SHORT NON-REFEREED PAPER

SOIL NEMATODE COMMUNITY ANALYSIS IN A LONG-TERM SUGARCANE TRIAL: AN INDICATION OF SOIL ECOSYSTEM STRUCTURE AND FUNCTION

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ABSTRACT

The BT1 long-term sugarcane trial, which was established at Mt Edgecombe in 1939, investigates the effects of residue retention and fertilizer application on crop yield and soil quality. Although these properties are routinely monitored, limited research has focused on the soil ecosystem and its functioning. The nematode community structure of BT1, which is frequently used to infer soil ecosystem health, was characterised shortly after the September 2019 harvest. The biggest difference was observed between the fertilized and non-fertilized treatments, with the nematode community composition of the unfertilized treatments being indicative of a more mature and stable ecosystem. In contrast, the nematode community in the fertilized treatments indicated a less mature and stressed ecosystem, particularly where maximum residue was retained. Fertilization significantly increased the yield, but it also significantly reduced the soil pH, which most likely altered the soil nematode community and potentially masked the effects of other soil factors on the soil nematode community.

Keywords: ecosystem functions and services; nematode community; soil health

INTRODUCTION

Soil health, described as “the continued capacity of the soil to function as a vital living ecosystem that sustains plants, animals and humans” (USDA-NRCS, 2012 https://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/health/), is characterised by the soil’s physical, chemical and biological properties. Biological properties are typically the most difficult to measure accurately. Previous biological studies at BT1 investigated the soil microbial community. Graham (2003) observed a positive effect of fertilizer and residue retention on microbial biomass. Mulching increased microbial activity and the activity of specific soil enzymes involved in nutrient turnover. Furthermore, microbial metabolic efficiency increased with increased residue levels. Wallis (2011) found that soil organic carbon (SOC) and magnesium had a significant association with bacterial structural diversity, while pH and total cations had a significant effect on fungal structural diversity. In addition, SOC, pH and total cations influenced the catabolic diversity of both the bacterial and fungal communities (Wallis, 2011). Mthimkhulu (2017) showed a positive effect of residue on total microbial abundance in the unfertilized plots. Bacterial abundance increased with residue retention, while the fungal abundance decreased. In addition, Mthimkhulu (2017) noted that bacterial and fungal evenness and richness increased in the unfertilized plots, with the increasing residue, but decreased in the fertilized plots.

These studies provided an insight into the effect of the different management practices on soil biology, structure and function. However, the referenced studies examined the total microbial community and did not differentiate between beneficial and non-beneficial microorganisms or identify key groups that could be contributing to this trend. Furthermore, little information was presented on the soil ecosystem health. The use of nematodes, as bioindicators of soil ecosystem health, can potentially bridge that gap. Nematodes occupy key trophic positions in the soil food web, present a broad range of sensitivity to disturbance and are cheaply and easily quantified (with the correct expertise) into different trophic groups (Ferris *et al*., 2001, Neher, 2001). Furthermore, nematode-specific indices are available for the in-depth measurement of soil ecosystem structure and functioning.

The aims of this study were: 1) to assess the soil ecosystem structure and function at BT1 (under different management practices), using nematodes as bio-indicators; and 2) to determine the association between the soil ecosystem and selected soil properties.

MATERIALS AND METHODS

The BT1 trial details and nutritional soil sampling methods (conducted soon after harvest) are described in Mthimkhulu and Miles (2017). Five samples each were taken from two guard rows for nematode extraction two weeks after fertilization. Further details of the extraction methodology can be found in Pillay and Ramouthar (2017). Nematodes were extracted, counted and identified to genus/family level (Marais *et al*., 2017) and the nematode-specific (biotic) indices were calculated, using the online NINJA tool (Sieriebriennikov *et al*., 2014). To compare the treatment effects, the linear mixed model (REML) analysis was used, and where the data were not normal, even after transformation, the non-parametric Man Whitney-U test was used (Genstat 19th edition). A redundancy analysis was performed, using the multivariate analysis tool Canoco 5 to study the relationship between the nematode-specific indices, treatments and soil properties.

RESULTS AND DISCUSSION

Higher total nematode numbers, driven by the herbivores, were observed in the fertilized treatments, when compared with the unfertilized treatments. This difference in herbivores, however, was only significant when residue was retained. Higher yields, and presumably higher root biomass, in the fertilized treatments served as a food source for herbivores. No significant trend was observed for the other trophic groups (Table 1). Higher maturity (MI), enrichment (EI, burnt treatments only) and structure (SI) indices were found in the unfertilized treatments, when compared with the fertilized treatments. Although a consistent trend was observed for all three indices, the only significant difference was observed for the maturity index when no residue was retained (Table 1). The association of omnivores and predators (Fig. 1), as well as the EI, MI and SI (Table 1 and Fig. 1) with unfertilized treatments, is indicative of a mature and stable ecosystem (Ferris *et al*., 2001). The absence of fungivores and omnivores and the low percentage of predators, as well as the low structure and enrichment index in the fully-mulched, fertilized treatment, indicated that the soil was stressed and that the soil food web had degraded (Ferris *et al*., 2001).

