Molecular analysis of soil nematode communities

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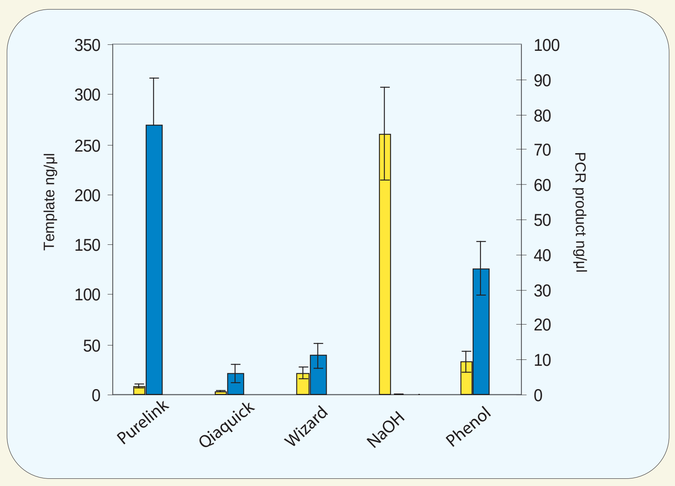
Intensive land use has been implicated in declining soil health, raising concern over the sustainability of agronomic production under current management strategies. Soil faunal communities may be used as indicators of soil health. However, identification based on morphology is time consuming and problematical.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

Disturbance trial at SCRI. 5 treatments are replicated x 3.



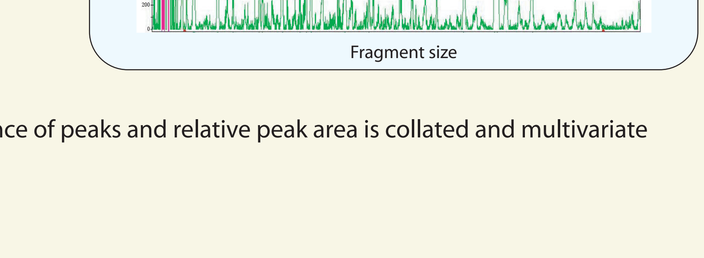
Mean concentration of template DNA and PCR product for 3 replicates of each of 5 habitat types. Bars indicate standard error, n=3. Concentration of PCR product and template DNA, meaned over 5 habitat types.

DNA extraction from whole nematode community

Nematodes were extracted from 5 habitats and DNA extractions performed using various techniques.
Quantification of PCR product is used to assess quality of the extract.
Tissue disruption followed by purification through the Purelink PCR Purification kit (Invitrogen, Paisley) was selected as the optimal method.

Amplification of small subunit (SSU) ribosomal DNA

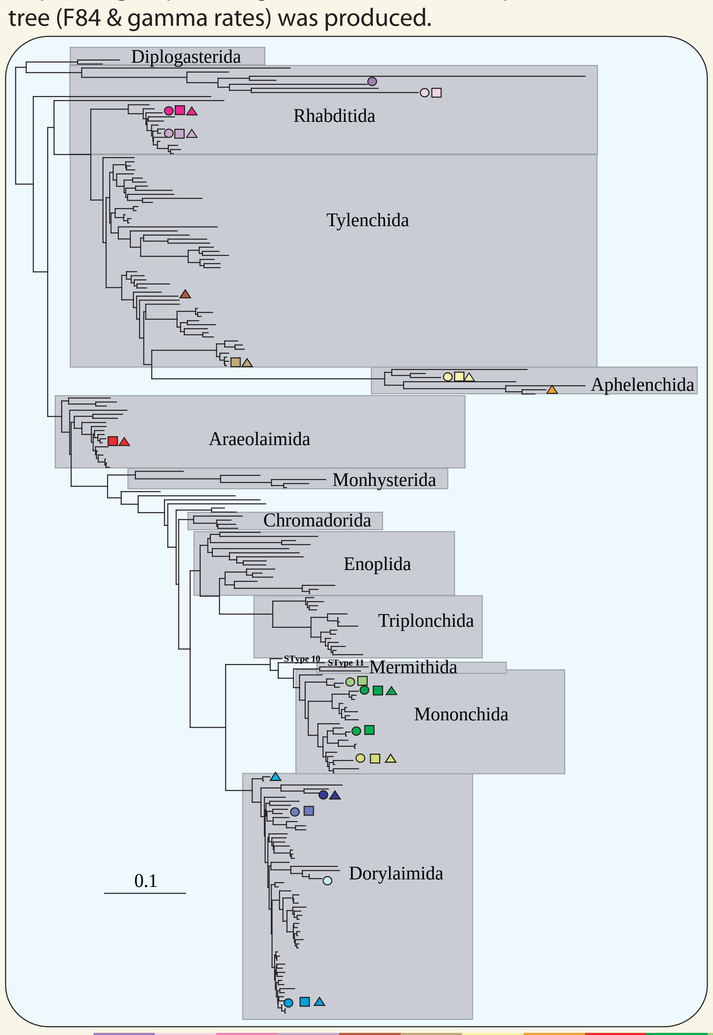
PCR products amplified from 3 arable samples were cloned, sequenced and assigned to taxonomic order by phylogenetic analysis.
100 nematodes from each sample were identified under the microscope to order level, and their biomass calculated.
Clone number corresponds more closely to biomass than with counts.



