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RESEARCH ARTICLE

Effect of long-term fertilization on free-living nematode community structure in Mollisols

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Abstract

The influence of long-term application of pig manure combined with chemical fertilizer (MCF) or chemical fertilizer (CF) on free-living nematodes was evaluated in this study. The application model of fertilizers lasted 14 years in Mollisols, and treatments included MCF, CF and no fertilizer (NF). A total of 26 free-living nematode genera belonging to seven functional guilds were found in maize fields, and the community structure of free-living nematodes was different in MCF, CF and NF. Pig manure increased the abundance of bacterivores, especially those belonging to c-p1 (Ba1) and c-p2 (Ba2) guilds. Channel index (CI) was higher in NF than in MCF and CF, but enrichment index (EI) was higher in MCF and CF compared to NF. The structure index (SI) was highest in NF among three treatments. Total bacterivores, Ba1 and Ba2 guilds were positively correlated to organic C, total N, available N, total P and available P, but fungivores only had correlation with organic C and soil moisture. The SI index was negatively related to organic C, total N, available N, total P and available P. Collectively, these results indicate that the pig manure or chemical fertilizer normally applied to increase soil nutrition also induce negative influence on soil food web structure as reflected from biological aspect, and that soil nematodes can enhance agroecological assessments of changes induced by long-term fertilizer application in maize field in Mollisols.

Keywords: Free-living nematodes, food web indices, long-term fertilization, Mollisols, soil physicochemical properties

1. Introduction

Amendments of organic manure or fertilizer are essential for increasing crop productivity, but they also induce environmental changes (Giola *et al*., 2012; Oloyede, 2012; Liu *et al*., 2013; Khan *et al*., 2014). The changes affect soil nematode communities and other soil microorganisms which live in soil and play important roles in nutrient cycling (Fließbach and Mäder, 2000; Yeates, 2003). Soil nematodes are ubiquitous, diverse, abundant, and form one of the dominant belowground communities in agroecosystems (Ritz and Trudgill, 1999). Nematode community attributes such as tropic groups, life history, ecological indices, and food web indices can be combined to form what many researchers consider as ideal bio-indicators for environmental assessment and soil quality assessment (Bongers, 1990; Ferris *et al*., 2001; Yeates, 2003; Liang *et al*., 2009).

Soil nematodes are usually assigned to five or eight trophic groups according to their feeding habits (Yeates *et al*., 1993), and this classification is particularly useful to characterize the positions of nematodes in soil food webs (Moore and de Ruiter, 1991). Free-living nematodes form a large proportion of total soil nematodes, and include bacterivores, fungivores, predators and omnivores. Different trophic groups of free-living nematodes closely correlate to other microorganisms and participate in fundamental ecological processes in soil directly or indirectly, such as decomposition of organic matter and nutrient cycling (Sochová *et al*., 2006). The population of bacterivores with short generations responds quickly to changes in the food resource (Bongers, 1990). Predators or omnivores can feed on other soil nematodes or other soil microfauna, and they are sensitive to disturbances or recovery of the soil environment (Yeates *et al*., 1993).

Soil nematode food web indices provide valuable information on soil processes in agroecosystems (Bulluck *et al*., 2002; Ferris *et al*., 2001; Papatheodorou *et al*., 2012). The development of the structural index (SI) provides a significant advance in measure of the number of trophic layers and potential for regulation of opportunists, the enrichment index (EI) successfully detects resource availability, and the channel index (CI) indicates the predominant decomposition pathway of soil organic matter (Ferris *et al*., 2001). These three indices have been widely used to present the ‘structural’, ‘enrichment’ and ‘decomposition’ conditions of the soil food web under different environments imposed by land use, fertilizer or heavy metal contamination (Ferris *et al*., 2001; Briar *et al*., 2007; Pan *et al*., 2012).