Fertilization with N, P and K significantly increased the K levels at all levels of residue retention, but it affected the P levels only where residue was added. No significant effect on N was observed. Soil pH was significantly lower in the fertilized treatments, when compared with the unfertilized treatments across all residue levels (Table 1).

Table 1. The effect of fertilizer on total nematode numbers, nematode trophic groups, biotic indices and soil properties

BIOTIC FACTORS									
Treatment	Nematodes (n)	Herbivores (%)	Bacterivores (%)	*Fungivores (%)	Omnivores (%)	Predators (%)	Maturity index	*Enrichment index	*Structure Index
				Burnt with tops removed					
Unfertilized	765^{ab}	95^{ab}	1.0^{a}	0.1^{b}	3.3^{ab}	0.7^{a}	4.2^{b}	25^{a}	98^{a}
Fertilized	924^{abc}	97^{b}	2.8^{ab}	0^{ab}	0^{a}	0.1^{a}	2.5^{a}	0^{a}	59^{a}
				Burnt with tops retained					
Unfertilized	708^{ab}	90^{ab}	1.8^{ab}	0.3^{b}	6.2^{ab}	1.4^{a}	4.1^{ab}	50^{a}	97^{a}
Fertilized	1574^{c}	98^{b}	1.2^{a}	0.25^{b}	0.4^{a}	0.1^{a}	2.8^{ab}	16^{a}	65^{a}
				Fully mulched					
Unfertilized	547^{a}	85^{a}	6.7^{b}	0^{a}	7.5^{b}	1.3^{a}	3.7^{ab}	0^{a}	98^{a}
Fertilized	1339^{bc}	99^{b}	1.1^{a}	0^{ab}	0^{a}	0.3^{a}	2.7^{a}	0^{a}	35^{a}
p value	0.02	0.002	0.002	<0.001	<0.001	0.05	0.003	>0.05	>0.05
ABIOTIC FACTORS									
Treatment	N (%)	P (mg/l)	K (mg/l)	Ca (mg/l)	Mg (mg/l)	Na (mg/l)	S (mg/l)	C (%)	pH (CaCl2)
				Burnt with tops removed					
Unfertilized	0.19^{ab}	2.65^{a}	43^{a}	3618^{c}	680^{b}	58^{c}	9^{a}	3.7^{ab}	5.1^{c}
Fertilized	0.19^{ab}	14.93^{ab}	104^{b}	2610^{abc}	453^{ab}	45^{abc}	33^{b}	3.7^{ab}	4.4^{b}
				Burnt with tops retained					
Unfertilized	0.18^{a}	2.59^{a}	46^{a}	3540^{bc}	628^{b}	59^{c}	8^{a}	3.6^{a}	5.2^{c}
Fertilized	0.21^{ab}	32.91^{b}	151^{b}	2261^{ab}	334^{a}	34^{ab}	28^{b}	4.0^{b}	4.3^{ab}
				Fully mulched					
Unfertilized	0.21^{ab}	3.29^{a}	44^{a}	3467^{c}	596^{b}	45^{bc}	10^{a}	3.7^{ab}	5.0^{c}
Fertilized	0.22^{b}	32.25^{b}	140^{b}	1656^{a}	340^{a}	32^{a}	36^{b}	3.9^{ab}	3.9^{a}
p value	0.007	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.015	<0.001

Nematodes are the total number of nematodes found in 200g of soil. Nematode-specific (biotic) indices were calculated using the online NINJA tool (Sieriebriennikov *et al*., 2014). Soil properties were analysed by the Fertilizer Advisory Service (FAS). Data were subjected to a linear mixed models (REML) (Genstat 19th edition). Treatments with the same letter are not significantly different at the 5% significance level using the Sidak post-hoc test. Where data were not normal, data were transformed using the $\log_{(x+1)}$ transformation. Back transformed means are presented. *Data were not normal and could not be normalised through transformation. Data were analysed using the non-parametric Man Whitney-U test and medians, instead of the means presented (Genstat 19th edition).

A redundancy analysis was used to further investigate the relationship between the nematode trophic groups, the calculated biotic indices and the measured soil properties. The biggest significant contribution to the variation in the nematode parameters was the application of fertilizer, compared with no fertilizer, which accounts for 47.2 % of the variation observed in the RDA biplots (Fig. 1). Consistent with the REML (Table 1), this was associated with higher total nematode numbers and herbivore occurrence (%). Bacterivore, omnivore and predator occurrence, as well as the nematode indices, were associated with no fertilizer application and a higher soil pH (Fig. 1).

Consistent with the previous biological studies at BT1, the lower soil pH, which is a consequence of long-term fertilisation without liming, was identified as a key driver of soil biological properties. The influence of soil pH on the soil nematode community has previously been described by Chen *et al*. (2015). These authors reported a substantial decrease in free-living nematode trophic groups in a semi-arid grassland, due to soil acidification, and as a result, a reduced food source. They concluded that the positive effects of N fertilization on aboveground biomass, and consequently on below-ground communities, were outweighed by the negative effects of the N fertilizer on soil pH. Zhao *et al*. (2015) showed that the addition of lime to *Eucalyptus* plantations increased soil pH and consequently significantly reduced herbivore densities, while the temporal trends indicated an increase in the bacterial index and a reduced presence of fungivores.

