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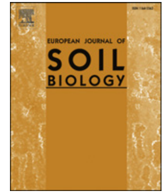

Original article

Bioindication potential of using molecular characterisation of the nematode community: Response to soil tillage

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The utility of a high-throughput molecular characterisation (directed - terminal restriction fragment length polymorphism – dTRFLP) of soil nematode community structure was evaluated in a field trial of the effects of tillage intensity. Replicated plots were established in a field previously used for continuous, conventional tillage of spring barley (*Hordeum vulgare*) and cultivated by: zero tillage; minimum tillage; conventional tillage; deep tillage and conventional tillage with compaction. Nematodes were sampled in spring, summer and autumn for three years after treatment began. Total nematode abundance and Nematode Channel Ratio indicated changes due to the zero and minimum tillage, more nematodes and a greater proportion of fungal feeders, but effects were secondary to those of year and season. The effects of tillage were far easier to interpret when other bioindicators were also taken into account. Increases in soil carbon and fungal biomass in the upper layers of the zero and minimum tillage treatments, observed in parallel studies, corroborated the nematode data. The high-throughput molecular method proved to be well suited for multiple measurements of nematode community structure, although limitations in the resolution of nematode taxa could obscure changes in generic and species composition.
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1. Introduction

Nematodes are widely recognised as bioindicators of the soil environment [1–7] and their practical use is increasingly based on analysis of the nematode community to calculate various ecological indices related to enrichment and trophic status [8,9] and more recently carbon and energy flows [10]. While still largely reliant on a microscopical analysis of selected individual nematodes extracted from the soil, there is increasing development of molecular based approaches which will allow analysis of the complete sample of extracted nematodes and a greater sample throughput [11]. However, nematodes are just one of a number of potential soil bioindicators [12,13] and a strategy using multiple indicators has been used in some schemes [14].

It is well known that tillage has a major effect on the biology of the soil system [15] and that the most significant effects are seen when grassland is converted to arable agriculture and tilled for the first time. Given the interest in soil sustainability, it is increasingly common to reduce the amount of tillage used in arable agricultural systems [16]. The environmental benefits can include reduced risk of soil erosion, increased organic matter in the upper part of the soil profile, improved soil structure, increased biodiversity [17–19] and reduced greenhouse gas emission [16,20]. Although, increased N_2O production [21] and reduced soil carbon [16] have been observed in some reduced tillage trials. A likely scenario is for conservation tillage to be applied to existing, conventionally cultivated arable land. Studies on the effects of altered tillage intensity on land that is already in tillage are less common and show variable results, particularly for nematode abundance with studies showing that conservation tillage had a negative [22–24], neutral [25–28]; or positive effect [29–33].

We describe the results of a field experiment in which a range of tillage treatments were applied to a conventionally tilled arable field (spring barley), in which changes in the nematode community over a three year period were monitored using a high-throughput molecular method [34,35]. Our objectives were to:

- Determine the time scale of any changes occurring after the change in tillage practice;
- Evaluate the utility of the high-throughput molecular characterisation of nematode communities by comparing results between different indicators of soil quality.

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2. Material and methods

The tillage trial was established in a field that had been used for continuous spring barley (*Hordeum vulgare* L.) cultivation for four years. The soil was a Cambisol [36] with a sandy-loam surface texture, 2.56% C and a $pH_{(CaCl_2)}$ of 5.7. To reduce in-field variability the entire site was initially ploughed to 20 cm depth, power harrowed and sown with a single spring barley variety (cv Optic) in 2003. Five cultivation treatments were established in triplicate in autumn 2003 that imposed different levels of soil disturbance ranging from light to heavy disturbance in the order: zero tillage, minimum tillage to 7 cm depth, conventional plough to 20 cm depth, plough to 20 cm followed by compaction and deep plough to 40 cm depth, as described by Sun et al. [37]. There were 15, 33 × 33 m plots in an even grid with five blocks in each of three north-south columns representing the three treatment replicates. The tillage treatments, applied annually, were: (1) conventional tillage (CT) by ploughing to 20 cm with a mouldboard plough and disking; (2) zero tillage (ZT) in which the seeds were directly drilled; (3) minimum tillage (MT) in which the soil was cultivated by disk to a depth of 7 cm before the seeds were drilled; (4) deep ploughing (DP) to 40 cm and disking; and (5) conventional tillage followed by compaction (CP) imposed by wheeling of the entire plot using a conventional Massey Ferguson 5850 tractor. Spring barley (cv. Optic) was combine-drilled with a compound fertiliser at a rate of 77 kg N, 14 kg P and 49 kg K ha^{-1} in early March, with an additional 33 kg N, 6 kg P and 21 kg K ha^{-1} at growth stage 22–24 (as defined by the UK Home Grown Cereals Authority) and was harvested in late August. Foliar diseases were not controlled. Crop yields were similar between all cultivation treatments in 2004 when the experiment began, but by 2008 ZT and MT had 72% and 78% of the yield of CT or DP [38].