Soil nematode communities and food web indices have been widely applied as ecological tools in various terrestrial ecosystems, especially in agroecosystems (Briar *et al*., 2007; Liang *et al*., 2009; Pan *et al*., 2010). However, the response of soil nematodes to disturbance by fertilizer application was different among different fertilizer regimes, vegetation or soil environments (Villenave *et al*., 2010; Sohlenius *et al*., 2011).

In our previous study in the soybean phase of a maize-soybean rotation in the black soil (Mollisols) zone in Northeast China, we found fertilizer application had significant effects on abundance of bacterivores, fungivores and nematode channel ratio (NCR), and that bacterivores were correlated to soil organic C, total N and available P (Pan *et al*., 2010). Combining our previous findings with those of other studies, we hypothesize that the long-term amendments of pig manure or chemical fertilizer would have a significant effect on free-living nematode community structure in a maize field in Mollisols, that free-living nematode and soil food web indices would be correlated with soil physicochemical properties. The objectives of the present study were to 1) assess the effect of long-term fertilization on free-living nematode community structure, 2) evaluate effect of long-term fertilization on soil nutrition and food web structure, and 3) determine the correlations among free-living nematodes, soil microorganisms, food web indices and soil physicochemical properties in Mollisols.

2. Materials and Methods

A brief overview of experiment and nematode sampling is given here; more details are provided in our previous papers on nematode community structure (Pan *et al*., 2010) at the same experimental site.

2.1. Experimental site

The experiment was conducted on an existing long-term fertilization plots at the National Observation Station of Hailun Agro-ecology System, Heilongjiang province, China (47°26′N, 126°38′E). The farmland was reclaimed from grassland and has been cultivated about 100 years. No fertilizer was applied to the soil in the region in the first 60 years of planting, livestock manures were applied for the next 20 years, and finally, nitrogen fertilizer was applied in the last 20 years. The soils in the region are typical black soil (Udic Mollisols). Annual precipitation is about 500-600 mm, and cumulative temperature (≥10 (^{o}C) is about 2400-2500 degree-days (^{o}C).

2.2. Experimental design and sampling

The long-term fertilization plots were established in 1994. Three fertilizer treatments were included in this study, pig manure combined with chemical N and P fertilizer (MCF), chemical N and P fertilizer (CF) and no fertilizer (NF). A 3-year rotation of wheat, corn and soybean was applied in the fertilization plots since 1994. No chemical fertilizer or manure was applied in NF. For CF, 150 kg N ha^{-1} and 32.8 kg P ha^{-1} were applied. The phosphorus fertilizer was in the form of diammonium phosphate. For MCF, pig manure was applied at 30,000 kg ha^{-1} in addition to above amounts of chemical fertilizers. Pig manure contained average total N, P and K concentrations of 22.1, 2.6 and 2.4 g kg^{-1}, respectively. Each treatment of the parent experiment had four replicates and 12 plots were randomly distributed over the experimental field. Each plot occupied 60 m^2. The plots were plowed to a depth of about 20 cm after crop was harvested in autumn. We sampled three of the four plots for each treatment. Soil samples were collected in corn field in June, July, August and September 2007. Ten soil cores, 5 cm diameter and 20 cm deep were collected from each plot with a manual soil coring tube. The soil samples from each plot were combined and thoroughly mixed by hand, and plant litter or large stones were removed from the soil samples by passing through a 6 mm mesh. All soil samples were kept in a refrigerator at 4 ^{o}C for measurements of soil nematode, soil microorganism and soil physicochemical properties.

2.3. Nematode extraction, identification and classification

Nematodes were extracted from100 g fresh soil by elutriation and centrifugation (Bulluck *et al*., 2002), and were fixed in a 4% formaldehyde solution. Then one quarter of each nematode suspension was observed under a Motic microscope (400 x) and each nematode was identified to genus using diagnostic keys. Free-living soil nematodes were assigned to three trophic groups according to Yeates *et al*. (1993): fungivores, bacterivores and omnivores-predators.

Soil food web condition of different fertilizer treatments was estimated using food web indices, enrichment index (EI), structure index (SI) and channel index (CI) calculated as described by Ferris *et al*. (2001). Bacterivores were assigned to four functional guilds, cp-1, cp-2, cp-3 and cp-4 following Bongers (1990).

