Using nematodes as bioindicators for soil health in bananas

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Abstract
Agricultural industries are increasingly recognising the link between soil health and the impact of farming operations on the surrounding environment. However, there are few tools available to diagnose and measure soil ecology and the interactions that occur. Soil nematodes integrate the biological, chemical and physical soil properties. Analysis of the nematode community structure may be affected by soil management practices and therefore indicate sustainability of land use. To determine the impact banana cultivation has on soil properties, six paired sites in Queensland were used to compare banana production to less intensively managed plant systems (forest or pasture). The nematode community structure and more conventional soil physical and chemical tests were analysed to detect differences due to soil management. The cultivation of bananas significantly reduced the diversity of nematode genera ($P<0.05$) compared to less intensive systems. Established banana plant systems had a distinct nematode community relative to less intensively managed systems. Nematode community indices were correlated with some chemical properties: total N, pH buffering and electrical conductivity and physical properties: bulk density, surface organic matter and infiltration rate of water. Additional nitrogen fertiliser added to the banana system changed the chemical properties in the soil altering the nematode community structure, increasing the enrichment index and decreasing the number of omnivores present. Similarly, an increase in soil bulk density led to changes in nematode community, increasing the dominance of single genera and increasing the number of omnivores. Soil nematodes demonstrated an excellent potential as bioindicators for soil health, and the analysis of nematode communities would complement information obtained from conventional soil testing.

Key Words
Channel index; Diversity; Enrichment Index; Musa spp.; Paired sites; Structure index.

Introduction
Considerable interest has been expressed in the development of bioindicators to help measure soil health, due to the intimate relationship of biological organisms with their surrounding environment (Neilsen & Winding, 2002). Soil health is defined as "the capacity of a soil to function within an ecosystem, to sustain biological productivity, maintain environmental quality and promote plant and animal health" (Doran & Parkin, 1996). Soil dwelling nematodes are well suited to the role of bioindicators for soil health, being numerous and diverse with a wide range of trophic survival specialisms and acting as integrators of soil properties (Neher, 2001). They are also easy to extract from soil relative to other microorganisms (Yeates & Bongers, 1999). Their main drawback is the problem of identification to species level, but this has been circumvented by the use of trophic group categories and functional guilds which require reduced taxonomic skills (Ferris et al., 2001; Yeates, 2001). The trophic categories and functional guilds have been used to develop a number of indices for assessing nematode diversity, being weighted in favour of the rarer nematode types, whereas other indices have a numerical weighting (Bongers, 1990; Ferris et al., 2001; Yeates, 1999). Abundance of nematodes, trophic classification, Shannon-Weiner diversity, dominance, enrichment index, structure index and channel index have all been used to varying degrees to assess soil management (Berkelmans et al., 2003; Bongers, 1990; Ferris et al., 2001; Hohberg, 2003; Stirling et al., 2004; Yeates, 1999). It is accepted that nematodes of certain functional guilds are present in degraded soils, which form a basal community to compare other nematode communities (Ferris et al., 2001; Ferris & Matute, 2003). The nematode community above the basal community is responsive to chemical and physical soil properties.

Soil factors influencing nematode communities include nutritional enrichment, carbon conservation and physical changes to the soil structure caused by agricultural operations (Gupta & Yeates, 1997). Nutrient enriched soils show a reduced biodiversity; under such conditions the populations of short-lived r-strategists (bacterial feeding Rhabditae, Pangrolaimidae and Diplogastridae) increase relative to other nematode groups (Ferris & Matute, 2003). Soil disturbance, either tillage or chemical, such as fertiliser application, alters the structure of the soil ecosystem, discriminates against larger nematodes, often predatory or omnivorous nematodes, that are slower to reproduce and have a longer life cycle (K-strategists). In general, agricultural operations tend to favour a higher proportion of plant-parasitic nematodes and the reduction in diversity and abundance of their predators (Kimpinski & Sturz, 2003; van Bruggen & Termorshuizen, 2003). Agricultural practices that have a reduced soil disturbance effect may be expected to show a more stable nematode community structure with long term implications for crop health and sustainability.

