T-RFLP approaches to nematode assemblage analysis

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Intensive land use has been implicated in declining soil health, raising concern over the sustainability of agronomic production under current management strategies. Soil faunal communities may be used as indicators of soil health. However, identification based on morphology is time consuming and problematical. Molecular methods offer an alternative.

Terminal Restriction Fragment Length Polymorphism (T-RFLP) is proposed as a means of nematode community analysis and will be used to profile nematode communities under different agricultural regimes.

DNA extraction

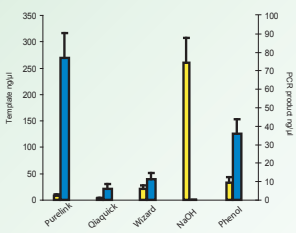


Fig 1. Mean concentration of template DNA and PCR product for 3 replicates of each of 5 habitat types. Bars show standard error, n=15

Nematodes were extracted from 5 habitats and DNA extractions performed using various techniques. Quality of the extract was assessed by quantification of PCR product. Tissue disruption, by bead beating, followed by purification through the Purelink PCR purification kit (Invitrogen, Paisley, UK) was selected as the optimal method.

Morphological vs molecular

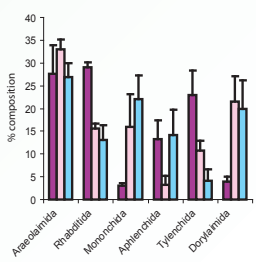


Fig 2. % community composition as calculated from individual counts, biomass and clone number.

PCR products amplified from 3 arable samples were cloned, sequenced and assigned to taxonomic order by phylogenetic anlysis.

100 nematodes from each sample were identified under the microscope to order level and their biomass calculated. Clone number corresponds more closely to biomass than to counts.

Effects of organic ammendment revealed by T-RFLP

Fig.3 Compost, slurry and no ammendment treatments were applied to spring barley, *Hordeum vulgare*, along with an irrigation treatment. Samples were taken in March 2006.

Nematodes extracted from 200g soil
↓
Community DNA extracted
↓
500bp of SSU rDNA amplified using fluorescently labelled primers
↓
Digestion of PCR product with HinfI
↓
Analysis of terminal fragments on capillary sequencer
↓
Multivariate analysis of relative abundance of fragments

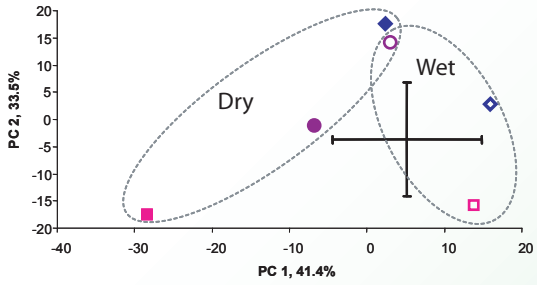
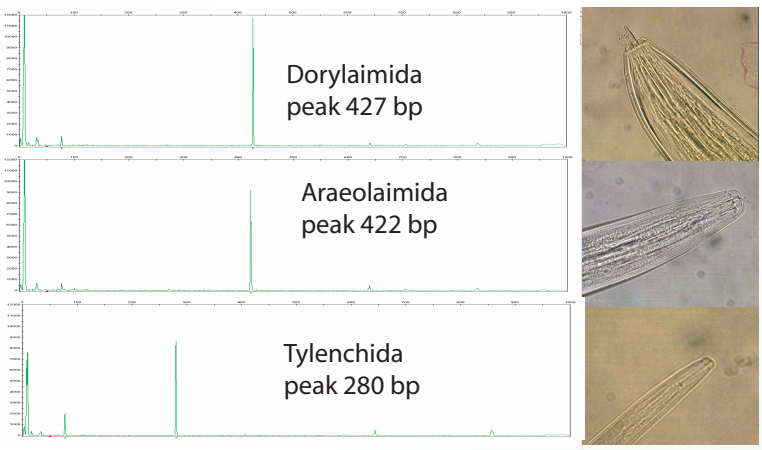


Fig.4 Communities extracted from compost treatments ■ were significantly different to the slurry ● and no ammendment ◆ treatments, PC2 ANOVA p=0.034. Open symbols represent irrigated treatments, PC1 ANOVA. p=0.029. Bar = L.S.D.

Directed T-RFLP



With careful design of the enzyme digest, from known nematode sequences, signature T-RFLP peaks may be assigned to taxonomic groups. This will allow us to integrate T-RFLP data with established nematode ecological indices.

References

Donn,S, Daniell,TJ, Neilson,R, Griffiths, BS, (2007) DNA extraction from soil nematodes. Applied Soil Ecology, *In press.*

Griffiths, BS, Donn,S, Neilson, R, Daniell,TJ, (2006) Molecular sequencing and morphological analysis of a nematode community. Applied Soil Ecology 32, 325-337.