2.4. Soil physicochemical property analyses

Soil samples collected in September 2007 were used for analysis of soil properties. Organic C, total N, soil available N, total P, available P, soil moisture and soil pH were determined by the methods described in our earlier paper at the same experimental site (Pan *et al*., 2010).

2.5. Statistical analyses

The abundance data of soil nematode were LN-transformed to meet assumptions of normality prior to statistical analyses. Repeated measures analysis of variance was used to test the overall effect of fertilizer and sampling time on abundance of free-living nematodes and food web indices. Separate one-way analyses of variance (ANOVA) were conducted for the effects of fertilizer on free-living nematode and food web indices at each sampling time. Principal component analysis (PCA) was done on the soil physicochemical properties to express the suite of properties with two independent components. Bivariate correlation analysis was used to test the significance of correlations between free-living nematode abundance, food web indices and soil properties. All statistical tests were conducted at a significance level of 0.05 in SPSS version 16.0 statistical software package (SPSS, Chicago, IL).

3. Results

3.1. Effect of long-term fertilization on community structure and abundance of free-living nematodes

A total of 26 free-living nematode genera belonging to seven functional guilds were found under the three different fertilizer treatments (Table 1). Among these free-living nematodes, *Acrobeloides, Chiloplacus, Protorhabditis and Mesorhabditis* were dominant genera in MCF, *Protorhabditis and Mesorhabditis* were dominant in CF, and *Mesorhabditis* was dominant in NF.

Fertilizer had significant overall effect on bacterivores but not on fungivores or omnivores-predators (Table 2); sampling time had effect on all these three trophic groups, but fertilizer x sampling time had an effect only on bacterivores. Abundance of bacterivores was always highest in MCF (Figure 1). The abundances of bacterivores, fungivores and omnivores-predators fluctuated with sampling times. Bacterivores and fungivores reached their highest abundance at the end of August, while omnivores-predators showed a general increasing trend over the growing season.

All bacterivore genera except *Alaimus* and *Prismatolaimus* belonged to two of the four functional guilds, c-p1 (Ba1) and c-p2 (Ba2) (Table 1). Fertilizer and sampling time both had significant effect on Ba1 and Ba2 (Table 2). Abundance of Ba2 was greater than that of Ba1 in same treatment for all sampling times. Abundance of Ba1was highest in MCF, and lowest in NF. The difference between MCF and CF and NF was greater for Ba1 than Ba2 (Figure 2).

