



Nematode faunal analysis in an aquic brown soil fertilised with slow-release urea, Northeast China

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Abstract

In this study, faunal analysis of nematode communities in an aquic brown soil (silty loam Hapli-Udic Cambosols in Chinese Soil Taxonomy) of Northeast China was conducted through a single wheat growth season, aimed to assess nematode faunal response to the application of slow-release urea fertiliser. Three treatments (conventional urea CU, slow-release urea SRU and control NU) were installed, and nematode ecological indices (enrichment index EI, basal index BI, structural index SI and channel index CI) were used to quantify the influence of various treatments on the nematode fauna. The results showed that soil C/N values were significantly greater in SRU than in CU at the wheat tillering stage, while soil urease activity exhibited a reverse trend. During the study period, SI values were significantly greater in SRU than in NU and CU, and CI had a negative correlation with $\text{NO}_3^- - \text{N}$ and $\text{NH}_4^+ - \text{N}$. Among the indices used in this study, SI was the only one that detected nematode community structural differences between SRU and CU during the wheat growth season, and indicated a greater food web diversity and structure in SRU than in CU, showing the positive effect of applying slow-release urea.

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1. Introduction

Urea is a nitrogen (N) fertiliser commonly used in world agriculture. After being applied to soil, it can be rapidly hydrolyzed to NH_3 and CO_2 by soil urease

(Gioacchini et al., 2002), followed by NO_3^- formation through nitrification. Therefore, ammonia loss and nitrate leaching are environmental concerns in regions where urea is applied. In Chinese agriculture, more than half of the N fertiliser applied is urea, and this comprises 40% of the global annual urea consumption. The N recovery by crops from urea is often as low as 30–40%, with a potentially high environmental cost associated with N losses via NH_3

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volatilization, NO_3^- leaching and N_2O emission (Zhou et al., 2003).

In order to improve urea-N recovery and reduce its loss, many forms of slow-release urea fertilisers have been developed and applied to different plant species under a range of environmental conditions. The products may be coated, chemically and biochemically modified, or granular (Jiao et al., 2004). As for biochemical modification, urease inhibitors are often incorporated in urea. Among these, hydroquinone (HQ) and thiophosphoric triamide (NBPT) are superior in their low incorporation rate and higher efficiency in retarding urea hydrolysis and reducing NH_3 volatilization loss (Zhao et al., 1993; Watson et al., 1994; Vittori et al., 1996; Chen et al., 1998). Such slow-release urea fertilisers can increase the efficiency of applied urea-N and are environmentally friendly, because their N release is in synchrony with plant N uptake, and in a single application, can provide sufficient N to satisfy plant N requirements while maintaining very low concentrations of mineral N in soil throughout the growth season (Bacon, 1995). Although the effect of urease inhibitors on reducing urea N losses has been assessed in field trials (Gilacchini et al., 2002; Zhou et al., 2003), little attention has been paid to their effect on nematode communities.

Soil nematode communities can provide unique insight into many aspects of soil processes. Because most nematodes are active in soil throughout the year, they can provide a holistic measure of the biotic and functional status of soils (Ritz and Trudgill, 1999). Laboratory experiments and field studies have suggested that soil nematodes feeding on bacteria and fungi played an important role in affecting the turnover of soil microbial biomass, and thus, the availability of plant nutrients (Bardgett et al., 1999; Liang et al., 1999, 2001; Yeates, 2003).

Nematode faunal analysis has been developed as a powerful bioindicator of soil conditions and of structural and functional attributes of the soil food web (Bongers, 1990; Neher, 2001). In recent years, new nematode faunal indices including enrichment index (EI), basal index (BI), structural index (SI) and channel index (CI) have been developed and applied in soil food web diagnostics and succession (Ferris et al., 2001; Wu et al., 2002; Berkelmans et al., 2003; Ferris and Matute, 2003; Ruess, 2003; Ferris et al., 2004).

Compared with conventional urea fertiliser, slow-release urea fertilisation may have a positive effect on the community structure of soil nematodes, but no information is available on this. The objectives of this study were to evaluate the nematode faunal response to the application of slow-release urea fertiliser in an aquic brown soil of Northeast China, to evaluate several ecological indices for their ability to assess nematode communities, and to determine the relationships between these ecological indices and soil chemical and biochemical properties.

