RESEARCH ARTICLE

Soil nematode responses to fertilization with ammonium nitrate after six years of unfertilized apple orchard

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Abstract

A nematode community was used as a bioindicator of changes in agroecosystems caused by fertilization. The effect of applying nitrogen (N) fertilizer on a soil nematode community structure was studied in a soil which had not been fertilized for six years in the Rio Negro Valley, Argentina. Treatments were: i) 100 kg N ha^{-1} (N100); ii) 200 kg N ha^{-1} (N200); in each case 50% of the dosage was applied at the time of petal fall and 50% at fruit harvest in 2004/2005, 2005/2006, 2006/2007 growing seasons; and iii) control with no fertilizer (N0). Soil samples were collected in the 0 to 30 cm soil layer in October, November, April and July in each growing season. The number of bacterivores increased in N200 compared to N0. Cephalobidae were present in greater numbers in N200 than in N100 and N0. Predator abundance was lower under N200, after the first N application in each growing season. The ratio of fungivores to bacterivores (F/B) was 0.21, 0.3 and 0.41 in N200, N100 and N0, respectively. N200 resulted in a community with a lower maturity index (MI) than N0. Structure index (SI) was lower in N200 than N100 and N0. The enrichment index (EI) was less sensitive at detecting fertilizer effects. In November and April, soil nitrate concentrations were higher in N200 than in N100 and N0. Soil nitrate concentration was positively correlated with EI and negatively with MI, SI and F/B. N200 affected the trophic structure of the nematode community consistent with a less stable soil system.

Additional key words: ecological indices; soil food web; bioindicator; nitrogen fertilizer; *Malus domestica*.

Introduction

In Argentina, the apple (*Malus domestica* Borkh) growing region is located in the Upper Valley of Río Negro and Neuquén Provinces, and in the Middle Valley of Río Negro province. This region concentrates 80% of production with an estimated 21,291 hectares of apple orchards. The most widespread apple variety is Red Delicious and clones (Altube *et al.*, 2007).

Nitrogen (N) fertilization is one of the most basic components of an apple orchard management program due to low nutrient levels in the Patagonian soils. Farmers normally apply it in excess, approximately 100 to 200 kg N ha^{-1} per growing season (Weinbaum *et al.*, 1992). The highest N requirement for apple trees is in

the spring, during cell division, at which time N application stimulates canopy growth and fruit enlargement. The application of N is also recommended in the summer or early in the fall, after fruit harvest, in order to raise the N content of the developing blossoms and tree reserves. An oversupply of N can lead to excessive vigor and poor fruit-quality characteristics (Silva & Rodríguez, 1995).

Nematodes have been evaluated for their ability as indicators of soil diversity and functioning due to several attributes they possess (Neher, 2001). They live in capillary water, their permeable cuticle providing direct contact with their microenvironment. They also occupy key positions in soil food webs. They are transparent and since their feeding habits are clearly rela-

Abbreviations used: Ba (bacterivores); Ca (carnivores); c-p (colonizer-persister); EI (enrichment index); Fu (fungivores); H (plant parasites); MI (maturity index); N0 (no fertilizer); N100 (100 kg N ha^{-1} yr^{-1}); N200 (200 kg N ha^{-1} yr^{-1}); Om (omnivores); PPI (plant parasite index); SI (structure index).

ted to oral structure, their trophic roles are inferred (Neher, 2001). Their specific diversity and numerical abundance places them among the favored organisms for assessing the environmental impact of anthropogenic changes (Yeates *et al.*, 1991; Yeates, 2004). Nematode community structure and function are known to change in response to land management practices such as nutrient enrichment through fertilization by inorganic N (Gruzdeva *et al.*, 2007; Li *et al.*, 2010), and nematode indices allow us to determine the effects of environmental stress and dominant decomposition channels (Ferris *et al.*, 2001; Vestergard, 2004; Forge *et al.*, 2005).

In previous studies the nematode community maturity index (MI) was lower in grassland soils with the application of 100 kg N ha^{-1} than in the control (Forge *et al.,* 2005). Li *et al.* (2010) observed that the soil food web was degraded due to the lower soil pH and high electrical conductivity induced by application of 600 kg N ha^{-1}. In the Upper Río Negro Valley the effects of multiple-year application of N fertilizers produced changes in bacterivores and therefore the fungivore-bacterivore ratio was lower in treated than in untreated plots. MI was negatively correlated with soil nitrate concentration and the ratio of plant parasite index to maturity index (PPI/MI) was higher in plots treated with 150 kg N ha^{-1} than in unfertilized plots (1.52 *vs* 1.17) (Azpilicueta *et al.,* 2008). At PPI/MI ratios of 1.6 the soil might be considered severely nutrient-enriched (Bongers *et al.*, 1997). After soil agricultural disturbance, the diversity and maturity of nematode communities generally increased with the age of abandoned fields (Hánel, 2010).

The objective of this study was to identify changes in the soil nematode community structure after the application of ammonium nitrate during three growing seasons, in a soil which had not been fertilized for 6 years. The hypothesis tested was that the effect of this land management practice could be detected by an increase in the abundance of soil free-living organisms such as bacterivores and fungivores, and a decrease in the omnivores-predators.

Material and methods

Experimental site and design

The research was conducted in an orchard located in the Rio Negro Valley, Argentina (38° 56' S, 67° 59' W). In 1996, apple trees *cv*. Red Delicious were planted at 3.7 × 2.7 m. The area used for the study had not received N fertilizer from 1998 to 2004.