There is increasing pressure on banana farmers to optimise their fertiliser use to avoid loss of nutrients from the root zone of their crops (Kleiese et al., 1997; Moody & Aitken, 1997; Rasiah & Armour, 2001). There may be other benefits from balancing agricultural inputs with crop needs, such as the reduction in plant-parasitic nematodes (Kimpinski & Sturz, 2003). However, there are few ecological tools sensitive enough to detect changes in soil management. Soil dwelling nematodes and the indices derived from analysis of their community structure may be able to demonstrate that changes in soil management are either beneficial or deleterious to the soil ecology. There is a need to ensure that nematode indices are related to physical and chemical measurement of soil properties. This paper investigates how soil dwelling nematodes can be used to investigate differences in plant systems and the relationship between soil nematodes and other soil physical and chemical properties in Queensland banana growing operations.

Methods

Survey sites

The soil properties from four conventional banana fields in north Queensland near Innisfail, Mission Beach, Tully and Kennedy and two in south-east Queensland, near Wamuran and Nambour were compared with different plant or management systems: organic banana production, pasture or forest between July and September, 2003 (Table 1). The sites represented the major soils and banana production regions occurring in Queensland with the soil classifications listed in table 1.

Table 1. Soil classification and soil texture of survey sites sampled.

Site	Location	Soil type	Vegetation	Texture classes 0-10 cm (%)		
				Clay	Silt	Sand
East Palmerston	North Queensland	Ferrosol	Banana	45	36	19
			Pasture	41	31	28
Kennedy	North Queensland	Dermosol	Banana	29	21	50
			Forest	20	21	59
Mission Beach	North Queensland	Ferrosol	Banana	49	27	24
			Forest	30	15	56
			Organic banana	43	30	27
Tully	North Queensland	Dermosol	Banana	25	22	53
			Forest	20	17	63
			Pasture	20	30	51
Nambour	S.E. Queensland	Dermosol	Banana	31	22	47
			Pasture	32	19	49
Wamuran	S.E. Queensland	Podsol	Forest	11	28	61
			Organic banana	19	31	50
			Plant banana	14	29	57

Sampling method

Four replicate soil cores were taken from each plant systems at each site, using a 50 mm soil corer. Samples were collected at depth intervals 0-10, 10-20, 20-30, 30-50, 50-70 and 70-90 cm below the soil surface. The soil samples were collected within the plant rows of banana plants, 15 cm from banana plants. Soil samples in the undeveloped plant systems were taken at random intervals along the same contour as the banana soil samples. Samples were stored in cooled foam boxes until they were delivered to the laboratory. Sub-samples were immediately extracted in a field moist state for nematode composition to avoid changes due to drying and storage.

Physical soil measurements
The silt and clay content of the soil was determined down the soil profile to ensure similar soil types at each paired site. Soil bulk density was determined down the soil profile, 0-5, 5-10, 10-15 and 15-20 cm increments at the three sites in north Queensland, Mission Beach, Tully and Kennedy. Further soil bulk density measurements, 0-15cm, were measured separately at Mission Beach, Tully, East Palmerston and Kennedy, using the method described by Sarrantonio *et al*., (1996). Water infiltration rates were determined using a double ring infiltrometer at Mission Beach, Kennedy and Tully. A single ring infiltrometer was used separately at Mission Beach, Tully, East Palmerston and Kennedy. However, only the single ring infiltrometer readings are reported in this paper. Surface organic matter was determined by collecting litter on the soil surface from a 0.1 m^2 quadrat. The litter was dried for 3 days at 70 ^oC before being weighed.

Chemical soil measurements
The remainder of the soil samples were air dried at 40°C and sieved to <2mm. Samples were analysed for pH (1:5 water and 0.01 M $CaCl_2$), electrical conductivity, mineral N (nitrate), organic carbon, labile carbon and exchangeable cations (Na, K, Ca and Mg). All methods of analysis were those described by Rayment & Higginson (1992). Analysis of C and N, were determined using a Leco analyser. For consistency, the soil chemical results reported are only from the top 10 cm depth.