2. Materials and methods

2.1. Experimental site and design

This study was conducted at the Shenyang Experimental Station of Ecology ($41^\circ 31' \text{N}$, $123^\circ 22' \text{E}$), Chinese Academy of Sciences, belonging to the Chinese Ecosystem Research Network (CERN) and established in 1990. The station is located in a continental temperate monsoon zone, with a dry-cold winter and a warm-wet summer. The annual mean temperature is $7.0\text{--}8.0^\circ \text{C}$, annual precipitation is 650–700 mm, and the annual non-frost period is 147–164 days. The soil at the study site is classified as an aquic brown soil (silty loam Hapli-Udic Cambosols in Chinese Soil Taxonomy) (CRGCST, 2001), with 9.55 g kg^{-1} total C, 0.862 g kg^{-1} total N, pH (H_2O) 6.7, 21.4% sand, 46.5% silt and 32.1% clay at 0–20 cm depth.

Nine experimental plots, $5 \text{ m} \times 6 \text{ m}$ each, were planted with monocultured spring wheat (*Triticum aestivum* L.) in 2003. The previous crop was maize (*Zea mays* L.). The plots were strip planted with wheat in a conventional tillage system, which did not receive residues from the previous crop. The spring wheat in all the plots relied on natural rainfall. Three treatments were installed: control (no urea, NU), conventional urea (CU), and slow-release urea (SRU) (a urea manufactured by Jinxi Natural Gas Chemical Co., Ltd., China, incorporating a liquid urease inhibitor). The experimental design was a randomized block with three replicates. Before planting, CU plots were treated with urea and SRU plots with slow-release urea, while both CU and SRU plots received super-

phosphate and potassium phosphate, at rates equivalent to 225 kg N ha⁻¹, 60 kg P ha⁻¹, 112 kg K ha⁻¹, respectively, while NU plots did not receive any fertilisers.

2.2. Sampling, extraction and identification of nematodes

During the wheat growth season, five soil cores (5-cm diameter) were collected from 0–15 cm depth in each plot on 1 May (tillering stage), 19 May (booting stage), 2 June (flowering stage), 16 June (filling stage) and 5 July (ripening stage), stored in individual plastic bags, and immediately transferred to a 4 °C cold room.

A sub-sample (300 g) of each sample was taken for nematode extraction by elutriation and sugar-centrifugation (Ingham, 1994). After counting the number of total nematodes, 100 nematodes per sample were selected randomly and identified to genus.

2.3. Soil chemical and biochemical analysis

Soil total C was analysed by dry combustion, using a Shimadzu TOC 5000 Total C analyzer. Soil total N was determined by Kjeldahl digestion, followed by NaOH distillation, and measured by titration with 25 mM H₂SO₄ in boric acid indicator (Bremner, 1996). Soils NO₃⁻-N and NH₄⁺-N were determined by extraction with 2M KCl, steam distillation and titration (Mulvaney, 1996). Soil urease activity was determined by using urea as substrate, incubating for 5 h at 37 °C, and measuring the remained urea with a colorimetric method (Tabatabai, 1994).

2.4. Community indices

Nematode faunal analyses were performed to provide indicators of food web structure, status, functionality, and resource availability (Ferris et al., 2001; Ferris and Matute, 2003; Ferris et al., 2004). To calculate the various nematode ecological indices, all genera were assigned weights for indices, according to their classification into functional guilds. Based on the relative weighted abundance of nematode guilds, the faunal profile represented the basal, structural and enrichment conditions of the soil food web (Ferris

et al., 2001). The ecological indices were calculated as follows:

$$EI = 100 \times \left(\frac{e}{e + b} \right) \quad (1)$$

$$BI = 100 \times \left(\frac{b}{b + e + s} \right) \quad (2)$$

$$SI = 100 \times \left(\frac{s}{b + s} \right) \quad (3)$$

$$CI = 100 \times 0.8 \times \frac{Fu_2}{3.2 \times Ba_1 + 0.8 \times Fu_2} \quad (4)$$

where *e* is the abundance of individuals in guilds in the enrichment component weighted by their respective *k_e* values, *b* is the abundance of individuals in the basal component weighted by their *k_b* values, and *s* is the abundance of individuals in the structural component weighted by their *k_s* values. The channel index CI indicates the decomposition channel through the soil food web, where Ba and Fu represented bacterivores and fungivores respectively, suffix numbers are cp values (Bongers, 1990) for the taxa. Low values of CI suggest a primarily bacterial decomposer community, while high values suggest fungal dominated decomposition (Ferris et al., 2001, 2004; Berkelmans et al., 2003).

