Free Living Nematodes – Liebe Soil Biology Trial

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BIOLOGICAL CROP PROTECTION

Key Messages

- Organic amendments improved 'soil health' in terms of free living nematode community structure.
- The Tilled + Organic Matter treatment had a more well balanced soil microbial system than other treatments.
- Numbers of omnivorous and predatory nematodes are quite low in comparison to other cropped soils around Australia, thus room for improvement.
- Soils have improved, as the number of omnivorous nematodes in 2013 significantly increased in organic amended treatments, in comparison to 2011.

Aim

Investigate the composition of the free living nematode communities in Australian grain-growing soils; determine whether the community responds to different organic matter inputs and to various tillage and stubble management regimes; and decide whether nematodes are a useful indicator of soil health.

Background

Most grain growers know that nematodes are important pests of cereal crops, but are probably not aware that their soils also contain non-parasitic free living nematodes. These free-living nematodes provide a wealth of information on a soil's biological status and are therefore useful indicators of soil health. Free-living nematodes are important in nutrient cycling within the soil as they feed on microbes (bacteria and fungi) releasing nitrogenous compounds and other compounds which are then available to plants.

Some nematodes can survive harsh, polluted, or disturbed environments better than others, and some have short life cycles and respond to environmental changes rapidly. Bacterial-feeding nematodes are relatively small, they complete their life cycles in only a few days and have high reproductive rates. Bacterial feeding nematodes that colonise habitats following disturbance or inputs of organic matter multiply rapidly to high population densities and then decline when their food source is exhausted; these are termed enrichment opportunists. Fungal feeding nematodes are also relatively small but have slightly longer life cycles. They live on the fungi associated with surface litter and decomposing organic matter, and also feed on mycorrhizal spores and fungal plant pathogens. At the other end of the spectrum, the omnivores and predators are much larger nematodes, and they take one to several months to complete their life cycles. These nematodes help regulate nematode populations but are sensitive to disturbance. Once they are lost from the soil food web, it may take months or years for them to return. In a healthy soil, the predominant nematodes will be free-living species, with a good balance between bacterial and fungal feeders and will also contain a diverse range of omnivorous and predatory nematodes

Liebe Group's Soil Biology Trial was chosen as a part of a GRDC supported Australian wide study on nematode communities in grain-growing soils. It is a long term trial (established in 2003) investigating how soil biology and carbon affect crop yield and soil health. The site has no significant chemical or physical soil constraints, and so there is a capacity to demonstrate that grain production can be increased through improved moisture conservation and enhanced contributions from the soil biota.

Some plots have now received a total of 80 t/ha of organic matter (chaff) across four separate applications (2003, 2006, 2010 and 2012).

Trial Details

Property	Long Term Research Site, west Buntine
Plot size & replication	10.5m x 80m x 3 replicates
Soil type	Deep yellow sand
Soil pH ($CaCl_2$)	Topsoil: 6, Subsoil: 4.6
EC (dS/m)	0.1
Paddock rotation	2010: wheat, 2011: wheat, 2012: canola

Table 1: Cropping history of the Soil Biology Trial at the Libe Group's Long Term Research Site.

Year	Crop rotation	Yield range	Treatment notes
2003	Lupin	None recorded	Set up phase: 20 t/ha barley chaff applied, lupin crop brown manured
2004	Wheat (cv. Wyalkatchem)	2.9-3.5 t/ha	Brown manuring and addition of 20 t/ha organic matter increased yield by 18-22%
2005	Wheat (cv. Wyalkatchem)	2-2.8 t/ha	Burnt plots yielded 25% higher than control.
2006	Lupins	None recorded	Set up phase: 20t/ha canola chaff applied, brown manure
2007	Wheat – sprayed out	None recorded	
2008	Wheat (cv. Wyalkatchem)	2.4-3.4 t/ha	Addition of organic matter increased yield by 23% compared to control.
2009	Lupins	1.5 t/ha	Set up phase
2010	Wheat (cv. Magenta)	2.5-1.9 t/ha	20 t/ha chaff applied. No significant yield difference between treatments.
2011	Wheat (cv.Wyalkatchem)	3-3.8 t/ha	No significant difference in yield
2012	Canola (cv. Tefler)	0.7-0.9 t/ha	20 t/ha chaff applied

