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SOIL BIOLOGICAL AND BIOCHEMICAL QUALITY OF WHEAT-MAIZE CROPPING SYSTEM IN LONG-TERM FERTILIZER EXPERIMENTS

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SUMMARY

Two long-term field experiments, each consisting of three treatments (organic compost treatment, chemical fertilizer treatment and an untreated control) were established in 1993 and 1997, respectively. Soil samples were collected from each plot in June 2004 and 2005 after crop harvest and were used to determine soil physical-chemical properties, biological and biochemical activity, and the nematode community. Soil physicochemical parameters, microbial biomass, biological activities and nematode communities were significantly influenced by long-term application of organic compost. In general, soil total organic carbon, dissolved organic carbon, total nitrogen, alkaline-hydrolysable nitrogen, available phosphorus, and available potassium, microbial biomass, basal respiration, urease activities, total number of nematodes and bacterial-feeding nematodes were significantly higher in the compost plots than in the chemical fertilizer and control plots at two experimental sites and two sampling dates. Soil bulk density and pH values were significantly lower in the compost plots. We conclude that soil physical-chemical properties, size and activity of soil microbial biomass, metabolic quotient (qCO_2), urease activity, total number of nematodes and bacteria-feeding nematodes could be used as indicators of soil quality.

INTRODUCTION

In order to meet the demands of food grain production, maintaining soil quality is essential (Mandal *et al.*, 2007). Soil quality does not only depend on soil physical and chemical properties, but it is also very closely correlated with its biological properties (Van Eekeren *et al.*, 2009).

Soil microbial biomass, the living component of the soil organic matter, which comprises 1–5% of soil total organic carbon (Nsabimana *et al.*, 2004), can be used as an important bioindicator of soil quality (Gil-Sotres *et al.*, 2005). Soil respiration is a well-established parameter to monitor soil organic matter decomposition, but it is also highly variable and can show wide natural fluctuation depending on substrate availability, moisture and temperature (Masto *et al.*, 2006). Soil enzymes are important components involved in soil nutrient transformations; furthermore, soil enzyme activity is considered to be a major contributor to soil quality (Masto *et al.*, 2006). Phosphatases are involved in the transformation of organic and inorganic

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phosphorus compounds in soil (Amador *et al.*, 1997) and soil ureases are closely associated with releasing inorganic nitrogen (N) (Bandick and Dick, 1999).

Soil nematodes are ubiquitous and are the most abundant metazoans; they are well adapted to a wide range of environmental conditions and respond rapidly to soil disturbance (Cheng and Grewal, 2009). The structure of the nematode community is an efficient instrument as a biological indicator of soil quality, because the soil nematodes belong to several trophic levels, namely, plant feeders, bacterial feeders, fungal feeders, omnivores and predators, and occupy central positions in the soil detritus food web (Neher, 2001; Villenave *et al.*, 2009). They are also linked closely to soil ecological processes such as decomposition of soil organic matter, N mineralization, nutrient cycling and plant growth (Wu *et al.*, 2002).

Some studies have suggested that the nematode community structure and trophic group diversity may be useful and readily measured bioindicators of soil quality (Ferris *et al.*, 1996; Fu *et al.*, 2009; Urzelai *et al.*, 2000). Analysis of nematode community structure can help to estimate responses of the soil environment to perturbation, such as, application of fertilizer (Vestergård, 2004), tillage (Fu *et al.*, 2000), irrigation (Porazinska *et al.*, 1998; 1999) and pesticides (Yardim and Edwards, 1998). Additionally, Bongers (1990) put forward a maturity index according to nematode life history characteristics; this is used as a tool to assess soil quality for references sites (Bongers and Ferris, 1999).

Although some studies have reported effects of long-term application of manure and fertilizer on soil biological and biochemical activities (Mandal *et al.*, 2007), only single measures of microbial biomass, enzyme activity or the nematode community were carried out. Information on biological processes, such as size and activity of soil microbial biomass, soil enzyme activities and the nematode community is limited. In the Northern China Plain, the integrated influence of long-term application of compost and chemical fertilizer on the soil microbial biomass, biochemical properties and nematode community are still poorly understood. The objective of this study was to determine the impact of long-term addition of organic and inorganic fertilizers on soil physical, chemical, biochemical, microbial characteristics, free-living nematode community and crop yields in a wheat-maize cropping system.

