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From Microscopy to Genomic Approach in Soil Biodiversity Assessment

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Abstract
Soil biota represents a major component of the earth's biodiversity and for over 200 years, the microscopy approach was the only way to explore it. In the last decade, the DNA-based technique has been adopted in soil ecology. Due to the rapid development of cutting-edge technology, the field is transitioning from barcoding individuals to metabarcoding communities. With the advent of next-generation sequencing and a rapid decline in sequencing cost, it has become feasible to assess soil biodiversity at species level. This review article summarizes current approaches in soil biodiversity research along with their advantages and disadvantages.

Introduction
Soil food webs are one of the most useful and challenging aspects of soil ecology. The networks of predator-prey interactions that occur in soil, provide an example of very complex adaptive systems. Relatively easy to sample, but difficult to describe and to model, soil food webs are nevertheless the focus of present and future practical and theoretical importance. The interactions between species on different trophic levels underlie the flow of energy and biomass in the ecosystem and provide information about species' responses to natural and human perturbations. Thus, understanding the principles of species' coexistence, ecology and mathematics of soil food webs means understanding the fate of ecosystem biodiversity in response to any kind of disturbance and their speed and time recovery following disturbance.

The impact of agricultural management on soil food web stability is huge in countries with a high density of population and less productive areas. Therefore, it is important to speed up the development of effective genomic approaches to assess the risk of low biodiversity in agricultural soils and avoid dominance of certain soil biota species.

Soil Food Web: From microscopy to metagenomics
For over 200 years, the microscopy approach was the only way to explore soil biota. Undoubtedly, there is a great acquisition of soil biodiversity data set based on morphological characterization of soil organisms, but there is much more to explore. Soil biota represents a major fraction of the earth biodiversity and the multitrophic interactions that occur in the soil food web are remarkably complex. Therefore, it is impossible to describe the soil food web at the species by species interaction level. By grouping the soil organisms in functional groups the soil food web diagram can be made more intuitive (Figure 1). However, given that a real soil food web is a dynamic multi-dimensional system, soil ecologists have been unable to capture the full breath of complexity by assessing individual-geographical locations as these do not represent changes in time. Therefore, there are multiple approaches to describe a soil food web: the connectedness, the energy flow, and the functional web description of the soil food web. Nowadays, soil ecologists are combining aspects of the community-based (Hanemaaijer et al., 2015) and ecosystem-based (Aitkenheada et al., 2011) modeling approaches. The importance of top-down and