Trial Design

Treatments sampled in 2013

1. Burnt (stubble burnt annually in March; minimum till)
2. Tilled (using offset discs)
3. Tilled + organic matter (chaff is applied once every 3 years last applied 2012 at rate of 20 t/ha; tilled with offset discs)
4. Tilled + organic matter run down (plots where chaff was previously applied in 2003 & 2006 but not since)

In this study, the four treatments above were sampled pre sowing after the initial opening rains in May (13/5/13). The same four treatments were also sampled in 2011 and 2012, but only the 2013 data is reported here.

Four plots from each treatment were sampled and consisted of 30 cores (about 1.2kg soil) collected with a

25mm diameter corer at a depth of 0–10cm. The soil was sub divided into three portions for chemical analysis by CSBP, manual nematode analysis and DNA nematode analysis.

Nematodes were extracted from the soil and free living community analysis was performed. A sub sample of 100-150 randomly-selected individual nematodes were identified to forty different groups at the family or generic level. They were then separated into groups based on their feeding habits and the number of plant associates (PA), enrichment opportunists (EO), bacterivores (BF), fungivores (FF), Omnivores (Dorylaimida) and Carnivores (Mononchida) per gram of soil were calculated.

Soil was also dried and DNA was extracted and analysed using PredictaB tests by the SARDI's Root Disease Testing Service. Predicta B testing quantified DNA levels of important soil nematodes and fungal crop pathogens.

Results

Table 2: Free living nematodes per gram of soil from the Liebe Soil Biology trial as determined by manual community analysis counts.

	EO/g	BF/g	FF/g	PA/g	Dorylaimida/g	Mononchida/g	FLN/g
Burnt	0.81^{b}	1.79^{b}	1.82^{b}	3.95^{a}	0.15^{b}	0.00^{a}	8.53^{b}
Tilled	0.71^{b}	3.28a^{b}	3.19^{b}	3.85^{a}	0.21^{ab}	0.00^{a}	11.23^{b}
Tilled + OM	5.62^{a}	4.50^{a}	12.17^{a}	20.54^{a}	0.34^{a}	0.00^{a}	43.17^{a}
Tilled+OM+RD	1.89^{b}	2.51^{b}	2.26^{b}	4.88^{a}	0.17^{ab}	0.00^{a}	11.71^{b}
Reduced Till	0.81^{b}	1.79^{b}	1.82^{a}	3.95^{b}	0.15^{a}	0.00^{a}	8.53^{b}
Conventional Till	2.74^{a}	3.43^{a}	5.87^{a}	9.76^{a}	0.24^{a}	0.00^{a}	22.04^{a}

PA - Plant associates, EO – Enrichment opportunists, BF - Bacterivores, FF - Fungivores, Dorylaimida –Omnivores, Mononchida – Carnivores, FLN – Free living nematodes total
Means that have no superscript letter in common are significantly different from each other

Table 3: Free living nematode community indices from the Liebe Soil Biology Trial as determined by manual community analysis counts.

	EI	SI	CI	MI	B/(B+F)
Burnt	60.11^{ab}	68.15^{a}	36.09^{ab}	2.07^{ab}	0.61^{ab}
Tilled	55.19^{b}	65.08^{a}	53.42^{a}	2.16^{a}	0.56^{ab}
Tilled + OM	69.89^{a}	58.83^{a}	35.04^{b}	1.90^{b}	0.47^{b}
Tilled+OM+RD	70.50^{a}	73.51^{a}	23.66^{b}	2.07^{ab}	0.68^{a}
Reduced Till	60.11^{a}	68.15^{a}	36.09^{a}	2.07^{a}	0.61^{a}
Conventional Till	65.20^{a}	65.81^{a}	37.37^{a}	2.04^{a}	0.57^{a}