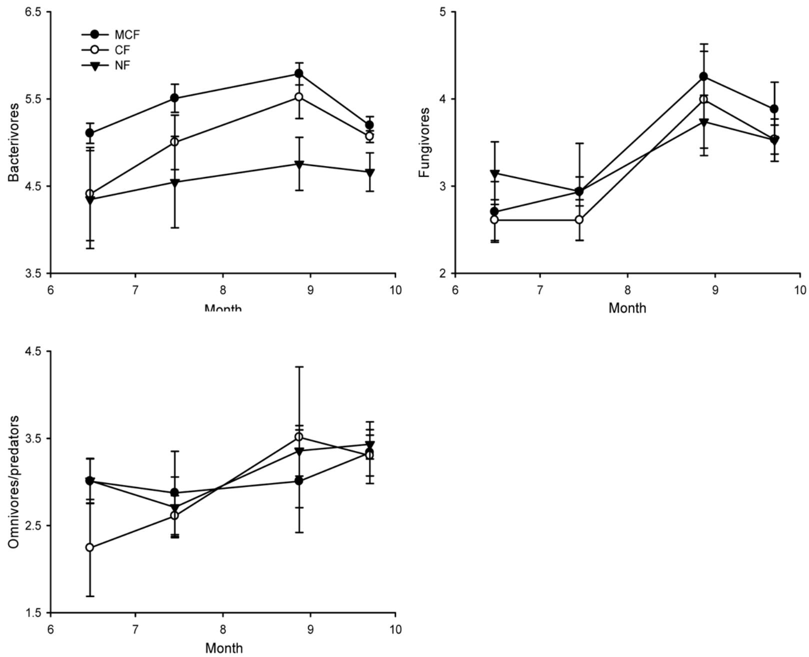

Figure 1. Abundances (LN individuals per 100 g of dry soil) of free-living nematodes in different trophic groups over maize growing season. MCF: pig manure combined with chemical fertilizer; CF: chemical fertilizer; and NF: no fertilizer. Bars in figure are standard errors.

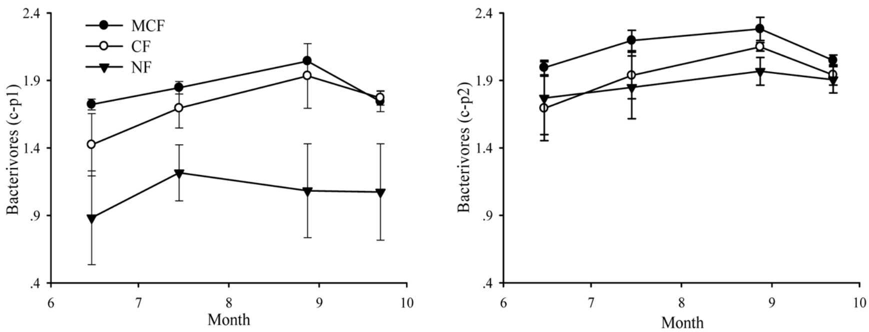

Figure 2. Abundance (LN individuals per 100 g of dry soil) of bacterivores belonging to c-p1 and c-p2 functional guilds in maize growing season. MCF: pig manure combined with chemical fertilizer; CF: chemical fertilizer; and NF: no fertilizer. Bars in figure are standard errors.

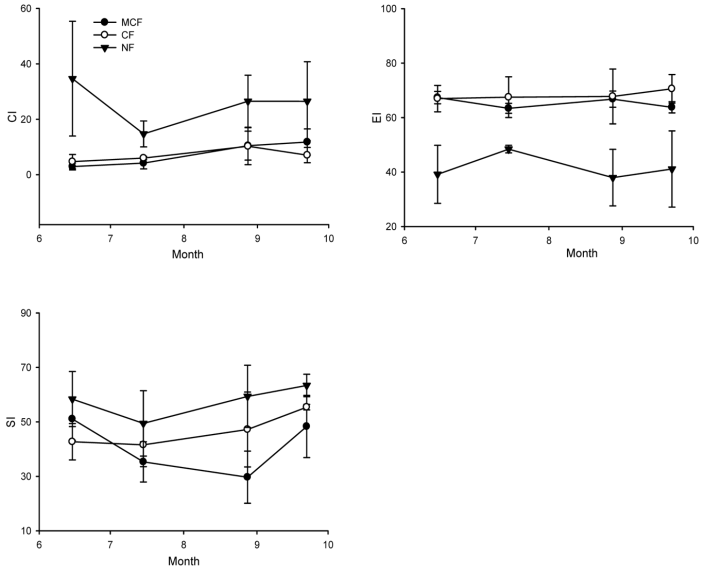

Figure 3. Nematode food web indices in the maize growing season under three fertilizer treatments. CI: channel index, EI: enrichment index, SI: structure index. MCF: pig manure combined with chemical fertilizer; CF: chemical fertilizer; and NF: no fertilizer. Bars in figure are standard errors.