2.5. Statistical analysis

All the data were analysed through a two-way ANOVA (treatment × date) to determine the between-subject effects. For soil chemical properties, the LSD was then performed as a post hoc test to assess treatment effects on each sampling date. All statistical analyses were performed by SPSS software package. Difference at *P* < 0.05 level was considered as statistically significant.

3. Results

3.1. Soil chemical and biochemical properties

During the study period, significant treatment effects were found for total soil C, and significant differences were observed for total soil N and C/N ratio between treatments and dates (Table 1). At the

Table 1
Variance of soil chemical-biochemical properties and nematode ecological indices under date and treatment effects

Indicator	Treatment		Date		Treatment × date	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Total C	6.083	0.006	0.558	0.695	2.340	0.044
Total N	26.609	<0.001	23.881	<0.001	8.589	<0.001
C/N	17.403	<0.001	4.216	0.008	2.818	0.019
NO ₃ ⁻ -N	790.077	<0.001	98.271	<0.001	21.660	<0.001
NH ₄ ⁺ -N	401.872	<0.001	26.099	<0.001	25.046	<0.001
Total mineral N	1772.083	<0.001	100.997	<0.001	50.467	<0.001
Urease activity	588.539	<0.001	228.325	<0.001	76.033	<0.001
EI	0.402	0.673	0.425	0.789	3.353	0.007
BI	2.961	0.067	0.490	0.743	1.766	0.124
SI	8.917	0.001	1.980	0.123	3.639	0.005
CI	9.429	0.001	2.028	0.116	1.478	0.207

F and *P* values, from two-way ANOVA are given.

wheat flowering stage, total soil N was significantly greater in SRU than in NU and CU ($P < 0.05$) (Table 2). During the whole growth season except at the ripening stage, available soil NH₄⁺-N was significantly less ($P < 0.05$) in SRU than in CU. Soil NO₃⁻-N at the booting, flowering and ripening stages was significantly higher ($P < 0.05$) in SRU than in CU and NU. Except at the booting and ripening stages, soil total mineral N was significantly less in SRU than in CU, but higher than in NU ($P < 0.05$) (Table 2).

Significant differences were observed in soil urease activity between different sampling dates and treatments ($P < 0.01$) (Table 1). Across tillering, booting and flowering stages, soil urease activity was less in SRU than in CU (Fig. 1).

3.2. Nematode faunal structure

Twenty-two genera of soil nematodes were identified in SRU during the course of the study

Table 2
Changes in soil chemical indices under three treatments during the wheat growing stages

Growth stage	Treatment	Total C (mg g ⁻¹)	Total N (mg g ⁻¹)	C/N	NH ₄ ⁺ -N (μg g ⁻¹)	NO ₃ ⁻ -N (μg g ⁻¹)	Total mineral N (μg g ⁻¹)
Tillering (1 May)	NU	9.51 ± 0.44 a	0.86 ± 0.06 b	11.0 ± 0.4 a	8.12 ± 1.80 c	7.99 ± 0.70 c	16.11 ± 1.93 c
	CU	8.72 ± 0.09 b	1.00 ± 0.02 a	8.7 ± 0.1 b	46.54 ± 0.84 a	22.58 ± 1.69 a	69.12 ± 2.18 a
	SRU	10.10 ± 0.40 a	0.95 ± 0.00 a	10.6 ± 0.4 a	21.45 ± 1.97 b	10.98 ± 0.02 b	32.42 ± 1.95 b
Booting (19 May)	NU	9.12 ± 0.47 a	0.81 ± 0.02 a	11.2 ± 0.5 a	18.24 ± 1.35 c	5.95 ± 0.50 c	24.19 ± 1.83 b
	CU	9.23 ± 0.22 a	0.83 ± 0.01 a	11.2 ± 0.2 a	42.91 ± 1.41 a	19.47 ± 3.05 b	62.38 ± 4.33 a
	SRU	9.55 ± 0.54 a	0.83 ± 0.05 a	11.5 ± 0.9 a	34.78 ± 1.85 b	31.96 ± 3.05 a	66.75 ± 1.38 a
Flowering (2 June)	NU	9.79 ± 0.99 a	0.86 ± 0.05 b	11.5 ± 1.6 a	14.85 ± 1.55 c	2.85 ± 0.57 c	17.70 ± 1.37 c
	CU	8.86 ± 0.08 a	0.90 ± 0.06 b	9.9 ± 0.7 a	50.26 ± 3.41 a	19.40 ± 2.05 b	69.66 ± 4.29 a
	SRU	9.93 ± 0.63 a	1.05 ± 0.03 a	9.5 ± 0.9 a	35.47 ± 3.49 b	24.44 ± 3.03 a	59.91 ± 2.59 b
Filling (16 June)	NU	10.12 ± 0.65 a	0.94 ± 0.05 a	10.8 ± 1.1 a	16.42 ± 0.87 c	2.85 ± 0.59 b	19.28 ± 0.99 c
	CU	9.38 ± 0.47 a	0.98 ± 0.05 a	9.6 ± 0.7 a	65.17 ± 1.78 a	22.28 ± 1.41 a	87.46 ± 0.55 a
	SRU	9.46 ± 0.57 a	1.00 ± 0.01 a	9.4 ± 0.5 a	57.97 ± 1.54 b	23.97 ± 2.53 a	81.94 ± 0.99 b
Ripening (5 July)	NU	10.30 ± 0.65 a	0.87 ± 0.03 c	11.9 ± 1.0 a	19.34 ± 1.16 b	2.05 ± 0.12 c	21.39 ± 1.25 b
	CU	9.35 ± 0.62 a b	1.08 ± 0.04 a	8.7 ± 0.8 b	56.29 ± 3.74 a	13.82 ± 1.46 b	70.11 ± 2.28 a
	SRU	8.99 ± 0.53 b	0.94 ± 0.02 b	9.6 ± 0.8 b	53.11 ± 6.15 a	16.46 ± 1.17 a	69.58 ± 4.99 a