MATERIALS AND METHODS

Experimental site and design

The experiment sites were located at Qu-Zhou experimental station of China Agricultural University in Hebei Province, Northern China (36°52′N; 115°01′E). The experimental station is in a continental temperate monsoon zone and the climate in the region is warm and semi-humid with summer rainfall and dry, cold winters. The mean annual temperature is 13.2 °C and ranges from a minimum of −2.9 °C in January to a maximum of 26.8 °C in July. The mean annual precipitation is 542.7 mm, of which 60% occurs from July to September, and the annual non-frost period is 201 days. Spring drought is very severe. The soil at the study site is an improved silt fluvo-aquic soil (Hu *et al.*, 2008).

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The long-term field experiment was conducted at two experimental sites, one was initiated in 1993 (experimental site A), and the other, in 1997 (experimental site B). The experiment was designed with three treatments and three replications, with nine plots laid out in a randomized complete block design at each experimental site. Plots, (3 m × 10.5 m each plot at site A and 4 m × 8 m each plot at site B), were planted with wheat (*Triticum aestivum*) in winter and with maize (*Zea mays*) in summer every year. Three treatments consisted of compost treatment (OF) (15 t ha^{-1}), chemical fertilizer treatment (IF) (265.5 kg N ha^{-1} and 90 kg P_2O_5 ha^{-1}) and a control (no fertilizer, NF). The compost was 60% straw, 30% livestock dung, 5% cottonseed-pressed trash and 5% bran (with a mean nutrient content of 100.5 kg N ha^{-1}, 36 kg P_2O_5 ha^{-1} and 196.2 kg K_2 O ha^{-1}). Before planting summer maize and winter wheat every year, the OF plots were treated with compost, and the IF plots were treated with chemical fertilizer respectively, while the control (NF) plots did not receive any soil amendment. The compost and chemical fertilizer were not applied during the crop growth stages during the experiment.

Sampling

Soil samples were collected from the 0–20 cm soil layer at both sites, using a 2.5 cm diameter soil auger after wheat harvesting and before maize sowing on 8 June 2004 and on 10 June 2005. Each soil sample consisted of 15 cores (2.5 cm diameter × 20 cm deep), which were mixed to form a bulk sample, and samples were collected from each plot. The soil samples were stored in insulated and tied plastic bags to prevent moisture loss and were transported to the laboratory as soon as possible. All soil assays were completed within a week of soil sampling.

Laboratory analysis

Soil bulk density was determined using the core volume and dry soil weight (stainless steel cylinders with diameter and height of 5 cm). The bulk density was expressed by dividing the weight of the dried soil by the volume of the core used. Soil moisture in each sample was determined gravimetrically by drying the soil samples at 105 °C for 48 hours. Soil subsamples were air-dried for 14 days at room temperature, sieved through a 1-mm screen and mixed, and sub-samples were assayed for alkaline hydrolysable N, available phosphorus (P), available potassium (K) and soil pH.

The air-dried sub-samples were ground to pass through a 0.25-mm sieve for determination of soil organic matter and total N content. The potassium dichromate external heating method (Blakemore *et al.*, 1972) was used to determine soil organic matter content. The semi-micro Kjeldahl method and the alkaline-hydrolysable diffusion method (Bremner, 1996) were used to determine total N and alkaline-hydrolysable N content.

Soil available P was extracted with 0.5 mol l^{-1} $NaHCO_3$ (soil: solution = 1:20) and measured with the Olsen method (Blakemore *et al.*, 1972). Soil available K was extracted with 1 mol l^{-1} NH_4 Ac (soil: solution = 1:10) and measured with the flame photometry method (Blakemore *et al.*, 1972). Soil pH was measured in 0.01 mol l^{-1}

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$CaCl_2$ slurry (soil: solution = 1:2.5) using a glass electrode. All the data were expressed on dry mass basis.

Soil basal respiration was determined by placing 50 g field moist soil samples into 50 ml beakers and incubating the samples in the dark at 25 °C in 1000 ml sealed jars along with a beaker contained 5 ml of 1 mol l^{-1} NaOH solutions, which captured respired CO_2. Then, the NaOH solution was removed and titrated to determine the amount of CO_2 evolved from the soil microbial respiration (Hu and van Bruggen, 1997). Microbial biomass C was analysed using chloroform fumigation-extraction based on the difference between carbon extracted with 0.5 mol l^{-1} K_2SO_4 from chloroform-fumigated and the unfumigated soil samples (Vance *et al.*, 1987). Microbial biomass C was calculated as the difference between fumigated and non-fumigated samples divided by the K_2SO_4 extract efficiency factor for microbial C ($Kc = 0.379$) (Vance *et al.*, 1987). The data were expressed on dry mass basis. The metabolic quotient (qCO_2) was calculated as the ratio of basal respiration to microbial biomass C (Anderson and Domsch, 1993). The qCO_2 is a specific parameter for evaluating the effects of environmental conditions on the soil microbial biomass. The metabolic quotient was expressed as basal respiration rate (mg CO_2-C h^{-1}) per g^{-1} of microbial biomass C.

