil fauna groups like springtails, mites, insects, spiders, ants, nematodes, enchytraeids and other organism groups according to their morphological characteristics. The different groups were separated into collection tubes with alcohol for further taxonomic determination and DNA analysis.

In the context of the ongoing MetaComp project at WSL (within the Keysom framework) comparing the meta-barcoding and morphological taxonomy of different groups of soil microarthropods, we tested different methods for DNA extraction from springtails and mites. The mites and springtails were first separated into different sub-groups, depending on equal morphological details (for control we described the mites and their morphological aspects and took pictures with an electronic binocular). Subsequently, individuals of each group were placed in eppendorf tubes containing sterile water and were mashed with a pestle before being frozen. This DNA extraction protocol worked for most of the springtails and mites tested, however, further development of the protocol need to be made to improve the extraction of a few recalcitrant

group of mites! In parallel, soil from the same site was extracted with a DNA extraction kit (PowerMax Soil DNA isolation kit, Qiagen). Afterwards, we quantified the DNA and performed the PCRs targeting general, mite- and collembolans-specific sequences. Although good amplifications were obtained with most of the primer tested, it is yet still not clear which primers are best to differentiate the different types of springtails and mites. Because of that we tested different primers. Amplified DNA (= amplicons) were subsequently inspected on agarose gel-electrophoresis. The DNA-sequencing will take place in the coming weeks.

This STSM was a very good possibility to learn from each other and to develop together a working-plan and a robust protocol for the processing of the samples of the MetaComb project. I particularly help to develop a sounded and well-established protocol for the soil fauna extraction and the sorting of the soil fauna into different groups. Because of the large amount of expected soil samples from different countries in this project it is advantageous to have a lot of experiences for dealing with the different soil organisms and to have an efficient and time-saving working protocol. In return, I got training in molecular biology including DNA extraction of soil and soil fauna, PCRs and gel electrophoresis. In summary, this STSM, although very short, was fruitful and productive, positive for both parties involved.

Cottbus, 28.04.2017 Cornelia Rißmann