Values within the same column followed by different letters (a, b, c) indicate significant differences ($P < 0.05$) between treatments for each sampling date (values are means ± S.D.). NU: control (no urea); CU: conventional urea; SRU: slow-release urea.

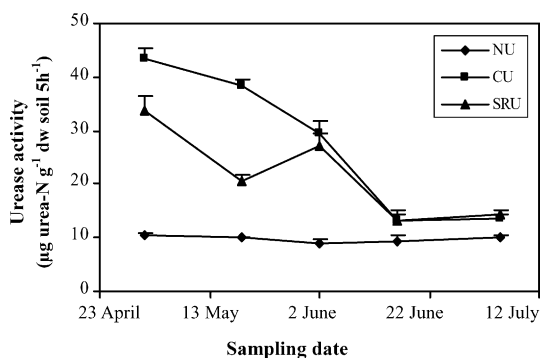


Fig. 1. Dynamics of soil urease activity with time (values are means \pm S.D.).

(Table 3). Of the four genera in the Ba₁ guild, *Protorhabditis* was predominant. Among the four genera in the Ba₂ guild, *Cephalobus*, *Brevibucca* and *Acrobeloides* were predominant, while of the three genera of the Fu₂ guild, *Aphelenchus* was most

Table 3

Nematode abundance (10^3 individuals m^{-2}) of each identified taxon across three treatments during the study period, expressed as mean \pm S.D.

Genus	Guild	NU	CU	SRU
<i>Monhystera</i>	Ba ₁	11.3 \pm 19.2	3.9 \pm 9.0	3.8 \pm 7.6
<i>Panagrolaimus</i>	Ba ₁	0.0 \pm 0.0	9.2 \pm 9.9	5.4 \pm 6.6
<i>Protorhabditis</i>	Ba ₁	23.3 \pm 29.0	30.9 \pm 20.5	29.9 \pm 12.3
<i>Rhabditis</i>	Ba ₁	15.2 \pm 18.0	8.2 \pm 13.3	7.1 \pm 9.7
<i>Acrobeloides</i>	Ba ₂	1.6 \pm 6.3	11.2 \pm 13.9	11.6 \pm 16.5
<i>Brevibucca</i>	Ba ₂	12.7 \pm 14.2	15.8 \pm 17.4	18.0 \pm 16.3
<i>Cephalobus</i>	Ba ₂	38.1 \pm 18.7	52.8 \pm 27.1	46.7 \pm 17.5
<i>Eucephalobus</i>	Ba ₂	6.4 \pm 12.1	9.2 \pm 9.4	3.7 \pm 5.7
<i>Aphelenchoides</i>	Fu ₂	14.5 \pm 21.3	6.1 \pm 17.9	6.2 \pm 10.7
<i>Aphelenchus</i>	Fu ₂	87.2 \pm 65.9	45.3 \pm 17.2	37.0 \pm 20.3
<i>Ditylenchus</i>	Fu ₂	17.3 \pm 16.9	7.1 \pm 9.7	13.3 \pm 13.1
<i>Mononchus</i>	Om ₄	0.0 \pm 0.0	0.0 \pm 0.0	1.2 \pm 4.1
<i>Aporcelaimus</i>	Om ₅	12.2 \pm 15.9	0.0 \pm 0.0	6.2 \pm 8.6
<i>Mesodorylaimus</i>	Om ₅	3.9 \pm 9.2	0.8 \pm 3.0	0.4 \pm 1.6
<i>Pratylenchus</i>	H ₂	9.2 \pm 21.6	10.6 \pm 13.3	6.8 \pm 13.6
<i>Psilenchus</i>	H ₂	7.3 \pm 11.7	9.8 \pm 16.7	6.9 \pm 10.4
<i>Tetylenchus</i>	H ₂	0.0 \pm 0.0	1.4 \pm 4.3	5.0 \pm 8.3
<i>Tylenchus</i>	H ₂	77.7 \pm 43.8	48.6 \pm 35.4	42.5 \pm 27.5
<i>Helicotylenchus</i>	H ₃	1.0 \pm 0.2	0.8 \pm 0.3	0.9 \pm 0.2
<i>Rotylenchus</i>	H ₃	66.5 \pm 56.9	38.0 \pm 30.4	40.7 \pm 35.1
<i>Tylenchorhynchus</i>	H ₃	8.8 \pm 13.2	0.7 \pm 1.8	4.1 \pm 6.5
<i>Longidorus</i>	H ₄	10.6 \pm 16.2	4.6 \pm 13.5	8.4 \pm 20.8