The climate of the zone is mild continental and arid with a mean annual temperature of 14°C and annual precipitation of 237 mm. The warmest month is January and the coldest is July. The original material of these soils is of alluvial. The moisture regime is aridic and the temperature regime is thermic. This shows that the zone suffers from water deficit during the year. The soil at the study site belongs to Aridisol classified as typic Aquicambid (Soil Survey Staff, 2006). In the upper 30 cm of soil the total N was 1.6 g kg^{-1}, the organic matter was 29 g kg^{-1}, and the pH(H$_2$O) was 7.1. The soil contained 15% sand, 58% silt and 27% clay.

Treatments were: (N100) 100 kg N ha^{-1}, (N200) 200 kg N ha^{-1}, both with half the dosage in each case applied at the time of petal fall (October 5th) and the other half at fruit harvest (March 15th) in 2004/2005, 2005/2006, 2006/2007 growing seasons; and (N0) control with no fertilizer. The N was applied as ammonium nitrate and was distributed on the soil surface in a band 20 cm wide on both sides of the plants, 50 cm from the trunk. The fertilizer was incorporated to a depth of 20 cm using a rotary tiller. Control plots were also tilled. Nine plots were arranged in a randomized complete design with three replicates per treatment. Each plot consisted of a row of 36 consecutive trees. They were irrigated by flooding and received water once a week, during spring, summer and fall. Weeds were removed by hand when necessary.

Sampling, extraction and identification of nematodes

A composite soil sample was collected in each row from the adjacent soil of the six central trees (observational unit). Each composite soil sample was made by combining six cores (2.5 cm in diameter and 30 cm in depth), which were taken alternately from each side of the trees. The samples were refrigerated and processed within 7 days of sampling. Soil samples were collected in October at the beginning of the experiment, 4 weeks after the first and second application of fertilizer (November and April) and 14 weeks after the last application (July), in the following growing seasons: 2004/2005 and 2005/2006. In 2006/2007, soil samples were collected in October, 3 and 4 weeks after the first and second applications of fertilizer, res-

pectively, and 14 weeks after the last application. Nematode populations were extracted from a subsample of 100 g (fresh weight) using the sugar flotation and centrifugation method (Caveness & Jensen, 1955). The nematode populations were expressed per 100 g dry weight soil. Soil moisture was determined gravimetrically by drying the samples at 105°C for 24 h. The recovered nematodes were counted and preserved in formalin. A total of 50 specimens per sample were randomly selected and identified to genus or family level. Nematode counts for each taxon were adjusted to the number of nematodes per 100 g dry soil. The classification of nematode trophic groups was assigned as: bacterivores (Ba), fungivores (Fu), plant parasites (H), omnivores (Om) and carnivores (Ca) according to Yeates *et al*. (1993). The characteristics of nematode communities were described using the following approaches: (1) absolute abundance of individuals per 100 g dry soil; (2) trophic groups; (3) F/B, ratio fungivores to bacterivores; (4) MI, maturity index, calculated as $MI = \Sigma v_i * p_i$, where v_i, is the colonizer-persister (c-p) rating of taxon i according to the 1-5 c-p scale (Bongers, 1990); (5) PPI, plant parasite index: $PPI = \Sigma v_i * p_i$, where v_i, is the c-p value of plant parasitic nematodes assigned by Bongers (1990) to the i-th nematode taxon and pi is the proportion of the taxon in the nematode community; (6) the ratio PPI/MI (Bongers *et al*., 1997), (7) the enrichment index (EI) and the structure index (SI) were calculated from the data on relative abundance of taxa with differing c-p rankings in different trophic groups, according to Ferris *et al*. (2001). EI assesses food web responses to available resources and SI is a measure of structural complexity of the nematode community, that is, the degree to which communities are represented by a diversity of functional guilds. These indices are based on functional guilds that represent nematode taxa with the same feeding habits, and inferred function, in the food web. Bax, Fux, Cax and Omx (where $x = 1$-5) are functional guilds of nematodes that are bacterivores, fungivores, carnivores or omnivores, where the guilds have the character indicated by x on the c-p scale.

Determination of soil nitrate

Soil nitrate (NO_3^--N) was measured in the same soil samples from which nematodes were extracted in October, November and April in the three growing seasons. A soil subsample of 100 g fresh weight was homogenized with 100 mL of 0.01 M $CaCl_2$ solution, shaken for 30 min and filtered through nitrate-free filter paper. Nitrate concentrations were measured reflectometrically (RQFlex, Merck). Soil NO_3^--N concentrations were expressed per 1000 g dry weight soil.

Statistical analysis

The number of individuals per trophic group and nematode taxa abundance were evaluated fitting generalized linear models (McCullagh & Nelder, 1989) with negative binomial random component, canonical link function and linear predictor accounting for effects of growing season, sampling time and treatment with the R software (http://www.r-project.org/). Soil nitrate concentration and the ecological indices were analyzed through an analysis of variance (ANOVA) model (Treatment × Sampling time × Growing season) to determine the between-subject effects. Differences obtained at the level of $p < 0.05$ were considered significant using Tukey test. A Pearson's correlation analysis was performed using Infostat (Di Rienzo *et al.,* 2009) to quantify the association between soil nitrate concentration and nematode community indices.

Results

Nematode faunal structure

During the study period, 25 nematode taxa belonging to 12 functional guilds were identified (Table 1). The total nematode density ranged between 60 and 411 individuals per 100 g dry soil. The maximum value occurred in N200 and the minimum in N0. Fertilizer × Sampling time × Growing season date interaction had a significant effect on the total number of nematodes (Table 2). The abundance of total nematodes was higher in N200 than in N0, in April 2005 and October 2006 (data not shown).