Soil alkali phosphatase enzyme activities (field moist soil samples) were determined using the *p*-nitrophenol (*p*NP) method (Tabatabai, 1994), and the activity was expressed as μg *p*-nitrophenol (*p*NP) per g dry soil and incubation time. Soil urease activity (field moist soil samples) was measured by using urea as the substrate, and the released ammonium was determined spectrophotometrically at 578 nm (Tabatabai, 1994). Urease activity was expressed as μg N-NH_4^+ produced per g dry soil and incubation time.

Nematodes were extracted from 100 g composite fresh soil samples using the sugar flotation and centrifugation method (Barker *et al.*, 1985). The nematodes recovered were counted and preserved in 4% formaldehyde. A randomly selected 100 specimens per sample were identified to genus level, using an inverted compound microscope. The nematode populations were expressed per 100 g dry soil (Pen-mouratov *et al.*, 2008).

Nematode index

The characteristics of the nematode communities were described by the following approaches: (1) total number of nematodes per 100 g dry soil (TNEM); (2) trophic groups, including bacteria-feeders, fungi-feeders, plant-parasites, omnivores-predators (Yeates *et al.*, 1993); (3) F/B, fungi-feeders/bacteria-feeders ratio (Twinn *et al.*, 1974); (4) T, trophic diversity, $T = 1/\sum pi^2$, in which *pi* is the proportion of the *i*-th trophic group in the nematode community (Heip *et al.*, 1988); (5) H', Shannon index, $H' = -\sum pi(lnpi)$, where pi is the proportion of individuals in the i-th taxon (Shannon and Weaver, 1949); (6) MI, maturity index, $MI = \sum vipi$, where *vi*, is the c-p value for free-living nematodes assigned by Bongers (1990) to the *i*-th nematode genus and *pi* is the proportion of the genus in the nematode community;

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Table 1. Soil physicochemical property under different treatments for two experimental sites in 2004.

Item	Experimental site A			Experimental site B		
	OF	IF	NF	OF	IF	NF
Total organic C (g kg^{-1})	12.10a	7.95b	7.46b	12.96a	7.68b	7.06b
Dissolved organic C (mg kg^{-1})	112.29a	65.76b	53.98b	107.40a	62.66b	59.10b
Total N (g kg^{-1})	1.20a	0.83b	0.81b	1.38a	0.81b	0.78b
Alkaline-hydrolysable N (mg kg^{-1})	103.71a	68.93b	68.43b	110.60a	68.87b	63.29b
Available P (mg kg^{-1})	36.29a	23.63b	4.07c	54.92a	20.12b	3.25c
Available K (mg kg^{-1})	161.75a	72.84b	80.86b	278.08a	94.23b	98.91b
pH value	7.26b	7.41a	7.53a	7.17b	7.44a	7.52a
Bulk density (g cm^{-3})	1.32b	1.47a	1.47a	1.23b	1.39a	1.37a

OF: organic compost treatment; IF: inorganic fertilizer treatment; NF: no fertilizer treatment (control). Different letters (a, b, c) indicate significant differences ($p < 0.05$) between treatments according to LSD multiple comparison.

(7) PPI, plant-parasite index, PPI = $\sum vipi$, where *vi*, is the c-p value for plant-parasitic nematodes assigned by Bongers (1990) to the *i*-th nematode genus and *pi* is the proportion of the genus in the nematode community; (8) MMI, modified maturity index, including plant-parasitic nematodes, MMI = $\sum vipi$, where vi is the c-p value for free-living and plant parasitic nematodes assigned by Bongers (1990) to the *i*-th nematode genus and *pi* is the proportion of the genus in the nematode community (Yeates, 1994).

Data analysis

All data were subjected to statistical analysis of variance (ANOVA) using the SPSS 11.5 software package and were used to evaluate differences between separate means. Difference obtained at $p < 0.05$ level was considered as statistically significant using the LSD (least significant difference) test.