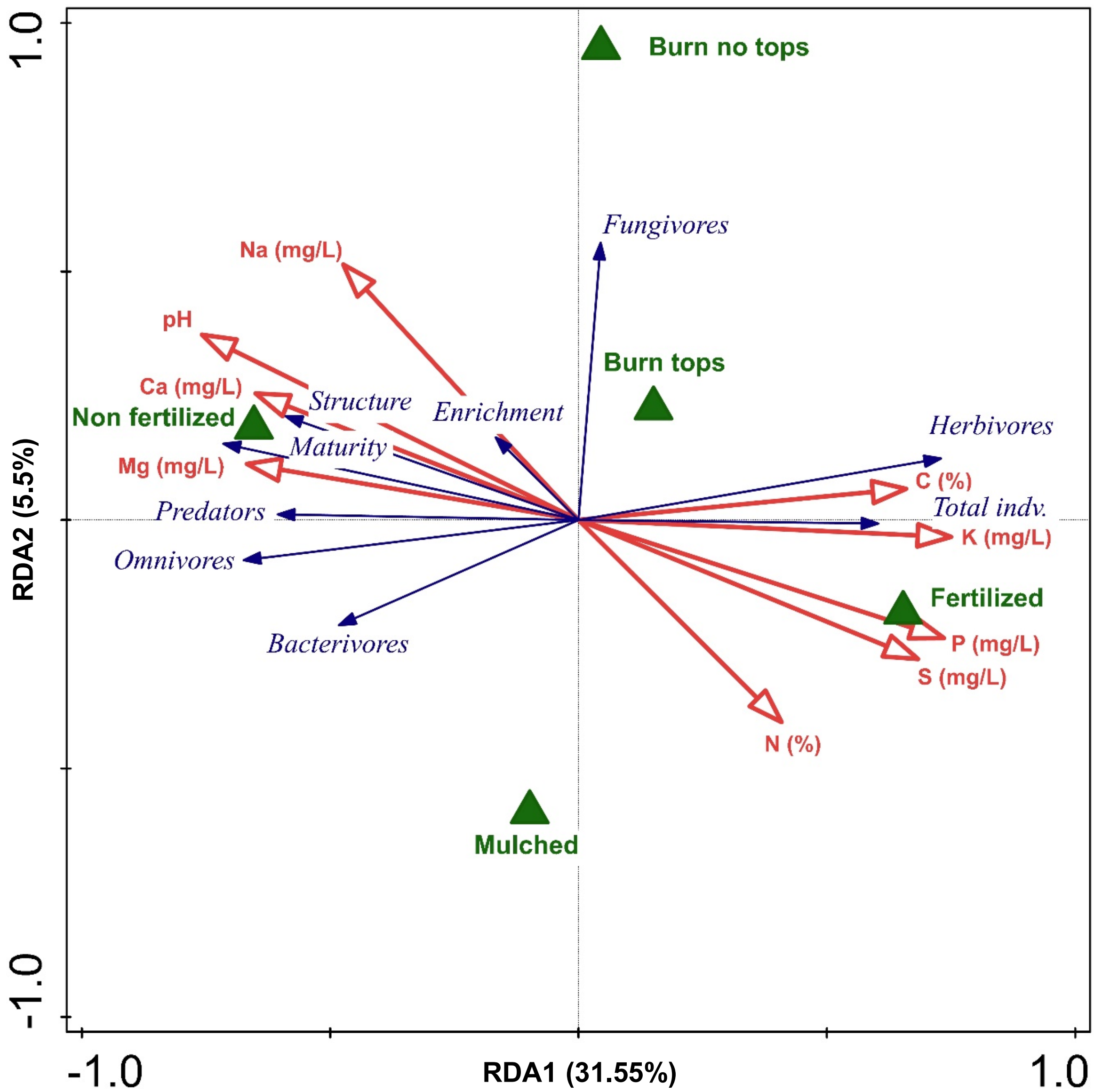

Figure 1. Redundancy-analysis ordination biplot illustrating the relationship between nematode trophic groups, nematode-specific indices and soil properties under different management practices in a long-term sugarcane trial. The RDA 1 and RDA 2 axes accounted for 31.55% and 5.5% of the explained variations, respectively. The green triangles represent treatment centroids, the red arrows

are vectors describing the soil properties and the blue arrows are vectors describing the nematode community properties.

CONCLUSIONS AND FUTURE WORK

This study represents the first assessment of soil health in the BT1 trial, using nematodes as bio-indicators. The results showed the potential of the selected indicators to identify properties that could be influencing the biological soil health. Consistent with previous studies, the dominant effect that was identified was the application of fertilizer, which negatively influenced the soil pH and the associated soil properties. However, it is important to consider that these findings were based on a single sampling event, thus representing a snapshot of the biological community at a certain point in time. Tracking the changes in the soil nematode community over time, with and without the addition of lime, could provide a greater insight into the factors, other than pH, that could also be influencing the soil ecosystem.

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