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Using nematode communities to test a European scale soil biological monitoring programme for policy development

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ABSTRACT

There is a current need to identify European biological indicators of biodiversity and ecosystem function that can be used for soil monitoring, in order to aid policy making. Europe, however, is subdivided into different bio-geographical (climate) zones, containing different soils and varying management practices. This work (as part of the EcoFINDERS project) set out to determine the range of variation in nematode community structure as a potential indicator across European bio-geographical zones, taking into account land use and soil characteristics. Nematodes have been suggested as biological indicators for the monitoring of soil quality due to their involvement in the delivery of functions such as carbon sequestration and recycling of nutrients as well as the provision of habitat for biodiversity. Using a molecular (directed-T-RFLP) approach for rapid nematode community structure assessment and a traditional morphological assessment at a feeding group level, we determined that nematode communities differ between bio-geographical zones and between different land uses within bio-geographical zones. Therefore, at the very large or trans-national level, the presence of any differing bio-geographical zones within the monitored area should be taken into account when sampling and analysing data. Care should be taken when making comparisons across different bio-geographical zones.
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1. Introduction

Since the development of the Soil Thematic Strategy (E.U., 2002) there has been increasing interest in the links between both soil biodiversity and soil ecosystem service provision, and soil quality (Ritz et al., 2009). Subsequent developments in environmental monitoring and risk assessment are moving toward the use of indicators and endpoints that are related to soil functioning and ecosystem services (Faber et al., 2013). Currently there is no comprehensive indicator of soil biodiversity that can combine all the different aspects of soil complexity in a single formula thus allowing accurate comparisons (Turbé et al., 2010). In response to this problem, it has been suggested that a suite of indicators should be used (Faber et al., 2013; Stone et al., 2016b). Soil nematodes are recognised as potentially useful indicators due to their high sensitivity to perturbations and disturbances (Chen et al., 2010). Nematodes are present in all trophic levels of soil food webs making them a good indicator for the functions of carbon sequestration and recycling of nutrients as well as involvement in the function of provision of habitat for biodiversity (Ritz and Trudgill, 1999; Chen et al., 2010; Griffiths et al., 2012).

Nematodes have been used as biological indicators across individual countries for some time (Faber et al., 2013; Stone et al., 2016b). However, a greater level of detail regarding the range of biodiversity present across all European bio-geographical zones, land uses and soil types is needed to aid European policy makers in the development of soil policy. In 2001, the OECD identified that to improve the interpretation of biodiversity indicators there was a need for information on their spatial and temporal coverage, including not only species presence, but also changes in species abundance and their distribution (OECD, 2001). This information should be as overall trends rather than absolute values. Specifically, if baselines could be established for the indicator measured, this could help improve the assessment of progress towards current goals and therefore the establishment of future targets. If nematodes are to be used as an indicator for soil biodiversity

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and ecosystem function across Europe, nematode communities need to be assessed across a range of European soil, land use and climate characteristics. The sensitivity of nematodes as an indicator should be able to reflect the influence of management and climate on long-term changes in soil quality (Breure, 2004).

Molecular methods of identifying soil dwelling nematodes for the purposes of assessing nematode communities are in an exciting period of development. The traditional method of morphological identification to genus or species by microscopic examination of a subset of the extracted community is still used, but there has been a recent increase in the development and use of molecular based approaches as the technology has advanced and become quicker and cheaper to use (Chen et al., 2010; Donn et al., 2012; Porazinska et al., 2012; Yang et al., 2013). To take account of this transition in the use of morphological and molecular methods, both types of analyses were performed on nematodes extracted from the sampled sites of the EcoFINDERS transect (Stone et al., 2016) to provide a pool of nematode community data that could be compared and used interchangeably.

Our hypothesis was that there were characteristic nematode communities according to bio-geographical zones, land management schemes and soil types. Such information would be relevant to inform the design of future, European scale, biological monitoring schemes.

2. Method

A transect of 81 sites were sampled across European climatic or bio-geographical zones (Fig. 1). Due to sampling constraints, one composite sample was collected from each site, with no replication A detailed outline of the sample sites is given in Stone et al. (2016). Each site was sampled following a pre-agreed standard operating procedure (SOP) whereby 20 cores of 5 cm diameter and 5 cm depth were collected at random within a 2 m^2 area chosen as typical for each of the 81 sites (Stone et al., 2016). Cores were transported to a central handling facility at 4 °C where a single, composite sample for each site was prepared from 12 of the 20 cores. The composite sample was broken up by hand and mixed using the cone and quarter method (Massey et al., 2014). From this composite sample, 100 g of fresh soil was subsampled for nematode elutriation. At the same time, a second subsample of 30 g was taken for moisture content determination. Nematode extraction with an Oostenbrink elutriator was performed following an adapted version of ISO 23611-4:2007(E) where the suspension of nematodes and small soil particles were passed through four sieves of decreasing mesh width (mesh width: 180 μm, 120 μm, 95 μm and 45 μm pore size respectively). The catch was then washed from each sieve onto tissue filters mounted on supporting sieves within Baermann funnels of water and left at room temperature for 48 h. During this time the nematodes separated themselves from the debris on the filter through active downward movement and were captured in water in 50 ml centrifuge tubes. Nematodes were allowed to settle for 24 h at 4 °C and the supernatant then removed by careful pipetting to leave 4 ml of nematode sample.