Functional guild classifications are represented by designated feeding habit (Ba: bacterivore, Fu: fungivore, Om: omnivore, H: plant feeder). Suffix numbers are cp values (Bongers, 1990) for the taxa.

prevalent. Three genera of nematodes were designated as omnivores (Om_{4,5}), and eight genera were designated as herbivores (H_{2,4}) which were relatively abundant in the study.

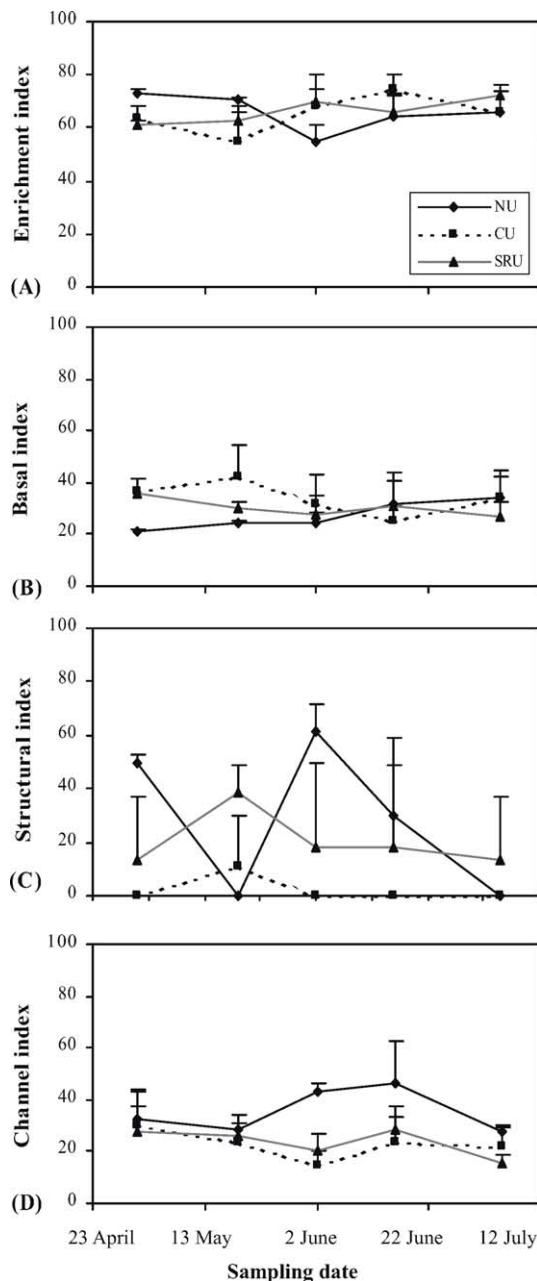


Fig. 2. Dynamics of nematode ecological indices with time: (A) enrichment index (EI), (B) basal index (BI), (C) structural index (SI), and (D) channel index (CI) (values are means \pm S.D.).