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

Cloning and sequencing

PCR products from the compaction, deep plough and no till treatments were cloned and sequenced. Near full length SSU rDNA sequence was obtained for samples of each sequence group and aligned with database sequences (Griffiths et al. 2006). A neighbor joining tree (F84 & gamma rates) was produced.



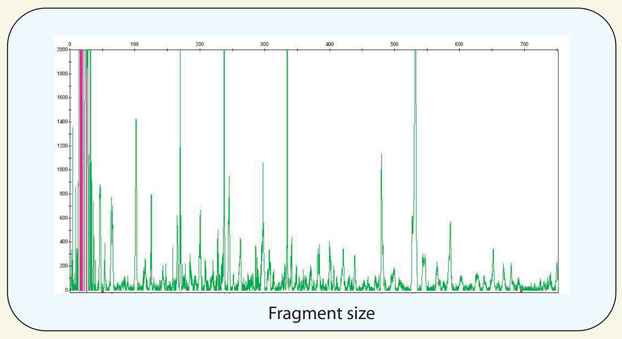
	Rhab.1	Rhab.2	Rhab.3	Rhab.4	Tylench.1	Tylench.2	Aphel.1	Aphel.2	Araeol.	Monon.1	Monon.2	Monon.3	Monon.4
compaction ●	1.12	0.56	1.12	0.56	0.00	0.00	0.00	0.56	0.00	22.35	37.43	3.35	8.94
deep plough ■	0.00	0.50	2.00	0.50	0.00	0.50	0.00	1.50	2.00	73.50	1.00	6.50	2.50
no till ▲	0.00	0.00	0.81	1.61	1.61	1.61	1.61	0.81	2.42	37.90	0.00	3.23	34.68

	Doryl.1	Doryl.2	Doryl.3	Doryl.4	Doryl.5	Doryl.6	total clones
compaction ●	6.15	0.00	3.35	1.68	1.12	11.73	179
deep plough ■	1.50	0.00	0.00	1.00	0.00	7.00	200
no till ▲	0.00	2.42	1.61	0.00	0.00	9.68	124

Nematode types from 3 treatments are assigned to orders by phylogenetic analysis. Single clones have been excluded. (Symbol colours on tree represent nematode types).

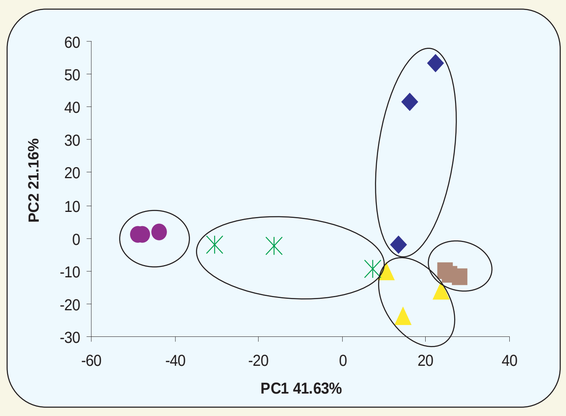
Terminal Restriction Fragment Length Polymorphism

Fluorescently labelled PCR product is digested with a restriction enzyme and the fragments analysed on a capillary sequencer.



Data incuding presence / absence of peaks and relative peak area is collated and multivariate statistical analysis performed.

Community fingerprints were created of nematode communities from five habitats.
A 500 bp fragment at the 3' end of SSU rDNA was generated using fluorescently labelled primers and digested with HinfI.



Principal components plot showing dimensions 1 and 2 representing over 60% of the total variation in the nematode communities extracted from:

- arable,
- dune,
- coniferous forest,
- pasture
- organic soil.

Directed T-RFLP

Sequence information will be used to design a directed T-RFLP approach, where peaks may be assigned to named nematode types.

Relative abundance of types, calculated from peak areas can then be used in conjunction with existing diversity indices to monitor nematode community composition.

References

Griffiths, B.S., Donn, S., Neilson, R., Daniell, T.J. 2006. Molecular sequencing and morphological analysis of a nematode community. Applied Soil Ecology 32: 325-337

Acknowledgements

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