Bacterivores were the most abundant trophic group in N100 and N200, averaging 39% and 42% of the nematode community, respectively. The proportion of plant parasites was higher than other trophic groups in N0. Omnivores plus predators made up 12-19% of the soil nematode community. Among the nematode taxa, Cephalobidae, *Pratylenchus* and *Aporcelaimellus* were dominant across all treatments while Rhabditidae

Table 1. Abundance of nematode taxa, and trophic groups per 100 g dry soil for three different levels of N fertilizer treatments. Each data (mean ± SE) was obtained by averaging across three years (2004-2005, 2005-2006, and 2006- 2007) with four sampling times per year and three replications per sampling time (n = 36)

Taxon	Guild[2]	Treatment[1]		
		N0	N100	N200
Bacterivores		**62 ± 5.2** (30%)	**75 ± 7.3** (39%)	**94 ± 10.1** (42%)
Cephalobidae	Ba_2	28 ± 2.8	27 ± 2.6	36 ± 2.6
Rhabditidae	Ba_1	27 ± 3.4	38 ± 5.4	50 ± 8.1
Panagrolaimidae	Ba_1	3 ± 1.0	7 ± 1.8	5 ± 1.5
Monhysteridae	Ba_2	2 ± 0.4	1 ± 0.8	2 ± 0.4
Plectidae	Ba_2	2 ± 0.4	1 ± 0.2	1 ± 0.3
Diploscapteridae	Ba_1	—	1 ± 0.2	0.2 ± 0.1
Diplogasteridae	Ba_1	—	—	0.4 ± 0.1
Fungivores		**17 ± 1.5** (8%)	**18 ± 2.0** (9%)	**17 ± 1.8** (8%)
Aphelenchus	Fu_2	7 ± 1.0	7 ± 1.4	6 ± 1.1
Aphelenchoides	Fu_2	5 ± 0.8	6 ± 0.9	8 ± 1.1
Diphtherophora	Fu_3	4 ± 0.9	4 ± 0.8	2 ± 0.5
Tylencholaimus	Fu_4	1 ± 0.0	1 ± 0.0	1 ± 0.1
Plant-parasites		**91 ± 7.1** (45%)	**63 ± 5.2** (33%)	**81 ± 5.9** (37%)
Boleodorus	H_2	38 ± 3.9	13 ± 1.4	28 ± 3.6
Pratylenchus	H_3	29 ± 3.5	21 ± 1.8	23 ± 2.9
Tylenchidae	H_2	10 ± 1.5	9 ± 1.7	13 ± 2.4
Criconematidae	H_3	7 ± 1.4	5 ± 0.8	5 ± 1.2
Hemicycliophora	H_3	4 ± 0.9	12 ± 3.2	7 ± 1.6
Paratylenchus	H_2	2 ± 0.7	2 ± 0.6	2 ± 0.7
Tylenchorhynchus	H_3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Helicotylenchus	H_3	1 ± 0.5	1 ± 0.9	3 ± 1.1
Meloidogyne	H_3	0.2 ± 0.5	0.1	0.1
Xiphinema	H_5	0.1 ± 0.5	0.3	0.1
Omnivores		**3 ± 0.7** (2%)	**6 ± 1.4** (4%)	**4 ± 0.8** (3%)
Dorylaimoidea	Om_4	3 ± 0.7	6 ± 1.4	4 ± 0.8
Predators		**30 ± 3.2** (15%)	**29 ± 3.2** (15%)	**25 ± 4.1** (11%)
Aporcelaimellus	Ca_5	28 ± 3.3	26 ± 3.1	22 ± 3.7
Mylonchulus	Ca_4	1 ± 0.3	3 ± 0.8	1 ± 0.5
Seinuridae	Ca_2	1 ± 0.3	0 ± 0.0	2 ± 1.3
Total		**203 ± 13**	**191 ± 13**	**221 ± 14**

[1] Treatment: N0 (no fertilizer), N100 (100 kg N ha^{-1}) and N200 (200 kg N ha^{-1}). [2] Functional guilds: Ba, bacterivores; Fu, fungivores; H, plant parasites; Om, omnivores; Ca, predators; numbers following the functional groups indicate the cp values (Bongers & Bongers, 1998; Ferris *et al.,* 2001). The numbers in brackets correspond to percentages of nematode trophic groups per treatment.

dominated in the N treatments and *Boleodorus* dominated only in N0 (Table 1).

Fertilizer × Sampling time × Growing season date interaction had a significant effect on the abundance of bacterivores (Table 2). This was higher in N200 than in N0, in April 2005, July 2005, April 2006 and November 2006 and presented an increasing trend in April 2007 (Fig. 1a). No significant difference was

observed in the abundance of bacterivores between N100 and N0, or between N100 and N200, except in November 2006 where this group was greater in N200. Among the bacterivores, Rhabditidae were more abundant in N200 than in N0 in April 2006, November 2006 and July 2007 while Cephalobidae were higher in N200 than in N0 in all growing seasons (Table 2).