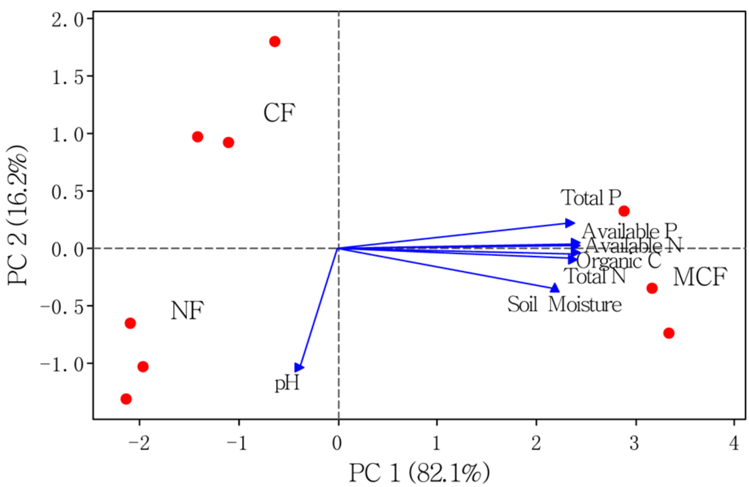

Figure 4. Principal component analysis (PCA) of soil physicochemical properties under different fertilizers. MCF: pig manure combined with chemical fertilizer; CF: chemical fertilizer; and NF: no fertilizer.

(1990). Ba, Fu and Op represent bacterivores, fungivores and omnivores/predators, respectively. Genus was considered as dominant when relative abundance>5%.

Table 1. Abundance and proportional contribution (%) of free-living nematode genera to soil nematodes in maize growing season

Family	Genus	c-p value	Trophic group	Abundance (individual 100^{-1}dry soil)			Relative abundance (%)		
				MCF	CF	NF	MCF	CF	NF
Alaimidae	*Alaimus*	4	Ba	0.4±0.8	0.0±0.0	0.9±1.5	0.1	0.0	0.0
Cephalobidae	*Acrobeles*	2	Ba	16.5±5.2	4.5±5.4	10.8±7.6	3.4	1.1	1.6
	Acrobeloides	2	Ba	36.9±21.5	16.3±12.2	9.7±7.5	7.7	3.8	3.6
	Cephalobus	2	Ba	19.0±4.4	17.3±9.2	4.2±3.4	4.0	4.1	2.7
	Cervidellus	2	Ba	4.9±3.2	3.5±4.2	2.1±3.0	1.0	0.8	1.2
	Chiloplacus	2	Ba	39.9±12.2	17.4±12.4	31.7±14.4	8.3	4.1	3.6
	Eucephalobus	2	Ba	19.9±7.5	19.3±10.9	15.4±10.2	4.1	4.6	3.2
Monhysteridae	*Monhystera*	1	Ba	1.4±2.7	0.0±0.0	0.0±0.0	0.3	0.0	0.0
	Prismatolaimus	3	Ba	0.4±0.8	0.0±0.0	1.2±1.5	0.1	0.0	0.0
Plectidae	*Anaplectus*	2	Ba	8.0±3.7	18.5±9.9	8.9±6.7	1.7	4.4	2.9
	Plectus	2	Ba	4.2±2.0	1.7±3.0	0.4±0.8	0.9	0.4	0.9
Rhabditidae	*Mesorhabditis*	1	Ba	44.1±13.1	40.1±20.5	8.2±6.8	9.2	9.4	6.0
	Protorhabditis	1	Ba	31.7±17.5	21.7±13.4	6.3±6.6	6.6	5.1	3.9
Anguinidae	*Ditylenchus*	2	Fu	7.2±9.0	3.9±4.0	2.6±3.8	1.5	0.9	1.2
Aphelenchoididae	*Aphelenchoides*	2	Fu	6.2±6.0	4.2±4.5	5.4±3.8	1.3	1.0	1.3
Aphelenchidae	*Aphelenchus*	2	Fu	15.3±12.2	11.7±4.8	8.0±7.3	3.2	2.8	1.4
Tylenchidae	*Filenchus*	2	Fu	11.6±5.3	11.2±3.3	13.7±4.2	2.4	2.6	1.0
Aporcelaimidae	*Aporcelaimus*	5	Op	13.5±1.7	11.0±5.6	16.9±4.8	2.8	2.6	1.6
Dorylaimidae	Mesodorylaimus	4	Op	0.0±0.0	0.0±0.0	1.7±2.3	0.0	0.0	0.0
Leptonchidae	*Doryllium*	4	Op	0.0±0.0	2.5±1.5	3.8±2.8	0.0	0.6	0.4
Mononchidae	*Mononchus*	4	Op	0.8±1.6	0.8±1.5	0.0±0.0	0.2	0.2	0.4
Nordiidae	*Enchodelus*	4	Op	0.0±0.0	0.4±0.7	0.4±0.8	0.0	0.1	0.2
Qudsianematidae	*Discolaimium*	4	Op	0.4±0.9	0.0±0.0	0.0±0.0	0.1	0.0	0.0
	Eudorylaimus	4	Op	0.4±0.8	0.4±0.7	0.4±0.8	0.1	0.1	0.2
	Labronema	4	Op	1.3±1.7	1.3±2.2	0.0±0.0	0.3	0.3	0.7
	Thorneella	4	Op	6.1±3.9	6.6±7.2	0.4±0.8	1.3	1.5	2.1

