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A comparison of molecular methods for monitoring soil nematodes and their use as biological indicators

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ABSTRACT

Soil fauna, especially soil nematode communities may be used as indicators for monitoring soil biodiversity and ecological processes. A major drawback facing ecologists is the specialised taxonomic knowledge and labour intensive nature of the work required for traditional morphological identification of soil fauna. We review rapid molecular methods, including: DNA Barcoding or sequencing, PCR-DGGE, PCR-TRFLP and real-time PCR, which could enable an empirical assessment of soil nematode assemblages, in relation to their use as monitoring tools. Based on advantages of: high-throughput; ease of comparison between samples; and rapid data analysis, we argue that PCR-TRFLP is well suited to monitoring purposes.

1. Introduction

1.1. Nematodes as biological indicators for soils

In recent years, interest has been shown by soil scientists and ecologists in measuring soil quality, particularly since the drafting of the Soil Framework Directive and increased national requirements for soil monitoring [17]. Soil quality is a combination of the physical, chemical and biological properties that contribute to soil function. Indicators of soil quality should be responsive to manage practices, integrate ecosystem processes, and be components of existing, accessible databases [41]. Such indicators must be quantified to document the improvement, maintenance or degradation of soil quality [42], represent different aspects of soil quality in different ecosystems [21], and strive to monitor or measure three basic functions or parameters: 1). soil structure development; 2). nutrient storage; and 3). biological activity [21].

Soil invertebrates are recognised as useful indicators as most are highly sensitive to perturbations and disturbances, for example, earthworms have been used to indicate soil properties [7] and soil pollution [68]; nematodes for environmental monitoring [11,12]; macroinvertebrates for soil heavy metal pollution [47,48]; and collembola for the restoration of environmental conditions [78]. Nematodes have been used as indicators of overall ecological condition because of the wide range of feeding types and the fact that they seem to reflect the successional stages of the systems in which they occur [11,22,24,76]. Furthermore, nematodes are sensitive to environmental insults and changes in their distribution and activity are diagnostic of changes in soil health [12,20,24,25,35,49,62,75], and they are the most abundant of the soil metazoa [20]. Nematode species occurring in soils encompass a wide variety of feeding strategies [77], including many free-living species that feed on soil microbes (bacteria or fungi). Microbial-feeding nematodes are among the most important consumers of bacteria and fungi in many systems [33], and their interactions with microbial decomposers affect ecosystem processes such as decomposition and nutrient cycling [28].

1.2. Limitations to routine monitoring

The identification of soil fauna often requires a high degree of taxonomic expertise [3,16]. Furthermore the time spent on identification (with the corresponding costs) makes it difficult to have results over a relatively short period of time with affordability. This is particularly true for the nematodes, identification of all individuals to the species level is time-consuming [43], so the characterisation of nematode communities continues to be resolved more coarsely than

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at the species level (i.e. genus, family, trophic group) [57,58], leaving ecological analysis potentially ambiguous or superficial [76]. There is also a constraint where identification of species is only possible from adult specimens which usually represent only a small percentage of the overall nematode assemblage [32].

So in spite of many advantages of using nematodes as biological indicators, identification even to functional group relies on highly trained experts [63]. A possible solution is to find an appropriate surrogate molecular method allowing empirical assessment of soil fauna biodiversity. This is especially the case as currently training in classical taxonomic techniques is in decline while that in molecular methods is increasing.

1.3. Molecular methods for fauna identification

Andre et al. (2002) highlighted the need for the development and consistency of methods in soil faunal monitoring; commenting that molecular techniques for community analysis are now widely used in soil microbiology and have greatly expanded our knowledge of soil microbes [2]. Molecular methods provide an alternative to traditional morphological identification for routine assessment of described species. Their application has enabled profiling of environmental samples of soil microbial populations, overcoming the need to culture and identify bacteria and fungi from complex mixtures [1] and similarly may reduce the taxonomic expertise currently required to characterise microfaunal communities. New, high-throughput sequencing technologies provide an opportunity to generate very large amounts of sequence data in a very short time and at low cost. One of most important applications of those molecular methods is the ability to identify large numbers of species from complex communities [54].