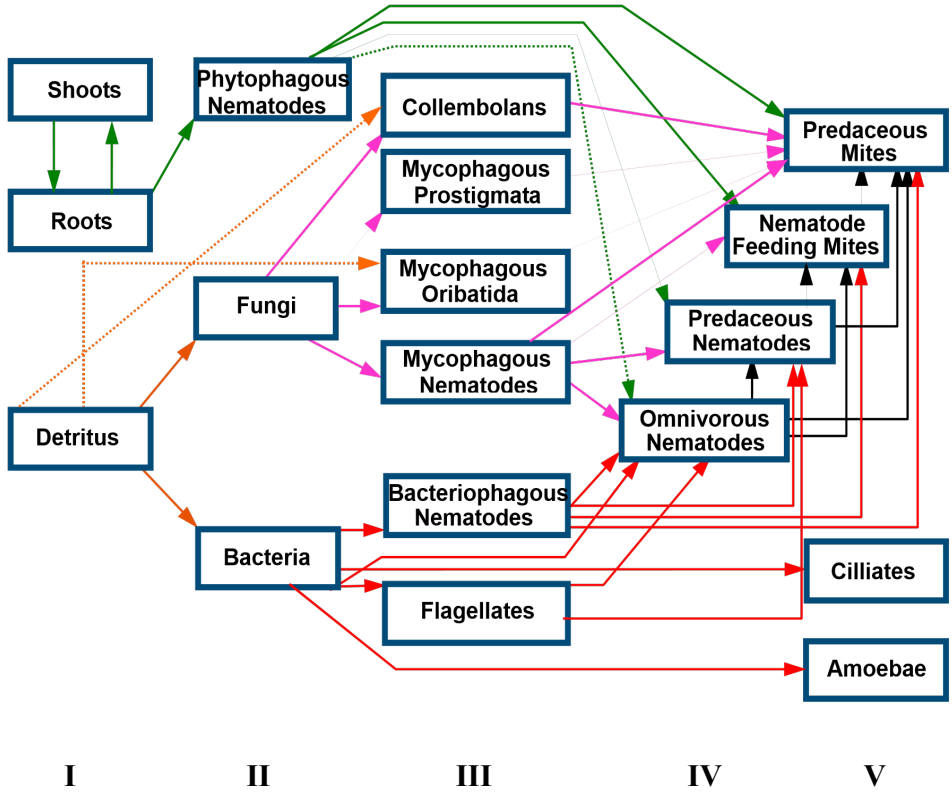


Figure 1. The connectedness descriptions of below-ground food web and five distinctions trophic positions. The dotted lines indicate potential feeding relationships that are not quantified.

bottom-up forces in regulating soil food web diversity and abundance of components is still a hot topic. Thus, structural equation modeling is used to test validated theory and generate new assumptions regarding the structuring forces in soil food web (Shao et al., 2015). Also, combining analytical and numerical modelling approaches can reveal the magnitude and interaction strengths at species level. In the last 20 years, the best approach to model the flow of energy and nutrients among soil food web components was to use the empirical diet data, stable isotopes or synchrotron radiation, but these data were not able to give information about demographic control or keystone species of the soil food web. Another, very common, less expensive, but time-consuming approach for describing soil food webs is the focus on one or a few groups of organisms (assumed to be the keystone species of the soil food web community), monitoring changes in their community structure, as indicators of changes in proprieties of the boarder soil food web. Thus, for instance, in the last three decades of microscopy approach several specific indices of nematode community structure (i.e. Maturity [Bongers, 1990; Bongers and Bongers, 1998], Enrichment, Structure and Chanel Indices [Ferris et al., 2001]) have been introduced and generally accepted as indicators of changes in soil food web proprieties.

Considering the rapid, on-going, environmental changes that occur everywhere around us is a real challenge for soil ecologists to model an "ideal" soil food web that will have/give an unanimous positive feedback. Identifying the species, constructing trophic interactions and describing their diet stability must be considered a good start, with regards to the fact that these sets of interactions are usually building blocks of any terrestrial or aquatic food web. Nevertheless, there are many biotic and abiotic factors, as well as contamination or disturbance factors (Brussaard et al., 2004), to be

considered while modeling the soil food web that can meet the standards of our best understanding of food web structure and functionality, with special concern to provide predictive ability regarding soil food web dynamics. It is not an easy task considering that few studies of complete (identification of all present species/functional groups) soil food webs have been published to date. Multiple variations in the composition of species communities occurs across different locations and hence there is high demand of trustable empirical evidence about real structure and functionality of soil food webs around the world. In fact, the soil food web is more about structure and functionality, rather than complexity. Over the years, soil food web research has revealed the impact of structure on dynamics. Therefore, it is strongly believed that any soil food web has its own keystone species that actually maintain the balance of food web survival characteristics: functional redundancy/structure and resilience. Yet, an intense collaboration between bioinformaticians and soil ecologists is required in order to model an "ideal" soil food web that will express as many as possible strong, along with weak, connections among species in real time and at real site/location. In other words, incorporating the newest ideas and lab techniques/devices, the most up-to-date IT modeling features can eventually help us to assemble a state-of-the-art soil food web based on real structure, diversity, and functionality of soil food web.