Table 2. Occurrence of significant effects of sampling time, fertilizer treatment, and growing season and their interactions on nematode taxa, and trophic groups. Level of significance is indicated (ns = not significant)

	Sampling time (S)	Fertilizer treatment (N)	Growing season (G)	S × N	N × G	S × G	S × N × G
Bacterivores	ns	ns	ns	ns	ns	ns	< 0.02
Rhabditidae	ns	ns	ns	ns	ns	ns	< 0.01
Cephalobidae	< 0.02	< 0.01	< 0.02	ns	ns	ns	ns
Fungivores	< 0.02	ns	ns	ns	ns	ns	ns
Aphelenchus	< 0.02	ns	ns	ns	ns	ns	ns
Aphelenchoides	< 0.01	ns	ns	ns	ns	ns	ns
Plant parasites	ns	< 0.05	ns	ns	ns	ns	ns
Pratylenchus	ns	ns	ns	< 0.05	ns	ns	ns
Boleodorus	< 0.05	ns	ns	ns	< 0.05	ns	ns
Predators	ns	ns	ns	< 0.01	ns	ns	ns
Aporcelaimellus	ns	ns	ns	< 0.01	ns	ns	ns
Total	ns	ns	ns	ns	ns	ns	< 0.03

As a trophic group, fungivores did not respond to fertilizer treatments (Table 2, Fig. 1b). The numbers of this group increased in July. Among the fungivores, the abundance of *Aphelenchoides* and *Aphelenchus* did not respond to fertilizer treatments. Fig. 1c shows the population dynamic of plant parasitic nematodes in all treatments during the three growing seasons. There was a significant difference among treatments (Table 2). This group was less abundant in N100 than in N200 and N0 (Table 1). Fertilizer × Sampling time da-
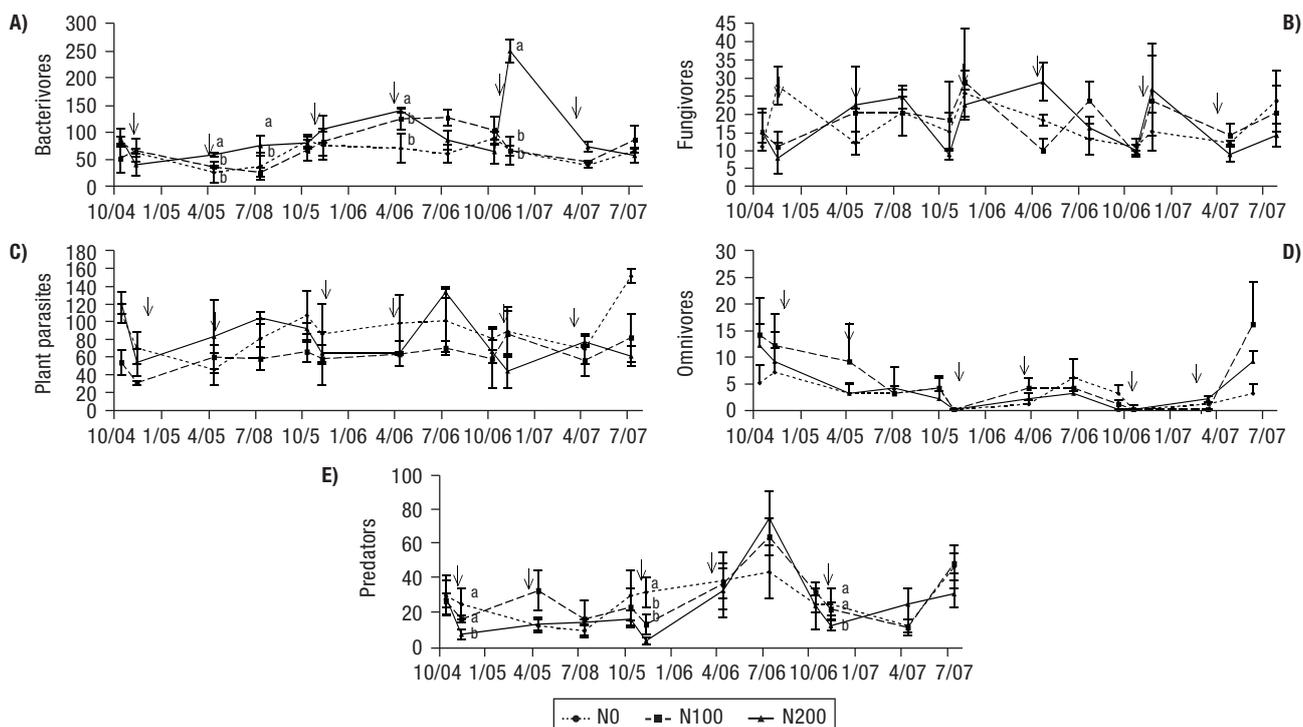
Figure 1. Mean abundance of bacterivores (A), fungivores (B), plant parasites (C), omnivores (D), and predators (E) per 100 g dry soil in N0 (no fertilizer), N100 (100 kg N ha^{-1}) and N200 (200 kg N ha^{-1}) treatments across three years (2004-2005, 2005-2006, and 2006- 2007). Values shown are the mean of three replications. Different letters indicate significant differences ($p < 0.05$) between treatments across the three growing seasons according to Tukey test. Bars indicate standard errors of the mean. Arrows mark fertilizer application dates.
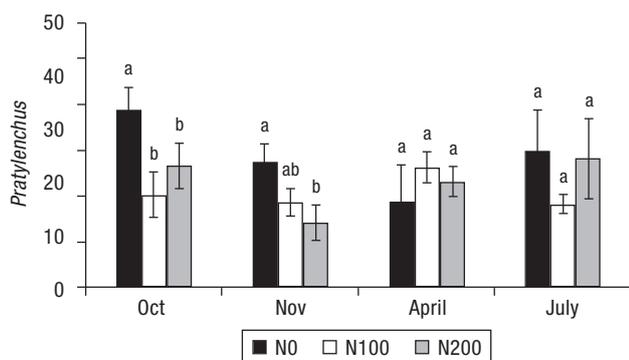
Figure 2. Mean abundance of *Pratylenchus* per 100 g dry soil in N0 (no fertilizer), N100 (100 kg N ha^{-1}) and N200 (200 kg N ha^{-1}) treatments in October (Oct), November (Nov), April and July. Each mean was obtained by averaging across three years (2004-2005, 2005-2006, and 2006-2007) the three replications per sampling time (n = 9). Different letters indicate significant differences ($p < 0.05$) between sampling times and treatments according to Tukey test. Bars indicate standard errors of the mean.