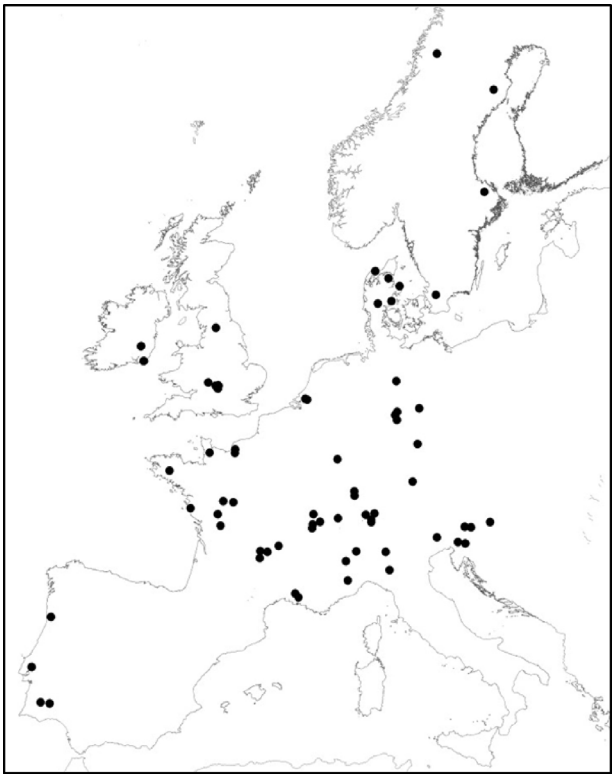

Fig. 1. Distribution of the 81 sites sampled across Europe.

Extracted nematodes were sub-divided into two samples (A and B) in separate micro-centrifuge tubes. Nematodes were once again allowed to settle for 6 h at 4 °C and the supernatant then removed by careful pipetting to leave 0.5 ml of nematode sample in each tube. Nematodes in sample A were frozen and stored for DNA extraction and terminal restriction fragment length polymorphism (T-RFLP) analysis. Nematodes in sample B were fixed in DESS, following the method of Yoder et al. (2006), for counting and morphological identification to trophic group level.

Genomic DNA was extracted from sample A using a Purelink® Genomic DNA Kit (Invitrogen) according to the manufacturer's protocol for Mammalian Tissue and Mouse/Rat Tail Lysate. DNA was eluted in 50 μl Tris Buffer (10 mM Tris–HCL pH 8.0) and then stored at −20 °C until used as a PCR template for directed-TRFLP as described by Donn et al. (2012).

DNA (18S rDNA) was selected for amplification using the primers: Nem_SSU_F74 (AARCYGCGWAHRGCTCRKTA) (Donn et al., 2011) and fluorescently labelled FAM-Nem_18S_R (GGGCGGTATC-TRATCGCC) (Floyd et al., 2005) (Eurofins MWG Operon, Ebersberg, Germany). PCR amplifications of 1.2 μl genomic DNA template were performed in 15 μl final volume reactions containing 1.5 μl of ×10 PCR buffer (Bioline, London, UK) with 2 mM $MgCl_2$ (0.6 μl 50 mM $MgCl_2$), 0.3 μl each of 10 mM dNTP mix and BSA, 0.45 μl of each primer (10 pmol/μl) and 0.12 μl of *Taq* polymerase (0.6 units). The volume of template DNA was as used by Wiesel et al. (2015) and yielded robust PCR amplification. All PCRs were performed on a G-STORM Thermal Cycler (Gene Technologies Ltd., Braintree, Essex, UK). The thermal cycling involved one initial denaturation cycle at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 51 °C for 30 s, and extension at 68 °C for 30 s. A final elongation step was performed at 68 °C for 10 min. Positive (DNA extracted from mixed nematodes, confirmed by preliminary study) and negative (distilled water) controls were included for each amplification series.

The amplified DNA then underwent T-RFLP analysis in a dual enzyme sequential digest. Firstly a PleI enzyme mix, made up of 1 × NEBuffer4 (20 mM Tris-acetate, 10 mM magnesium acetate, 50 mM potassium acetate, 1 mM dithiothreitol (pH 7.9)), 100 μg ml^{-1} BSA (supplied with the enzyme) and 2 units PleI per μl (all reagents from New England Biolabs, Hitchin, UK), was added to 10 μl PCR products which were digested at 37 °C for 60 min, followed by 65 °C for 20 min, to denature the enzyme. Digested products were then digested in a BtsCI enzyme mix, made up of 1 × NEBuffer4 (as above), 100 μg ml^{-1} BSA (supplied with the enzyme) and 2 units BtsCI per μl, with incubation at 50 °C for a further 1 h. Products were then frozen at −20 °C to inactivate the

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