Soil nematodes
Soil nematode community analysis was determined by placing 200 g of field moist soil on a single layer of tissue, contained within a mesh basket. The basket was placed in 200 mL of water within a tray. After 24 hours nematodes contained within the water of the tray were collected on a 25 µm sieve (Whitehead and Hemming, 1965). The nematodes were backwashed from the sieve and collected in a 30 mL vial. The total number of nematodes extracted from 200 g of soil was determined. Nematodes were identified to genera and plant-parasitic nematodes identified to species using a compound microscope and assigned to trophic groups according to Yeates et al. (1993). The Shannon-Weiner diversity index and dominance index were calculated from identification data (Yeates & Bongers, 1999). Additionally, the weighted faunal analysis concept was applied, without plant feeders, to determine the basal, structure and enrichment conditions of the soil food web (Ferris et al., 2001). This concept uses the structure, enrichment and channel indices. The structure index is a measure of the number of trophic layers in the soil food web and the potential for regulation by predators. The enrichment index is an indicator of the resources available to the soil food web and response by primary decomposers to those resources. The channel index, is an indication of the decomposition channel of nutrients, a low value suggested a primarily bacterial decomposer community and high value a fungal dominated decomposer nematode community (Hohberg, 2003).

Statistics
Means from sampling sites were analysed using REML and separated using χ^2 test using Genstat 5. Total nematode numbers and number of nematodes in each trophic group per g of soil were transformed using ln(x+1), prior to analyses, to comply with assumptions of normally distributed data. Back transformed means were presented. The number of nematodes in each trophic group was investigated with a multivariate cluster analysis. This allowed the construction of a dendrogram using the group average method to determine the similarity between crops and sampling sites. Sites linked at a Euclidean distance of 0.85 were considered to be in the same group. Simple linear correlation of soil properties and nematode measurements were determined using the statistical package Genstat 5.

Results and discussion
An analysis of the soil texture down the soil profile at all sites revealed no large differences in the clay and silt content, except at the Mission Beach site (Table 1). The undeveloped Mission Beach site had a different silt and clay content relative to conventional bananas and the organic banana sites down the soil profile (data not shown). This suggested that there was a change in soil type, which may have affected the soil properties.

The type of plant system strongly influenced the trophic group and diversity of soil nematodes. Conventional banana production had a significantly lower diversity of nematode genera than less

intensive land uses, such as pastures or forest (Table 2). There were significantly more plant-associated and omnivorous nematodes in the pasture soil relative to other plant systems, with the exception of the newly planted (plant) banana crop (Table 2). This may have been due the differences in root structures under the different plant systems and also due to weed growth in the plant crop of bananas.

The similarity between nematode communities using a cluster analysis of the nematodes in each trophic group suggested that there were two similar groups of plant systems, with greater than 85% similarity (Figure 1). One group contained all but one of the banana farms surveyed and included conventional and organic crops, but excluded the plant banana crop (Figure 1). However, first group contained the forest soil at Tully. This suggested that there may have been some contamination of the forest soil or the forest was significantly disturbed. The second group contained the remaining pasture and forest sites in north and south-east Queensland, except the forest soil at Wamuran (Figure 1). The forest soil at Wamuran had a nematode community that was not similar to either of the main groups (Figure 1). This result suggested that the type of plant systems strongly influenced the nematode community in the soil. The intensively managed monoculture of bananas had a distinct nematode community relative to the less intensive, heterogenous plant systems.

Table 2. Nematode diversity and number of plant associated and omnivorous nematodes per g of soil in three banana crops compared to less intensive plant systems.

Crop	Number of sites	Shannon-Weiner Diversity index		Nematodes per g soil			
				Plant associated		Omnivorous	
Banana	5	1.35	a	0.19	a	0.26	a
Organic banana	2	1.48	ab	0.27	ab	0.20	a
Plant banana	1	1.74	ab	0.94	bc	0.47	ab
Pasture	3	1.97	b	1.30	c	0.90	b
Forest	4	2.07	b	0.47	ab	0.46	a

(Means with the same subscript are not significantly different at the 5% level)