EI – Enrichment Index, SI – Structural Index, CI – Channel Index, BF - Bacterivores, FF – Fungivores, PA - Plant associates
Means that have no superscript letter in common are significantly different from each other

In general, the addition of organic amendments improved 'soil health' in terms of free living nematode community structure. The Tilled + Organic Matter treatment had significantly higher numbers of omnivourous nematodes compared to treatments with no amendments. These omnivourous nematodes are only present in soils that have well balanced soil microbial systems.

Manual community analysis found that the Tilled + Organic Matter treatment had significantly more total free living nematodes than the other treatments (Table 2). Most treatments were generally well balanced in terms of numbers of bacterial and fungal feeding nematodes, as the bacteria to fungal (B/(B+F)) ratio was generally around 0.5. However, the Tilled + Organic Matter was predominately fungal based (High FF/g and low B/(B+F). The channel index (CI) describes the channel or pathway of organic matter decomposition in the soil. The low CI in the Tilled + Organic Matter treatment further shows a fungal decomposition pathway with lots of enrichment opportunists. The fungal feeders are generally more dominant in the early stages of decay of surface residues due to the high C:N ratio of such residues. Treatments with organic matter amendments were dominated by enrichment opportunistic nematodes (High EO/g and EI) that were taking advantage by feeding on the flux of bacteria associated with the decomposition process.

The conventionally tilled, stubble retained soils had more enrichment opportunists, bacterial and fungal feeding nematodes than the burnt, reduced tillage soils. No difference was observed in numbers of omnivores (Dorylaimida) and predatory (Mononchida) between the two tillage treatments. However, this is an opposite trend to that observed from several other sites (SA, QLD, VIC), where reduced or no till has provided a more structured free living nematode community.

In general, the numbers of omnivorous and predatory nematodes were quite low in comparison to soils sampled from other cropped soils around Australia. However, the number of omnivorous Dorylaimida in all treatments, and especially those with organic amendments, has significantly increased in comparison to numbers present in 2011 (data not shown). A significantly higher number of ominvores (Dorylaimida) were present in the Tilled + Organic Matter treatment compared to the burnt treatment. The predatory Mononchida nematodes, only present in well balanced and structured populations, were not observed in any of the treatments. In addition, the low numbers of the maturity (MI) and structural indices (SI, which are

measures of disturbance and balance within a community, suggests that the structure and maturity of free living nematode communities in these soils could be improved.

Table 4: Predicta B DNA quantification of parasitic nematodes in soil from the Liebe Soil Biology Trial. Pathogen levels are reported in terms of picograms of DNA per gram of soil, except for stem nematode which is per 100g soil, which correlates to disease risk categories. Risk categories should be used as a guide only as they may be subject to regional and seasonal differences.

	CCN eggs	CCN Risk	P. neglectus	P. neglectus Risk	P. thornei	P. thornei Risk	P. penetrans	P. teres	Stem nematode	Stem nematode Risk
Burnt	0.00	BDL	0.50[a]	Low	0.00	BDL	0.00	0.00	0.00	BDL
Tilled + OM	0.00	BDL	1.03[a]	Low	0.00	BDL	0.00	0.00	0.00	BDL
Tilled+OM+RD	0.00	BDL	1.14[a]	Low	0.00	BDL	0.00	0.00	0.00	BDL
Tilled	0.00	BDL	1.33[a]	Low	0.00	BDL	0.00	0.00	0.00	BDL
Reduced Till	0.00	BDL	0.50[a]	Low	0.00	BDL	0.00	0.00	0.00	BDL
Conventional Till	0.00	BDL	1.17[a]	Low	0.00	BDL	0.00	0.00	0.00	BDL

BDL - Below Detected Level

CCN – Cereal cyst nematode, P. neglectus- *Pratylenchus neglectus*

Means that have no superscript letter in common are significantly different from each other

Table 5: Predicta B DNA quantification of common fungal pathogens in soil from the Liebe Soil Biology Trial. Pathogen levels are reported in terms of picograms of DNA per gram of soil and log (picograms of DNA per gram of soil +1), which correlates to disease risk categories. Risk categories should be used as a guide only as they may be subject to regional and seasonal differences.