te interaction had a significant effect on the abundance of *Pratylenchus* (Table 2, Fig. 2). The numbers of this endoparasite were lower in October and November in N200 and in October in N100 than in N0. *Boleodorus* was less abundant in N100 than in N0 in the first and the last growing season. Numbers of this ectoparasite were lower in N200 than in N0 for the last growing season (Fig. 3). The number of omnivores was low across the three growing seasons (Table 1, Fig. 1d). The abundance of predators was lower in N200 than in N100 and N0 in November for the three growing seasons (Fig. 1e). *Aporcelaimellus* numbers declined in N200 in November (Table 2).
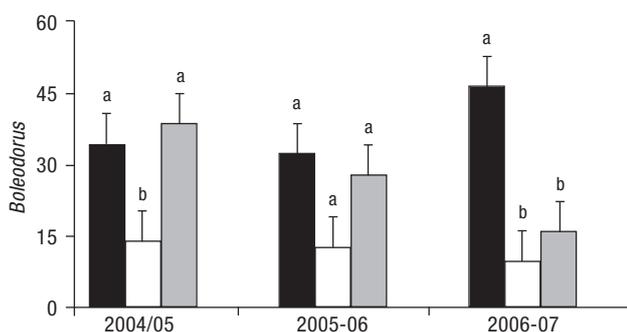

Figure 3. Mean abundance of *Boleodorus* per 100 g dry soil in N0 (no fertilizer), N100 (100 kg N ha^{-1}) and N200 (200 kg N ha^{-1}) treatments. Each mean was obtained by averaging in each growing season four sampling times and three replications per sampling time (n = 12). Different letters indicate significant differences ($p < 0.05$) between treatments and growing seasons according to Tukey test. Bars indicate standard errors of the mean.

Nematode ecological indices

The ratio of fungivores to bacterivores was lower in N200 than in N0 (Table 3) with the maximum values of F/B occurring in July, in all treatments, and ranging from 0.53 to 1.14.

Maturity index was lower in N200 than in the control plot during the three growing seasons. No significant difference in the MI was found between N100 and N0 (Table 3). Differences in MI were found between sampling time dates. The mean value of MI was highest (MI = 2.74) in July (data not shown). The PPI was not affected by the treatments (Table 3).

The Sampling time × Growing season date interaction and main factor effect of treatment were significant for PPI/MI ratio. PPI/MI was significantly higher in N200 than in N0. The mean value of the PPI/MI ratio in November was 1.31 in the third growing season, whereas in July of the same growing season it was 0.71.

The EI was not different between treatments (Table 3). The Sampling time × Growing season date interaction was significant. The EI was highest in April in the second growing season and lower in November in the first growing season. The values of SI were found to be lowest under N200 than in N100 and N0 (Table 3). The Sampling time × Growing season date interaction was significant. The SI was highest in July in the second and, the third growing seasons and lowest in November in the second growing season. Nematode faunal analyses based on plotting enrichment and structure indices across the sampling times revealed that the mean values were in quadrant B (Fig. 4), in all treatments. According to the plot, in November (Fig. 4, dot = 10) and only for treatment N200, SI showed a tendency to decrease, although it was not significantly different (Table 3).

Soil NO$_3^-$-N dynamics and its relation to the nematode ecological indices

At the time of the establishment of this experiment soil nitrate concentration was 31.4 mg kg^{-1}. The Treatment × Sampling time interaction and main factor effect of growing season were significant ($p < 0.0001$) for soil nitrate concentrations. Soil NO$_3^-$-N was greater in November and April in N200 than in N100 and N0 (Fig. 5a). There were no significant differences in soil NO$_3^-$-N in the plots N100 and N0. Soil NO$_3^-$-N was greater in the second and third growing seasons (Fig. 5b).

Table 3. Mean nematode ecological indices for different levels of N fertilizer treatments, sampling time, and growing seasons