In addition to more rapid high-throughput discrimination requiring less specialised skills, molecular techniques may also readily allow identification of cryptic species and juveniles [10,59], although care needs to be taken with identifications made from a single gene target especially where taxa are currently represented by few confirmed sequences. Amplification and sequencing of diagnostic regions (i.e. rapidly evolving regions of SSU rDNA and LSU rDNA coding for the small and large subunit of rRNA) of single nematode specimens has resulted in the development of extensive public DNA sequence databases that are available for blast-match searching [74] and phylogenetic comparison [9,34]. Although DNA-based databases are strongly biased towards plant-parasitic nematode taxa [18], the utility of these searches for identification of free-living taxa that comprise the majority of soil nematodes is continuously improving. Recent publications on the phylogeny of terrestrial nematodes now make the identification of nematodes, and their ecological function, far more robust [38].

2. Molecular methods for nematode community analysis

Vanderknapp et al. (1993) used an arbitrarily primed PCR technique to differentiate closely related bacterial-feeding nematode species (from agar culture) that could not be morphologically distinguished, and suggested that the technique could be used in an ecological context [70]. It would, however, require PCR amplification of individual nematodes with at least three different primer sets and could not identify the nematodes without considerable calibration. Since that early example, more practical solutions have been developed.

2.1. DNA barcoding or sequencing

DNA barcoding, based on the sequencing of a small segment of the genome, in the form of a specific sequence carries both the species-specific and phylogenetic information of an organism, which provides taxonomic identification for a specimen, is a technique that should be applicable to all organisms [8,26,36,60]. DNA barcodes can be used in the identification of unknown specimens, to assist the phylogenetic placement of unknown taxa through comparison with known reference sequences, and to enable the definition of molecular operational taxonomic units (MOTUs). Theoretically, this should allow rapid and high-throughput identification, either of individual organisms or of sequences isolated from an environmental DNA sample.DNA barcoding has been used to perform surveys of nematodes, tardigrades and other meiofauna in terrestrial and marine habitats [8,26,36,60,71]. For example, Floyd et al. (2002) used PCR amplification products from 166 individual cultured specimens to analyse MOTUs of 74 randomly sampled individuals from their study site of a hill farm grassland ecosystem [26]. And they developed a simplified system that would permit diversity and abundance estimation of nematodes in soils, and then suggested modifications to make the method applicable to community analysis [26].

Hamilton et al. (2009) extracted faunal DNA directly from soil samples, and then used PCR with metazoan specific primers and sequencing to characterise micro- and meso-faunal community composition [36]. The technique captured the more abundant faunal groups (nematodes, Collembola, Acari, tardigrades, enchytraeids) and provided sufficient taxonomic resolution to describe the overall structure of the soil faunal communities, although the nematodes were only separated into two major taxonomic classifications (Chromadorea and Enoplea) [36]. Powers et al. (2009) estimated nematode diversity and nematode distribution among soil, litter, and understorey habitats based on MOTU analysis in a tropical rainforest [60].

The effectiveness of barcoding is dependant on the identity of the standardised gene region that is selected. To date, barcoding has been tested most extensively in the animal kingdom using a 648 bp region of the cytochrome *c* oxidase 1 (*CO1*) gene [67]. Much of the research undertaken on soil and marine nematodes has used the 18S rRNA gene although in the case of Bhadury ey al., (2006) a range of gene targets were used, including *CO1*, but reliable PCR amplification was only obtained from the 18S rRNA gene [5,26]. More recently the *CO1* gene was used successfully to identify filarial nematodes [23]. An advantage of the 18S rRNA gene is the large amount of sequence data available, but there may be future developments to align nematode barcodes with those used for other animal phyla.

The next step in using a sequencing approach is the application of pyrosequencing or other of the so-called 'next generation' sequencing technologies. This approach is almost untried for the analysis of nematode communities, although Porazinska et al. (2007) presented results strongly supporting the suitability of 454-technology for identification of all nematode individuals from environmental samples [58]. At this point, however, the use of the distribution reads for inferring the relative abundances of species within a nematode community is premature [58]. However, the use of such technology for monitoring is probably inappropriate as their real strength lies in the ability to extract enormous amounts of information from relatively few samples [56], whereas the requirements for biological monitoring are for relatively little information from enormous numbers of samples.

2.2. PCR-DGGE

Several research teams have already attempted to analyse soil or marine nematode communities using denaturing gradient gel electrophoresis (DGGE) [5,27,53,71]. PCR-DGGE has been used to estimate nematode diversity in soil, by detecting nematode taxa as