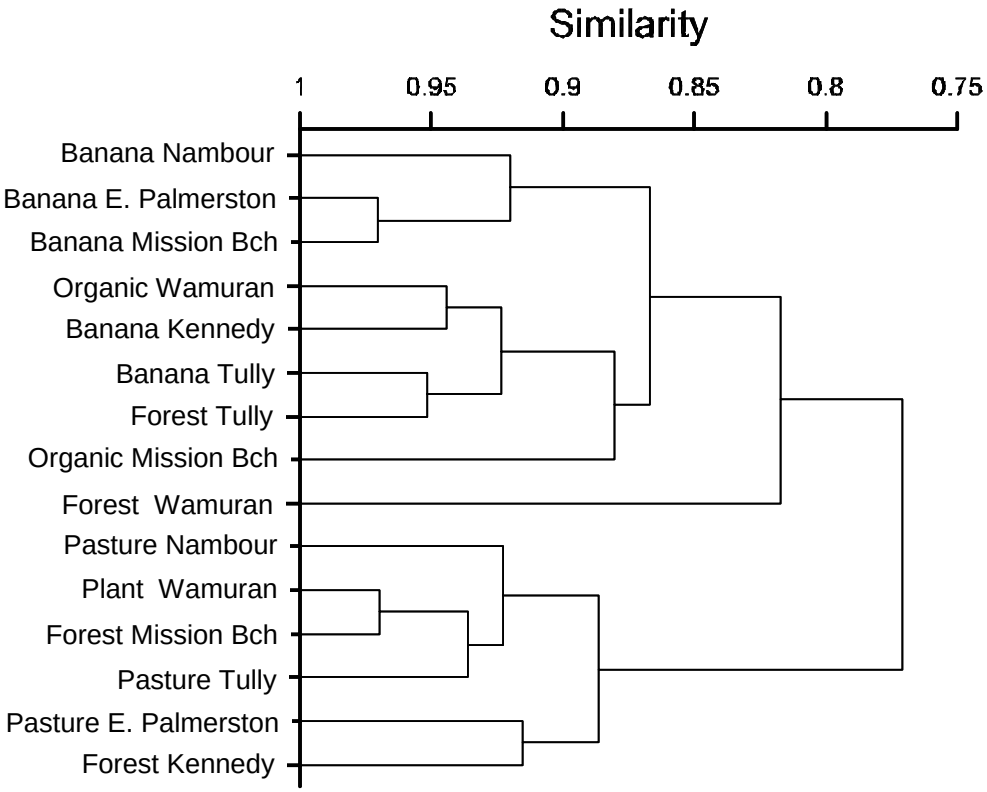

Figure 1. Cluster analysis dendrogram of nematode trophic groups comparing banana fields to less disturbed plant systems at four sites in north Queensland and two in south-east Queensland.

The enrichment index, an indicator of the resources available to the soil food web and the response of primary decomposing nematodes to those resources, was significantly lower at the two south-east Queensland sites, Nambour and Wamuran, relative to the four north Queensland sites (Table 3). The lower enrichment index suggested that there were fewer nutrients available for soil microorganisms at the

two sites in south-east Queensland, which was consistent with growing systems using lower nutrient inputs less organic matter turnover relative to tropical production systems. The East Palmerston site similarly had a lower enrichment index relative to other north Queensland sites.

The structure index, a measure of the number of trophic layers in the soil food web and the potential for regulation by predators, was lower at the East Palmerston and Kennedy sites relative to the other sites, with the Tully site being intermediate (Table 3). The lower structure index suggested a more disturbed and simplified food web, with less regulation of nematode numbers by predators relative to the other sites (Table 3). However, the survey was not balanced in regards to the intensification of land use. The Mission Beach and Tully sites may have had a higher structure index due to there being only one site of conventional banana production and two sites of less intensive plant productions systems sampled at Mission Beach and Tully. At the other sites conventional banana production was compared to only one other plant system.

Table 3. Nematode food web indices, enrichment and structure index, determined by analysis of nematode genera and life strategies averaged over different plant systems, bananas, forest and pasture.

Site	Enrichment index		Structure index	
East Palmerston	57.0	b	55.8	a
Kennedy	73.1	bc	55.7	a
Mission Beach	72.2	c	80.5	b
Tully	76.6	c	72.5	ab
Nambour	29.1	a	87.4	b
Wamuran	14.8	a	75.0	b

(Means with the same subscript are not significantly different at the 5% level)

The soil environment significantly impacts on soil dwelling nematode communities. No single nematode index was universal in indicating the difference in soil health, but rather soil health requires a more in-depth understanding of the nematode community composition, both trophic groups and life strategies. A multivariate analysis of nematode communities and the use of indices would prove most useful in determining the soil health status using nematodes as bioindicators (Ritz & Trudgill, 1999). Soil nematodes, as bioindicators of soil health, would not replace current soil chemical and physical tests, but would supplement information obtained and increase the understanding of the soil ecology and the effects of soil management.