Note: c-p value referred to Bongers

Table 2. Repeated measures ANOVA for effects of fertilizer, sampling time and interaction of fertilizer x sampling time on free-living nematodes and food web indices

Nematode trophic	Fertilizer		Time		Fertilizer × time	
group and index	F value	df	F value	df	F value	df
Bacterivores	22.5**	2	18.2**	3	3.1*	6
Fungivores	1.6	2	13.8**	3	0.9	6
Ba1	44.0**	2	10.5**	3	2.7	6
Ba2	12.7**	2	13.5**	3	1.8	6
Omnivores-predators	0.1	2	4.1*	3	1.0	6
CI	35.4**	2	1.3	3	86.5**	6
EI	204.9**	2	0.1	3	0.57	6
SI	8.7*	2	3.9*	3	1.3	6

Note: Ba1 and Ba2 represent bacterivores belonging to c-p1 and c-p2 functional guild. CI, EI and SI mean channel index, enrichment index and structure index, respectively. "*"means significance at the level of $p<0.05$, "**" means significance at the level of $p<0.01$.

3.2. Food web evaluation

Fertilizer treatment had significant overall impact on food web indices CI, EI and SI, but sampling time had a significant effect only on SI (Table 2). The value of CI was higher in NF than in MCF and CF (Fig. 3). Higher value of EI was observed in MCF and CF compared to NF. There were no significant differences in the value of CI or EI between MCF and CF. The value of SI was highest in NF among the three treatments, but the difference was not significant at each sampling time. Indices CI and EI had slight fluctuation across sampling time, especially in MCF and CF.

3.3. Correlations between free-living nematodes and soil properties

The first two principal components PC1 and PC2 explained 98.3% of the variation of the seven parameter soil properties (Fig. 4). A biplot showed strong positive correlation among organic C, total N, available N, total P, available P and soil moisture, and they all negatively correlated to soil pH. Organic C, total N, available N and available P had a small contribution, total P and soil moisture had an intermediate contribution, and soil pH had a large contribution to PC2 (Table 3). The three fertilizer treatments formed three distinct and widely separated clusters on the PC1 vs. PC2 plot (Figure 4) which demonstrates the effect of the long term fertilizer treatments on soil physiochemical properties.

The abundance of bacterivores, Ba1 and Ba2 significantly positively correlated to organic C, total N, available N, total P and available P, and the correlation coefficient of Ba2 with all soil physicochemical properties except soil moisture were correspondingly stronger than those for Ba1 (Table 4). Fungivores only had significant positive correlation with organic C and soil moisture. Omnivores-predators had negative correlation with all soil properties except pH, but not all of the correlations were significant. SI was negatively correlated to organic C, total N, available N, total P and available P, but none of correlations between the other two soil nematode food web indices, CI and EI and soil properties were significant.