Averages	Nematode ecological indices[1]					
	F/B	MI	PPI	PPI/MI	EI	SI
Fertilizer treatment[2]						
N0 (n = 36)	0.41 ± 0.1[a]	2.57 ± 0.1[a]	2.40 ± 0.0[a]	0.94 ± 0.0[a]	61.7 ± 2.7[a]	79.1 ± 1.7[a]
N100 (n = 36)	0.30 ± 0.0[ab]	2.46 ± 0.1[ab]	2.48 ± 0.0[a]	1.03 ± 0.0[ab]	67.9 ± 3.0[a]	79.6 ± 2.0[a]
N200 (n = 36)	0.21 ± 0.0[b]	2.23 ± 0.1[b]	2.39 ± 0.0[a]	1.10 ± 0.1[b]	66.6 ± 2.3[a]	64.7 ± 3.5[b]
Growing season × Sampling times (n = 9)						
Oct-04	0.20 ± 0.0[b]	2.65 ± 0.1[ab]	2.53 ± 0.1	0.96 ± 0.1[abc]	65.3 ± 3.8[abc]	74.1 ± 2.2[ab]
Nov-04	0.33 ± 0.0[b]	2.50 ± 0.1[ab]	2.75 ± 0.0	0.99 ± 0.1[abc]	50.8 ± 7.4[c]	73.4 ± 3.4[ab]
April-05	0.44 ± 0.0[ab]	2.60 ± 0.1[ab]	2.61 ± 0.0	1.00 ± 0.1[abc]	54.8 ± 2.9[bc]	78.5 ± 2.3[ab]
July-05	1.01 ± 0.3[a]	2.45 ± 0.1[ab]	2.48 ± 0.1	1.00 ± 0.1[abc]	54.3 ± 4.9[bc]	67.6 ± 5.2[bc]
Oct- 05	0.19 ± 0.0[b]	2.22 ± 0.1[bc]	2.57 ± 0.1	1.11 ± 0.1[ab]	68.7 ± 4.1[abc]	73.0 ± 4.4[abc]
Nov-05	0.30 ± 0.0[b]	2.08 ± 0.0[bc]	2.75 ± 0.0	1.22 ± 0.1[ab]	62.5 ± 3.1[abc]	49.6 ± 9.1[c]
April-06	0.19 ± 0.1[b]	2.21 ± 0.1[bc]	2.71 ± 0.1	1.15 ± 0.1[ab]	79.6 ± 3.6[a]	79.4 ± 4.2[ab]
July-06	0.39 ± 0.0[ab]	2.78 ± 0.1[ab]	2.56 ± 0.0	0.90 ± 0.0[c]	69.7 ± 4.0[abc]	87.7 ± 1.1[a]
Oct-06	0.14 ± 0.0[b]	2.29 ± 0.1[abc]	2.57 ± 0.0	1.08 ± 0.1[abc]	73.7 ± 5.2[ab]	78.5 ± 4.7[ab]
Nov-06	0.22 ± 0.1[b]	2.03 ± 0.2[c]	2.69 ± 0.0	1.31 ± 0.1[a]	75.0 ± 4.3[ab]	69.4 ± 5.5[abc]
April-07	0.26 ± 0.0[b]	2.22 ± 0.0[bc]	2.54 ± 0.1	1.10 ± 0.1[ab]	74.4 ± 3.5[ab]	79.1 ± 3.7[ab]
July-07	0.37 ± 0.1[ab]	3.00 ± 0.1[a]	2.70 ± 0.0	0.90 ± 0.1[c]	55.6 ± 6.2[bc]	87.5 ± 1.3[a]
GLM[3]						
Fertilizer treatment (N)	< 0.05	< 0.01	ns	0.03	ns	< 0.01
Sampling time (S)	< 0.01	< 0.01	ns	< 0.01	0.03	< 0.01
Growing seasons (G)	< 0.01	ns	ns	ns	< 0.01	ns
N × S	ns	ns	ns	ns	ns	ns
G × N	ns	ns	ns	ns	ns	ns
G × S	0.03	0.02	ns	0.02	0.02	< 0.01
N × S × G	ns	ns	ns	ns	ns	ns

[1] F/B: ratio of fungivores to bacterivores; MI: maturity index for the free-living nematodes; PPI: plant parasites index, PPI/MI: ratio of plant parasitic index to maturity index; EI: enrichment index; SI: structure index. [2] Fertilizer treatment: N0 (no fertilizer), N100 (100 kg N ha^{-1}), and N200 (200 kg N ha^{-1}). [3] GLM: generalized linear models. Different letters indicate significant differences ($p < 0.05$) among treatments or Growing seasons × Sampling times interaction according to Tukey test.

Soil nitrate concentration was positively correlated with EI and PPI/MI ratio, and negatively with MI, SI and F/B (Table 4).

Discussion

In this study, the composition of the nematode fauna in an Aridisol soil was comparable to the one pre-

Table 4. Table 4. Pearson's correlation coefficients between nematode ecological indices[1] and soil nitrate concentration (NO$_3^-$-N) (n = 108)

	MI	PPI	PPI/MI	EI	SI	F/B
NO$_3^-$-N	−0.45**	0.16 ns	0.53**	0.29*	−0.43**	−0.23*

[1] See Table 3. * $p < 0.05$. ** $p < 0.01$. ns: non-significant.

viously reported for an apple orchard in the Rio Negro Valley, Argentina (Bergna, 1976). The climatic and edaphic factors may have a significant part in determining the composition of the nematode fauna which develops in a particular soil (Yeates, 1984).

Bacterivores and plant parasites were the most abundant trophic groups present in the fertilized plots. Similar results were reported by Neher (1999) for conventionally managed soils. In agricultural soil, omnivores and predators are generally less abundant trophic groups; however, in this study the abundance of predators was greater compared with other studies (Wang *et al.*, 2006; Azpilicueta *et al.*, 2008). Our results may be due to the fact that the experimental site used for the study had not received N fertilization and had reduced tillage from 1998 to 2004. Intermittent fallow and standard tillage supported high abundances
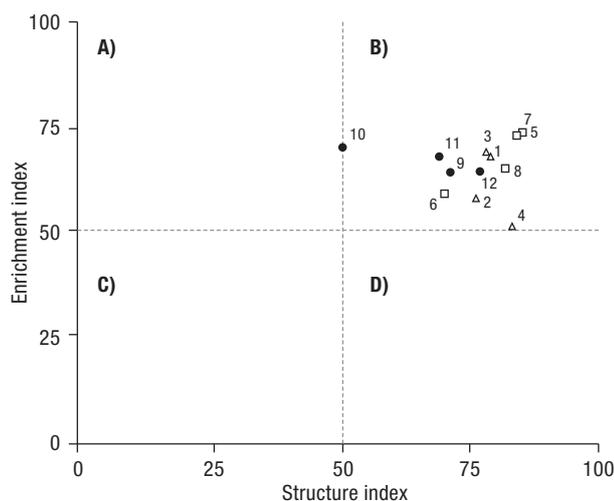
Figure 4. Temporal change in the soil food web indicated by nematode faunal analysis at different levels of N fertilization treatments: no fertilizer (△), 100 kg N ha^{-1} (□), and 200 kg N ha^{-1} (●). Numbers 1-12 represent the progression of change in soil food web across the sampling times: October (1, 5, 9), November (2, 6, 10), April (3, 7, 11), and July (4, 8, 12). The plotted values are the average of 9 replications. Quadrats A, B, C and D refer to faunal ordination in the faunal profile.