	Take-all	Take-all (log)	Take-all Risk	R. solani	R. solani (log)	R. solani Risk	F. pseudo	F. pseudo Risk	F. culmorum	F. culmorum (log)	F. culmorum Risk	Bipolaris	Pythium Clade f	Pythium Clade l
Burnt	1.58[b]	0.40[b]	BDL	0.00[a]	0.00[a]	BDL	0.00[a]	BDL	0.00	0.00	BDL	19.15[ab]	13.99[ab]	0.00
Tilled + OM	8.47[a]	0.97[a]	Low	0.00[a]	0.00[a]	BDL	0.67[a]	BDL	0.00	0.00	BDL	7.57[b]	21.53[a]	0.00
Tilled+OM+RD	2.07[b]	0.48[b]	BDL	0.87[a]	0.19[a]	BDL	0.00[a]	BDL	0.00	0.00	BDL	38.47[a]	10.22[b]	0.00
Tilled	1.19[b]	0.31[b]	BDL	0.00[a]	0.00[a]	BDL	0.27[a]	BDL	0.00	0.00	BDL	22.18[ab]	13.37[ab]	0.00
Reduced Till	1.58[a]	0.40[a]	BDL	0.00[a]	0.00[a]	BDL	0.00[a]	BDL	0.00	0.00	BDL	19.15[a]	13.99[a]	0.00
Conventional Till	3.91[a]	0.59[a]	BDL	0.29[a]	0.06[a]	BDL	0.31[a]	BDL	0.00	0.00	BDL	22.74[a]	15.04[a]	0.00

BDL - Below Detected Level

R. solani – *Rhizoctonia solani* AG8, F. pseudo – *Fusarium pseudograminearum*

Means that have no superscript letter in common are significantly different from each other

The SARDI Predicta B DNA analysis found below detectable levels of cereal cyst nematode (*Heterodera avenae*), stem nematode (*Ditylenchus dipsaci*) and the root lesion nematodes *Pratylenchus thornei*, *P. teres* and *P. penetrans*. The root lesion nematode, *P. neglectus*, was found in low levels in all treatments. Similarly below detectable levels of Rhizoctonia (*Rhizoctonia solani* AG8) and Crown Rot (*Fusarium pseudograminearum* and *F. culmorum*) were detected in all treatments. A low level risk of Take All (*Gaeumannomyces graminis var tritici* (Ggt) and *G. graminis var avenae* (Gga)) was detected in the Tilled + Organic Matter treatment. Risk categories should be used as a guide only as they may be subject to regional and seasonal differences. For soils that have been predominately cropped with wheat there is a very low level of common wheat root pathogens present.

Nematode communities are analysed by a trained nematologist which is laborious as it involves microscopic examination of individual nematodes extracted from soil samples and places severe limitations on the number of samples that can be assessed. DNA tests have been developed for some of the most important free-living nematodes in cereal soils that enable nematode communities to be quantified using Predicta-B technology that is now used widely as a diagnostic tool for plant-parasitic nematodes and other soil-borne pathogens. Once these new tests are available, growers will receive more balanced information on the biological status of their soils, as data on beneficial organisms as well as pathogens will be provided.

Comments

The Liebe site free living nematode communities are well balanced in terms of numbers of bacterial and fungal feeding nematodes. However, the site lacks the larger more fragile omnivorous and predatory nematodes which only are present in soils which are well structured and have not suffered from physical and chemical disturbances. A positive point is that the number of omnivorous Dorylaimida in all treatments, and especially those with organic amendments, has significantly increased in comparison to numbers present in 2011.

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