Nematodes respond differently to soil disturbance and therefore changes the nematode community composition (Gupta & Yeates, 1997; Yeates & Pattison, 2004). The structure index is an indicator weighted to increase the impact of long living, slow reproducing nematodes (K strategists) in the community (Ferris et al., 2001). There was no significant impact of cropping systems on the structure index. However, the structure index was significantly different between sites sampled. The East Palmerston and Kennedy sites had a significantly lower structure index, which suggested that the soil food web was more disturbed than the other sites.

The environments that bananas are grown in is typically wet and warm, which influences the organisms present in the soil. The tropical environment is not commonly reported in soil health studies, which tend to investigate soil ecology in temperate environments, and as a result soil ecology in the wet tropics may develop unique relationships between soil parameters that are not typical for other climates. For example, soils in north Queensland had a higher enrichment index relative to south-east Queensland. This may be due a more continual turn over of organic matter in north Queensland with continual decomposition of organic matter and a greater rate of recycling within the soil environment (Hu et al., 1997).

There was a significant correlation between nematode indices and trophic groups and a number of soil chemical and physical soil properties (Table 4). Total N in the soil was positively correlated with the enrichment index and negatively correlated with the number of omnivores in the soil. This was not unexpected as the enrichment index is a measure of the available resources for soil organisms. Conversely, the number of omnivores was reduced with increasing total soil N. Omnivores tend to be larger nematodes, with longer generation times (K strategists) and their reduction with increasing total N may be due the impact of intensive agricultural activities and an increase NO_3-N contributing to the total N pool.

The number of omnivores in the soil was positively correlated with increasing soil bulk density in the top 15 cm of soil (Table 4). This was unexpected due to the large size of the nematodes, but may reflect food resources available to the nematodes in the more compacted soil. In soils with a higher bulk density there tended to be a stronger dominance of nematode genera (Table 3). This is presumably due to nematodes that are able to survive in compacted soils, either due to changes in habitat or food resources, being able to out compete other nematodes and therefore dominating the nematode community composition.

The pH buffering capacity was negatively correlated with the structure index and the diversity of nematodes (Table 4). Conversely pH-buffering capacity was positively correlated with the dominance index of nematodes and the ratio of bacterial feeding to fungal feeding nematodes. This may be an indirect correlation as pH-buffering capacity is dependent primarily on clay content, clay type and organic matter (Moody & Aitken, 1997). Therefore, the correlation between pH buffering capacity and nematodes indices may be viewed as a fortuitous relationship or due to an indirect correlation possibly with soil organic matter.

The amount of surface organic matter was positively correlated to a high ratio of bacterial to fungal feeding nematodes (Table 4). This is not unexpected, as bacteria would presumably initially decompose organic matter added to the soil surface in the wet environment where bananas are grown. The more recalcitrant organic matter would be decomposed by fungi following the loss of labile nutrients. The higher surface organic matter suggested there is a continual deposition of organic matter, which would require a continual degradation by bacteria of the surface residue.

Electrical conductivity was positively correlated to a high ratio of bacterial to fungal feeding and herbivorous nematodes in the soil, and negatively correlated to the channel index, an indication of the pathways which nutrients are decomposed (Table 3). This is probably due to electrical conductivity being an indicator of fertiliser use in high rainfall regions where bananas are grown commercially. The higher electrical conductivity suggested higher fertiliser use and availability of nutrients, leading to an increase in the proportion bacterial feeding nematodes. Similarly, there was a negative correlation with the channel index, as a lower channel index is indicative of a bacterially dominated pathway in the decomposition of nutrients. The increased number of plant-parasitic nematodes with increasing electrical conductivity in the soil may be due to higher number of plant-parasitic nematodes, which are found on banana farms where fertilisers are used. Whereas, in less intensive plant systems, pastures and forest, there is less fertiliser usage, resulting in a lower electrical conductivity and a greater mix of plant systems, which reduce the number of plant parasitic nematodes.

There was a positive correlation between the infiltration rate of water and the number of fungal feeding nematodes (Table 4). Poorly drained soils may be less favourable for fungal growth, whereas well-drained soils appear more favourable for fungal growth and grazing by fungivores.