of predators and omnivores (Sánchez-Moreno *et al.,* 2006). Hánel (2010) found that *Aporcelaimellus* increased in abandoned fields, and this taxon was one of the most common at the beginning of our study.

　　There were no consistent differences in the abundance of bacterivores after the first N200 fertilizer application during 2004/2005 and 2005/2006 growing seasons but this trophic group responded positively in November 2006/2007 (Fig. 1a). This last sampling time occurred 1 week earlier than in the other growing seasons. Perhaps the lack of association observed for concurrent measures supports the hypothesis of asynchronous peaks in bacterivores and their food resource (Neher, 2001). However, after the second N200 fertilizer incorporation in autumn, bacterivores were more abundant, or at least followed this trend during the growing seasons. Fluctuations in total number of bacterivores in N200 plots were determined by Cephalobidae. Rhabditidae showed an immediate and short response to N200 fertilizer in the last growing season, while Cephalobidae were more persistent over time in N200. Rhabditidae seem to be affected predominantly by sudden flushes of food resources (Porazinska *et al.*, 1999) whereas Cephalobidae occur under food-rich conditions, for example, bacteria blooming in enriched soils, as well as food-poor conditions (Bongers & Bongers, 1998).

　　The pathogenicity of *Pratylenchus penetrans* to apple is well documented (Jaffee *et al.*, 1982). Early in each growing season (October and November), the abundance of *Pratylenchus* sp. was lower in N200. This decrease coincided with the highest rates of apple root growth (Silva & Rodríguez, 1995). Other studies have shown that inorganic fertilization increased the abundance of root feeding nematodes (Wang *et al.*, 2006; Van Eckeren *et al.,* 2009).

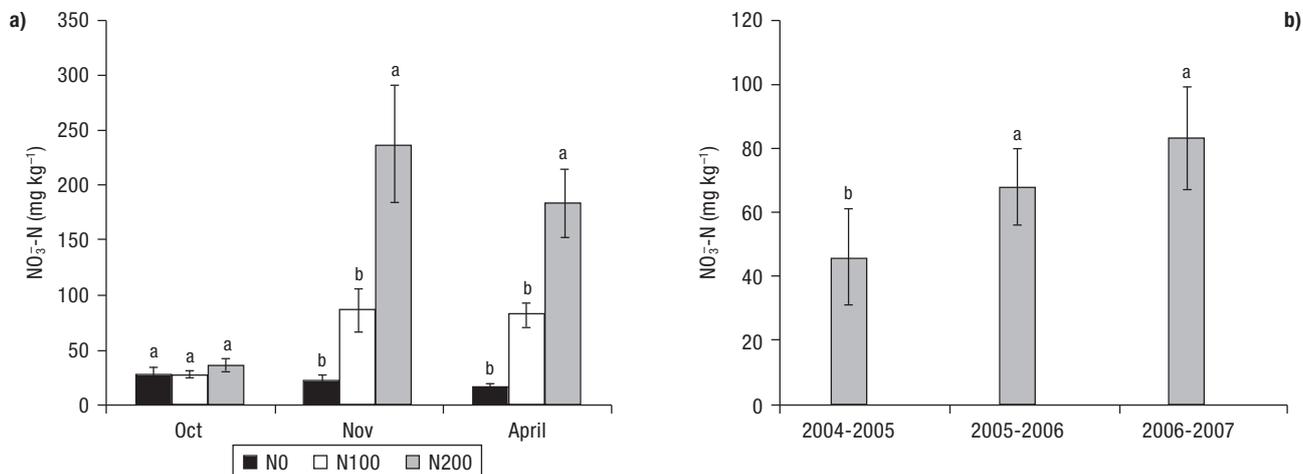　　Following the first N200 fertilizer application in each growing season, predators decreased (Fig. 1e).

Figure 5. Soil NO$_3^-$-N concentration: (a) in N0 (no fertilizer), N100 (100 kg N ha^{-1}), and N200 (200 kg N ha^{-1}) treatments and in October (Oct), November (Nov), and April. Each mean was obtained by averaging across three growing seasons (2004-2005, 2005-2006, and 2006-2007) the three replications per sampling time (n = 9); (b) each mean was obtained by averaging, in each growing season, the three treatments, three sampling times and three replications per sampling time (n = 27). Different letters indicate significant differences (*p* < 0.05) between treatments (a) or growing seasons (b) according to Tukey test. Bars indicate standard errors.