The first steps in DNA sequencing have been done forty years ago, when two different protocols for DNA sequencing were independently reported (Maxam and Gilbert, 1977; Sanger et al., 1977), but only in the last decade has the DNA-based technique been adopted in soil ecology (Figure 2). DNA barcoding means the identification of species in soil using standardized DNA fragments, such as internal transcribed spacer, and 16S and 18S rDNA. Recently, the field of soil ecology opened a new chapter moving from barcoding individuals to metabarcoding communities or metagenomics presumably linked to the advent of next-generation sequencing platforms, such as Illumina HiSeq, MiSeq and more recently NovaSeq, and a steep decline in sequencing cost. The relatively new approach involves new sequencing technologies, bioinformatics pipelines, along with developed IT skills and experimental designs. In the last several years, a metabarcoding approach has played a key role in the accurate identification among microbial communities and has made a promising start with applications in soil eukaryotes from fungi to soil mites. Thus, to obtain biodiversity data from the desired site in a relatively short time, the genomic approach based on the DNA-barcoding concept, and using next-generation sequencers will be

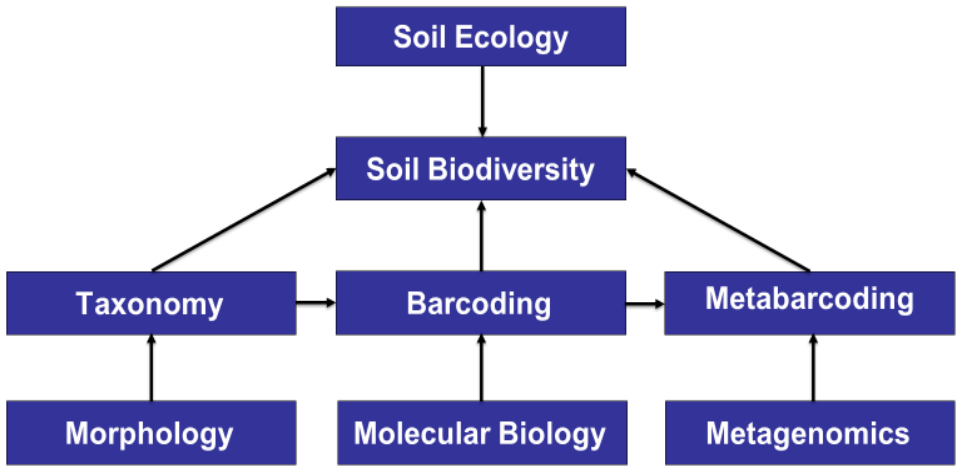


Figure 2. Current approaches in soil biodiversity research.

considered. This will require a single sampling time in the field, followed by a quite simple protocol of DNA extraction in the lab; DNA samples concentration and purity determination using Nano Drop, QUBIT, or agarose gel electrophoresis; selection of proper primers used in amplifying targeted regions of genes (such as variable regions of 16S and 18S rDNA) for the organisms we are interested in; massively parallel DNA sequencing on the next-generation sequencing platforms and data analysis using high-performance computing. These aforementioned steps will lead to a complete biodiversity assessment at the sampling site. However, there are many steps to be considered when we decide to choose the metagenomics approach. As with any other technique, metagenomics has not only its advantages, but also disadvantages. First, it is a relatively expensive technique (despite the steep decline in sequencing cost although in the future it might become even cheaper), and metabarcoding projects are hard to replicate. Second, working with a complex mixture containing degraded DNA and consequently, only dominant genomes are well-represented, it being hard, or even impossible, to estimate the total abundance of individuals representing the dominant genera in soil samples. Third, it cannot identify novel species/genera. Forth, sequence data focused on a single group (bacteria, fungi, protist etc.) are not the entire community of the environmental sample (i.e. soil food web). Fifth, lack of specific primers that could bring accurate data of group diversity. Sixth, the thechique works well only for communities with low diversity. In order to amplify equally the different target DNAs, the selected primers for use must be more versatile. Also, it still remains difficult to evaluate the pipeline of data processing while a standard framework widely accepted for eukaryotes data does not really exist. At present, many soil ecology projects are using metagenomics to evaluate the biodiversity using markers that are still not standardized, and are not associated with a reference DNA library.

A genomic approach can be a useful tool in describing food web species by species, but there are still unidentified species of soil organisms. Future steps are to integrate traditional approaches and effectively implement emerging technologies, that will lead to identify/read correctly the organism diversity information and be able to give practical advice regarding functional sustainability and resilience of disturbed ecosystems. Without these improvements the benefits of metagenomics are open to question.

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