Table 4. Correlations between soil physical and chemical properties and soil nematode poulations and indices

Soil parameter	Nematode parameter	R^2
Total N	Enrichment index	0.61
Total N	Omnivores	-0.61
Bulk density (5-10 cm)	Dominance index	0.71
Bulk density (0-15 cm)	Omnivores	0.75
pH buffering	Structure index	-0.69
pH buffering	Diversity	-0.84
pH buffering	Dominance	0.60
pH buffering	B:F ratio	0.63
Surface organic matter	B:F ratio	0.63
Electrical conductivity	B:F ratio	0.73
Electrical conductivity	Channel index	-0.64
Electrical conductivity	Plant-parasites	0.59
Infiltration rate	Fungivores	0.84

Bananas grown in monoculture tend to favour a higher population of plant-parasitic nematodes, with a decreased diversity relative to less intensive plant systems such as pastures and forest. This maybe due to

the pastures and forest sampled containing a heterogeneous mix of plant species and therefore not allowing the dominance of the nematode community by plant-parasites. Also, the low nutrient regimes, high organic matter and minimal soil disturbance may contribute to lower populations of plant-parasitic nematodes. The nematode trophic groups under bananas at different locations were closely related and distinct from the nematode communities under pastures and rainforest. There were a few exceptions to this relationship in this study, which could be explained by the cropping history, the plant banana crop having been recently established and the forest site at Tully through contamination by the neighbouring banana crop. The higher diversity under the less intensively managed plant systems relative to bananas suggested there is a more complicated food web and greater interaction between organisms in less intensive plant systems relative to monoculture bananas. Therefore, there would be a greater reliance on nutrient recycling in less intensive plant systems.

Nematodes communities can be used to develop a number of indices that are able to infer the health of the soil ecosystem (Bongers, 1990; Ferris et al., 2001; Yeates, 1999; Yeates & Bongers, 1999). Nematode communities are related to physical and chemical soil properties, due to the effects the soil properties have on the nematodes directly, such as habitat or due to the influence on the nematode food supply (Neher, 1999). There are also a number of indirect relationships between nematodes and the soil environment. The application of nutrients, such as nitrogen, may affect the electrical conductivity of the soil (Sarrantonio et al., 1996), which in turn is related to a number of nematode indices, such as the ratio of bacterial to fungal feeding nematodes. The increase in the availability of nutrients tends to favour a bacterially dominated environment, which favours bacterial feeding nematodes as consumers of soil bacteria. However, excessive nutrient availability increases the number of nematodes that are capable of responding quickly to the increased food supply and therefore increases the enrichment index. Increased nutrient availability may increase root growth, which in turn may increase the resources available for plant-parasitic nematodes and reduce population controls by predation and parasitism.

Caution must be exercised when investigating the simple correlations between soil properties as they may be due to indirect relationships. A direct relationship between pH buffering capacity and nematode community indices is not expected. However, there are a number of soil factors such as organic matter and proportion of clay that may influence pH-buffering capacity and the composition of the nematode community. The significant correlation between pH buffering capacity and nematode indices is most likely due to soil properties that are able to influence both indices. This study was able to demonstrate changes in nematode community structure in different plant systems and the relationship between plant systems and the nematode communities, however, it was not able to determine cause and affect relationships. This would require more intense replicated studies.

Interpretation of soil ecology measurements in agriculture must ensure that differences in parameters are due to changes in land management when comparing paired sites. Differences in soil type will affect many soil properties, so it is important to establish soil type similarities. Soil nematode ecology is a factor of soil type, vegetation, soil chemical and physical properties and is responsive to changes in these properties.

Conclusion

The indices that are calculated from analysis of nematode genera provide an excellent and responsive indication of the effects of soil management on soil ecology. The nematode community that develops under an agriculturally managed monoculture such as bananas is distinct from that of less intensive plant systems such as pasture and forest. The management of the soil will also impact on the nematode community structure. Increased fertiliser use may increase total N and also the electrical conductivity in the soil. This results in an increase in the nematodes that are able to respond to the increased resources and increases the proportion of bacterial feeding nematodes in the community. Nematode community analysis is a powerful tool that can be used together with more conventional soil physical and chemical tests to develop a deeper understanding of how soil management impacts on the health of the soil.

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