Nematodes were sampled (Table 1) from the top 10 cm using a grass plot sampler (Eijkelkamp, Giesbeek, Netherlands, 2.3 cm diameter × 10 cm deep) to take 15 individual soil cores per plot which were combined to form a composite sample. Samples were stored at 4 °C for up to 10 days and nematodes extracted from 200 g composite soil samples using a Baermann funnel extraction over 48 h [39]. The nematode suspension collected was left overnight to settle then reduced to a volume of 5 ml, of which 2 ml was transferred to a micro-centrifuge tube for DNA extraction and the remaining 3 ml used for the estimation of nematode abundance. Nematodes for counting were heat killed at 60 °C for 2 min, fixed in 1% formaldehyde and transferred to a counting dish where total numbers were counted under a low power microscope. Nematodes for DNA extraction were immediately stored at −20 °C. Subsequent DNA extraction by bead-beating and clean-up using a Purelink PCR Purification kit (Invitrogen, Paisley, UK) was as described by Donn et al. [34]. The extracted DNA was used for directed-terminal restriction fragment length polymorphism (dTRFLP). Near full length SSU rDNA was amplified by PCR using the primers Nem_SSU_F74 [40] and Nem_18S_R [41] with the addition of the fluorophore VIC, digested with *Ple*I and *Bts*CI (New England Biolabs, Hitchin, UK) and fragments were analysed on an ABI 3730 capillary sequencer, according to Donn et al. [42].

Table 1
Sampling dates for extracting nematodes from spring barley grown under different tillage treatments. (P) represents samples taken post-harvest.

Year	Spring sample	Summer sample	Autumn sample
2004	9th April	14th July	18th October (P)
2005	28th March	1st June	1st August
2006	9th March	8th June	1st September (P)

Data were analysed using standard analysis of variance (ANOVA) and multivariate procedures with GenStat 12th edition (VSN International, Hemel Hempstead, UK) using year, season and treatment as factors. For dTRFLP analysis an undigested PCR product was run and any peaks in this were assumed to be artefacts and removed prior to calculating the relative peak area. Principal component analysis was performed on relative peak area. Peaks were ascribed to nematode taxa and thence to trophic group (bacterial-, fungal- and plant- feeding or omnivore or predator) [42,43] to calculate the Nematode Channel Ratio [7], using the equation:

NCR = % bacterial feeders/(% bacterial feeders + % fungal-feeders) to accommodate the resolution of the dTRFLP peaks.

3. Results

3.1. Nematode abundance

There were significant interactions between: cultivation and sampling time (F 3.62; $P < 0.001$); cultivation and year (F 2.15; $P < 0.05$) and sampling time and year (F 5.42; $P < 0.01$) (Fig. 1). There were significant ($P < 0.001$) effects of: year (2004 > 2005 > 2006. F 154.01); sampling time (autumn > summer > spring. F 39.88); and cultivation (ZT = MT > CP = CT = DP. F 12.33).

3.2. Nematode community structure

As with abundance, the main effects were between years. Although there were significant treatment effects there was no strong general trend in the pattern of differences. Most taxa that showed a difference varied according to sampling time and year (i.e. *Panagrolaimus*, Rhabditoides, *Pelodera*, Plectida, Cephalobidae, *Aphelenchoides*, Mononchida, Pratylenchoides, *Longidorus*, Tylenchidae and *Helioctylenchus*, Table 2), while tillage effects were seen in: Monhysterida, Rhabditoides, *Pelodera*, Cephalobidae, *Aphelenchoides*, Tylenchidae and *Longidorus* (Table 2). Principal Component (PC) analysis of the dTRFLP peaks representing nematode families showed that PC1 varied significantly (F 7.30, $P < 0.001$, Table 3) with year and sampling time, the difference between sampling times was more evident in 2004 than 2006. For PC2 there was a significant (F 3.03, $P < 0.01$, Table 3) interaction with year and treatment showing that: in spring the nematode community in the minimum tillage plots differed from that in the zero tillage and compacted plots; in the nematode community in the minimum tillage plots in summer differed from that in spring and also from

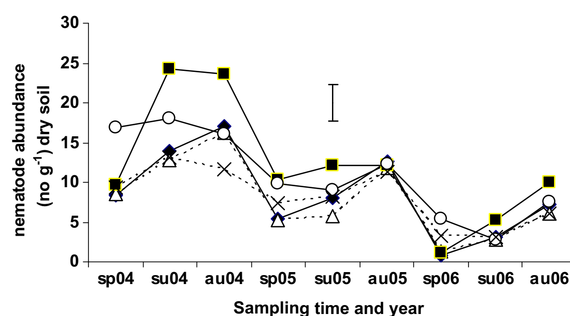

Fig. 1. Nematode abundance in samples taken in spring, summer and autumn (sp, su, au) of 2004, 2005 and 2006 (04, 05, 06) from compacted (◆), deep plough (△), minimum tillage (■), zero tillage (○) and conventional tillage (×) treatments. Bar represents least significant difference ($P < 0.05$, $n = 3$).

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