Table 3. Component score coefficient matrix for first two principal components of soil physicochemical properties

Component	PC1	PC2
Organic C	0.414	-0.038
Total N	0.411	-0.079
Available N	0.416	0.033
Total P	0.404	0.197
Available P	0.415	0.024
pH	-0.069	-0.925
Soil Moisture	0.382	-0.311

Table 4. Correlation coefficients among free-living nematodes, food web indices and soil properties

	Organic C	Total N	Available N	Total P	Available P	pH	Soil moisture
Bacterivores	0.76*	0.67*	0.76*	0.82**	0.77*	-0.57	0.53
Ba1	0.79*	0.74*	0.8**	0.81**	0.83**	-0.12	0.85**
Ba2	0.88*	0.86**	0.90**	0.91**	0.88**	-0.28	0.81**
Fungivores	0.67*	0.63	0.6	0.55	0.62	-0.17	0.72*
Omnivores-predators	-0.15	-0.19	-0.11	-0.16	-0.06	0.07	-0.04
CI	-0.32	-0.26	-0.37	-0.47	-0.37	0.43	-0.11
EI	0.41	0.36	0.45	0.56	0.43	-0.53	0.15
SI	-0.75*	-0.69*	-0.69*	-0.72*	-0.69*	0.43	-0.57

Note: Ba1 and Ba2 represent bacterivores belonging to c-p1 and c-p2 functional guilds. CI, EI and SI mean channel index, enrichment index and structure index, respectively. "*"means significance at the level of $p<0.05$, "**" means significance at the level of $p<0.01$.

4. Discussion

Among 26 free-living nematode genera found in this experiment, *Acrobeloides*, *Chiloplacus*, *Mesorhabditis* and *Protorhabditis* were dominant genera in MCF, and *Mesorhabditis* and *Protorhabditis* was the only dominant genus in CF, which indicates that pig manure and chemical fertilizer favored different genera. Ferris *et al*. (1997) also found *Acrobeloides* was predominant in the cropping systems with organic inputs. Relative abundance of *Mesorhabditis* was high in the two treatments with chemical fertilizer, MCF and CF, which indicates that *Mesorhabditis* was insensitive to the environmental change induced by fertilizer amendment in Mollisols. Fertilizer treatments had significant effect on bacterivores. The abundance of bacterivores was highest in MCF, intermediate in CF, and lowest in NF. Our results are in agreement with previous findings that bacterivores were more prevalent in organic than conventional chemical fertilizer treatments (Li *et al*., 2010). Based on the life strategies, four guilds of bacterivores were found, and the abundance of Ba2 was the largest among of them. The abundances of Ba1 and Ba2 were both significantly increased after organic amendments were applied to the soil, and while the abundance of Ba1 increased more than Ba2, Ba1 was still lower than Ba2. Ba1 and Ba2 are enrichment-opportunists and general opportunists, respectively, and the abundance of enrichment-opportunists will respond more rapidly than general opportunists when food resources are changed. Our results are in agreement with the findings of Villenave *et al*. (2004), but contrasts with the results of Liang *et al*. (2009) who found higher abundance of Ba1 than Ba2 under organic amendment condition. The different findings are likely due to different soil environment or nematode genera. Ba1 was mainly composed by three genera in our study, but it was composed by seven genera in the research of Liang *et al*. (2009).

We did not find significant effect of long-term pig manure application on fungivores in this study. This is inconsistent with previous findings that organic materials resulted in abundance increase of fungivores (Bulluck *et al*., 2002; Liang *et al*., 2009). The different results are likely due to different organic materials, dosage or crops used in the different research.

Most studies found omnivores–predators are sensitive to disturbance of fertilizer or tillage, and need longer time to recover than fungivores and bacterivores (Bulluck *et al*., 2002). We did not observe effect of fertilizer on omnivores–predators. This result is in agreement with Villenave *et al*. (2010) who found the effects of organic or fertilizer amendments on composition and abundance of omnivores–predators were minimal. In our experiment, most omnivores–predators belonged to c-p4 guild and only one genus belonged to c-p5 guild, which likely lead to this result because nematodes more sensitive to fertilizer disturbance are generally in a higher c-p guild than in a lower c-p guild (Tenuta and Ferris, 2004).