Some studies suggested that nematodes in c-p groups 4 and 5 are more sensitive to N solutions than nematodes representing lower c-p groups (Tenuta & Ferris, 2004). Fertilization with ammonium nitrate at 100 kg ha^{-1} reduced the percentage and abundance of omnivores (Wang *et al.*, 2006). However, in this study, at the end of each growing season, predators increased. This result is important because an increase in the abundance of predators can have a regulatory effect on lower trophic levels, including plant feeders (Sánchez-Moreno & Ferris, 2007).

Nitrogen fertilization influences detrital pathways. The ratio of fungivores to bacterivores was affected by 3 years of fertilization with N200. The low values of the ratio in N200 suggest that the breakdown of organic matter was assisted by bacteria during all growing seasons. Bacteria based food webs exhibit higher decomposition rates than fungal based webs (Ferris *et al.*, 2004). Todd (1996) reported that chronic additions of N lowered this index, suggesting a shift toward the bacteria dominated detritus food web typical of agricultural soils. In the present investigation, the increase in F/B ratio values in winter, in all treatments during all growing seasons, reflects the changes in the abundance of bacterivores and fungivores. Koenning & Barker (2004) demonstrated that the time of year when samples are collected might affect the FB ratio.

The MI and PPI of soil nematodes are sensitive indicators for assessing fertilization disturbance of soil ecosystems (Bongers *et al.*, 1997). The MI values obtained (2.23-2.57) are comparable to values in agriculture systems under the application of inorganic fertilizers (Gruzdeva *et al.*, 2007; Hu & Qi, 2011). A lower MI value indicates a disturbed environment, whereas a higher value indicates more stable conditions (Bongers, 1990). In this study, the MI for free living nematodes detected differences between the highest dose of N treatment and the control. The lower MI in N200 was associated with bacterivore dominance and a reduction in predators. Different levels of N fertilizer decrease the abundance of omnivores-predators (Li *et al.,* 2010). Sarathchandra *et al.* (2001) observed a significant decrease in MI in the 400 kg N ha^{-1} yr^{-1} treatment, suggesting a disturbance of the nematode community structure leading to a relative decrease in the persister community; however, MI did not change at a rate of 200 kg N ha^{-1} yr^{-1}. The nematode MI can provide useful information on the direction of change within a particular soil (Yeates & Bongers, 1999). In this study, the values of MI were found to be lower under N200 than N0 and MI values increased in July in all treatments. Furthermore, a gradual increase was found in MI from high-input plots to unfertilized control. This result was in accordance with the conclusion of Bongers *et al.* (1997) who found that MI decreases with increasing nutrient status.

Bongers *et al.* (1997) reported that the PPI was related positively to the level of nutrient enrichment that typically occurs in agroecosystems, and Nombela *et al.* (1999) also found that the PPI value decreased as recovery time after human intervention increased. However, in this study PPI did not differ between treatments during the three growing seasons.

In this work, the higher PPI/MI ratio at N200 treatment indicated a slight disturbance. In another study, in an apple orchard fertilized with 100-175 kg N ha^{-1} annually since 1994, a value for PPI/MI ratio of 1.52 showed the effects of nutrient enrichment (Azpilicueta *et al.,* 2008). The PPI/MI ratio does not exceed 0.9 in natural habitats where higher plants use nutrients at the optimum. The effects of slight nutrient disturbances are followed by an increase in the ratio to 1.2 (Bongers *et al.,* 1997).

Enrichment index reflects the availability of resources to the soil food web and the responses of primary decomposers to the resources (Ferris *et al.*, 2001). In this study, the EI values were similar in all treatments; EI does not always appropriately reflect soil fertilization (Okada & Harada, 2007). Liang *et al.* (2005) reported that the concentration of soil mineral N was not enough to change the numbers of bacterivores and fungivores. However, frequent soil fertilization with N200 decreased the SI in the current study. The N200 treatment appears to have resulted in a less structured nematode community than the others.

Plotting EI *versus* SI provides a model framework for nematode faunal analysis as an indicator of the likely conditions of the soil food web. At the establishment of the experiment food webs were moderately enriched and structured in all the plots. The lower values of SI in November in N200 may be due to a decrease in predators, which are sensitive to pollution and other disturbances in agroecosystems (Bongers & Bongers, 1998). Nematode communities in perennial crop systems may be expected to ordinate in quadrants B and C of plots of EI and SI (Ferris *et al.*, 2001). The results obtained in this investigation agree with the observation made by Ferris *et al.* (2001).

In this study, the nitrate concentration correlated positively with the PPI/MI ratio and negatively with MI.

A similar finding was reported by Neher (2001) who found that an increase in the availability of nitrate and ammonium is associated inversely with the successional maturity of nematode communities in mineral soils cultivated for agricultural purposes. Liang *et al*. (2005) found that SI had a negative correlation with soil nitrate concentration with slow-release urea fertilization. Also, in the present study, the negative correlation suggests soil nematode structure was affected by soil nutrient status. Soil nitrate content may be considered as a surrogate indicator of agricultural disturbance (Sánchez-Moreno *et al.,* 2011).

Subsequent to 6 years of no fertilization, fertilization management under field conditions changed temporary soil nitrate concentration which affected the abundance of nematode trophic groups. The nematode community structure rapidly changed within the first year of fertilization. The application of 100 and 200 kg N ha^{-1}, during 3 growing seasons, supported the same number of bacterivores. The highest level of N fertilizer caused a disturbance to the soil ecosystem and had an effect on the trophic structure of the nematode community consistent with a less stable soil system. Our findings underscore the potential benefits of reducing the use of high levels of N fertilization for developing long-term sustainable agroecosystems.

Acknowledgements

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