Table 4
Correlation coefficients between nematode ecological indices and soil chemical-biochemical properties

	Indicator											
	A	B	C	D	E	F	G	H	I	J	K	
Total C (A)	1.000											
Total N (B)	0.089	1.000										
C/N (C)	0.616**	-0.724**	1.000									
NO ₃ ⁻ -N (D)	-0.250	0.499**	-0.557**	1.000								
NH ₄ ⁺ -N (E)	-0.175	0.250	-0.315*	0.673**	1.000							
Total mineral N (F)	-0.243	0.449**	-0.514**	0.963**	0.847**	1.000						
Urease (G)	-0.133	0.115	-0.189	0.258	0.495**	0.366*	1.000					
EI (H)	-0.254	0.154	-0.309*	0.057	0.000	0.041	-0.322*	1.000				
BI (I)	0.144	0.056	-0.066	0.179	0.087	0.160	0.425**	-0.809**	1.000			
SI (J)	0.207	-0.262	0.354*	-0.448**	-0.116	-0.364*	-0.236	-0.051	-0.490**	1.000		
CI (K)	0.174	-0.172	0.265	-0.473**	-0.423**	-0.493**	-0.241	-0.436**	0.204	0.293	1.000	

*, ** Significant at $P < 0.05$ and $P < 0.01$, respectively.

3.3. Nematode ecological indices

EI and BI values fluctuated slightly across all sampling dates in the three treatments (Fig. 2). During the wheat growth season, significant treatment effects were observed on the SI and CI values ($P < 0.01$) (Table 1). The CI indices were higher in NU than in SRU and CU, while the SI indices were lower in CU than in NU and SRU (Fig. 2).

3.4. Correlations of nematode ecological indices with soil chemical and biochemical properties

No significant correlations were observed between nematode ecological indices and soil total C and N (Table 4), but the soil C/N ratio was negatively correlated with EI and positively with SI, soil NO₃⁻-N was negatively correlated with SI and CI, and soil NH₄⁺-N was negatively correlated with CI. Soil total mineral N was negatively correlated with SI and CI, and soil urease activity was negatively correlated with EI and positively with BI.

4. Discussion

The amount of fertiliser N supplied at the time of application often exceeds crop N needs at that time, and hence, excessive N may have adverse effects on crops and soil organisms, or may be lost through leaching or denitrification (Poudel et al., 2001; Ferris et al., 2004). In this study, our hypothesis was that the

application of slow-release fertiliser could maintain a lower concentration of mineral N in soil throughout the wheat growth season, which might positively affect the soil nematode community.

Ecological indices such as basal index (BI), channel index (CI), structured index (SI) and enrichment index (EI) may provide insight into the nematode community structure in stressed, enriched, stable structured and decomposition environments, and provide information on the dynamics of the soil food web (Ferris et al., 2001).

EI reflects the availability of resources to the soil food web and the response of primary decomposers to the resources (Ferris et al., 2001). In our study, the EI values were similar among the three treatments, which supported the finding of Ferris and Matute (2003) who reported no effect of fertiliser N on EI values in comparison with a control treatment (no N). It seems that the lower concentration of soil mineral N in SRU was not enough to change the numbers of bacterivorous and fungivorous nematodes.

The CI value was greater in NU than in SRU and CU, which was inconsistent with Ferris and Matute (2003) who observed no difference in succession from bacterivore to fungivore predominance between plots fertilized with ammonium fertiliser and control. The CI values obtained in our study suggested that, compared with the control, the bacterial decomposition pathway in SRU and CU was more important than the fungal decomposition one. In this study, soil C/N value may partly explain the variation of CI values in different treatments. The absence of significant

difference in CI values between SRU and CU supported the conclusion that nematodes were not N limited, and C was more important in ecological succession (Ferris and Matute, 2003).

In contrast to other ecological indices, the SI values were greater in NU and SRU than in CU. The abundance of *Mononchus* and *Aporcelaimus* in SRU was partially responsible for the difference in SI values. The negative correlations between SI values and soil total mineral N confirmed that the SRU treatment could positively affect the nematode community structure during the study period. Greater values of SI suggest a complex community structure with many linkages in the food web (Ferris et al., 2001), which potentially provides more biological control to regulate or suppress plant parasitic nematodes (Berkelmans et al., 2003). Because nematodes are usually numerically dominant in soil environments, changes in their community composition can suggest the changes in functional structure of other soil fauna exposed to a similar type of disturbance (Wu et al., 2002).

In conclusion, the application of slow-release fertiliser could positively affect the nematode community structure with more linkages in the soil food web and with greater resilience to the disturbance, which may benefit plant growth.

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