Fertilizer had significant effect on soil nematode food web indices CI, EI and SI. The index CI was lower in MCF and CF than in NF, which suggests pig manure or chemical fertilizer stimulate bacterial decomposition and increase nutrient cycling rate (Ferris *et al*., 2001). The EI index positively correlated to available resources of food web, and in combination with CI, provides a powerful tool for assessing soil fertility and nutrient availability (Ferris *et al*., 2001). The amendment of manure or chemical fertilizer resulted in higher EI in MCF and CF compared to NF, suggesting that more nutrition is available in MCF and CF than in NF. This result is consistent with previous findings that organic matter (crop cover) or human input enhanced EI (DuPont *et al*., 2009). The

SI index can be used to assess the structure of the soil food web from faunal profile, and the higher SI suggests a complex community structure with many linkages in the soil food web (Ferris *et al*., 2001). The application of pig manure decreased the value of SI in our study. This result is consistent with Villenave *et al*. (2010) who found lower SI in organic amendment than in un-amended control. This is mainly due to pig manure increasing the abundance of bacterivores, especially those belonging to lower c-p guilds, rather than omnivores-predators; the SI index is primarily determined by higher c-p guilds of bacterivores and omnivores–predators.

The three fertilizer treatments occurred in three clearly separated clusters in the PCA biplot, which means that fertilizer treatment clearly had an effect on soil physicochemical properties measured in this study. Bacterivores, Ba1 and Ba2 have positive correlations with organic C, total N, available N, total P and available P. This is consistent with previous findings that bacterivores positively correlated to NH_4^+, potentially mineralizable N, organic C and available P (Sánchez-Moreno *et al*., 2008; Pan *et al*., 2010). Our results support previous findings that bacterivores have important contribution to nutrient cycling (Ingham *et al*., 1985; Fu *et al*., 2005).

Many studies show that the EI index is correlated with soil nutrition (Sánchez-Moreno *et al*., 2008), but in our study, correlation of EI with organic C, total N, available N, total P and available P was not significant. This is likely due to the different soil type and range of physicochemical properties among the three fertilizer treatments. A negative correlation between SI and organic C, total N, available N, total P and available P was observed in our study. Similar results were also found previously where SI was negatively correlated to content of NH_4-N and NO_3-N in the soil (Sánchez-Moreno *et al*., 2006). This might due to relative abundance of bacterivores belonging to higher c-p guilds and omnivores-predators, which are positively related to SI (Ferris *et al*., 2001) were decreased after amendment of pig manure or chemical fertilizer; as the relative abundance of Ba1 and Ba2 are quickly increased in the environment where soil nutrition is enriched.

5. Conclusions

The results of this study showed that long-term fertilization had significant effect on free-living nematode community structure in maize phase of a maize-soybean rotation field in Mollisols. Amendment of pig manure or chemical fertilizer increased the abundance of bacterivores, but had little effect on fungivores and omnivores-predators, suggesting that bacterivores are more sensitive to environmental changes caused by amendment of fertilization. Nutrient cycling was promoted by pig manure or chemical fertilizer as indicated by lower value of CI in MCF or CF compared to NF. Higher value of EI was found in MCF and CF, indicating amendment of pig manure or chemical fertilizer resulted in more available resource. The application of pig manure decreased the value of SI in our study, indicating that soil food web was disturbed. Bacterivores, Ba1 and Ba2 were all positively related to organic C, total N, available N, total P and available P. A negative correlation between SI and soil physiochemical properties (organic C, total N, available N, total P and available P) was observed, implying that the pig manure or chemical fertilizer may induce negative influence on soil food web structure reflected from biological aspect. The relationships among the nematode indices, fertilizer treatments, and soil properties illustrate that soil nematodes can provide insights in agroecological assessments of changes induced by long-term fertilizer application in maize field in Mollisols.

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