RESULTS

Soil physical-chemical properties

The long-term application of organic and inorganic fertilizers caused significant changes in soil physicochemical properties (Table 1). Soil bulk density and pH values were significantly ($p < 0.01$) lower in the compost plot than in the chemical fertilizer and control plots for the two sites. Soil total organic carbon, dissolved organic carbon, total N, alkaline-hydrolysable N, available P and available K content were significantly ($p < 0.001$) higher in the compost plots than in the chemical fertilizer and control plots for both sites. Moreover, these parameters, except available K content, were higher in the chemical fertilizer plots than in the control plots for both sites. All these parameters were significantly ($p < 0.01$) influenced by fertilizers, but only soil bulk density and available K content were significantly ($p < 0.05$) affected by experimental sites and only soil total organic carbon, available P and available K content were significantly ($p < 0.05$) affected by interaction effects.

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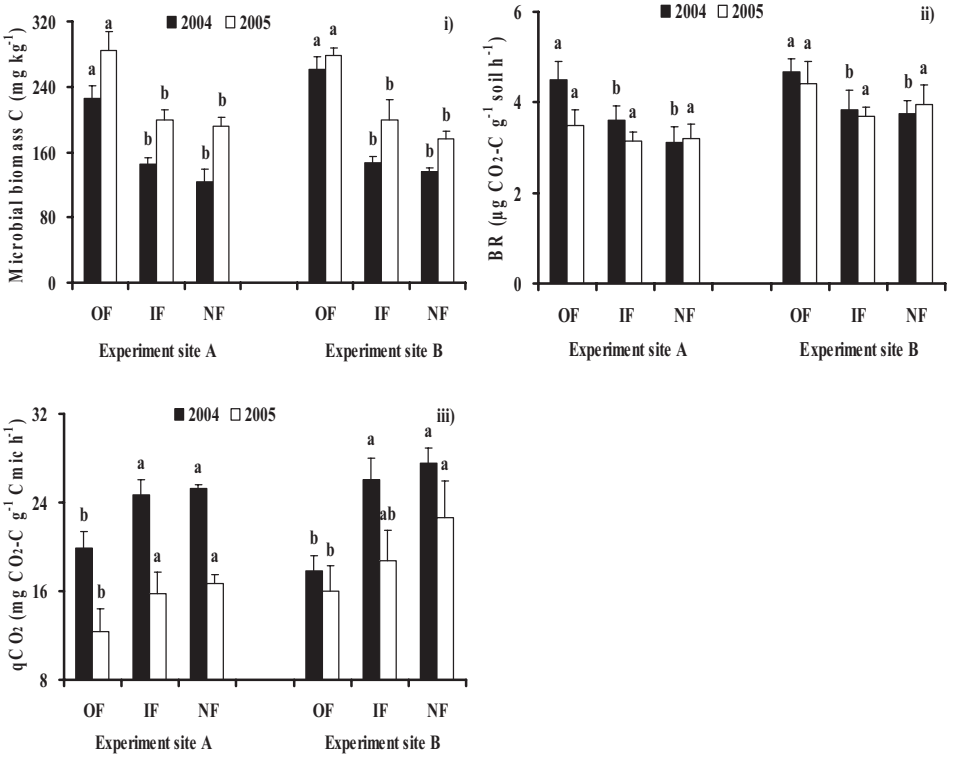

Figure 1. Soil microbial parameter under the long-term fertilizer experiment in 2004 and 2005. Different letters (a, b, c) indicate significant differences ($p < 0.05$) between treatments according to LSD multiple comparison. Bars indicate standard errors ($n = 3$). i) soil microbial biomass C; ii) soil basal respiration; iii) metabolic quotient (qCO_2). OF: organic compost treatment; IF: inorganic fertilizer treatment; NF: no fertilizer.

Soil microbial biomass carbon and basal respiration

Soil microbial biomass carbon (MBC) content was significantly ($p < 0.001$) higher in the compost plots than in the chemical fertilizer and control plots for both sites in 2004 and 2005 (Figure 1i). Furthermore, soil MBC content in the chemical fertilizer plots was higher than in the control plots for both sites in both years. The soil MBC was significantly ($p < 0.05$) influenced by fertilizers and experiment sites in 2004, but significantly ($p < 0.001$) influenced only by fertilizers in 2005; the interaction effect was not significant.

Soil basal respiration (BR) showed similar trends to those for soil MBC content. The soil BR was significantly ($p < 0.001$) higher in the compost plots than in the chemical fertilizer and control plots for both sites in both years (Figure 1ii). Moreover, the soil BR was higher in the chemical fertilizer plots than in control plots for both sites in 2004, but this was reversed in 2005. The soil BR was significantly ($p < 0.05$) influenced by fertilizers in both years, and significantly ($p < 0.01$) influenced only by experimental sites in 2005; the interaction effect was not significant.

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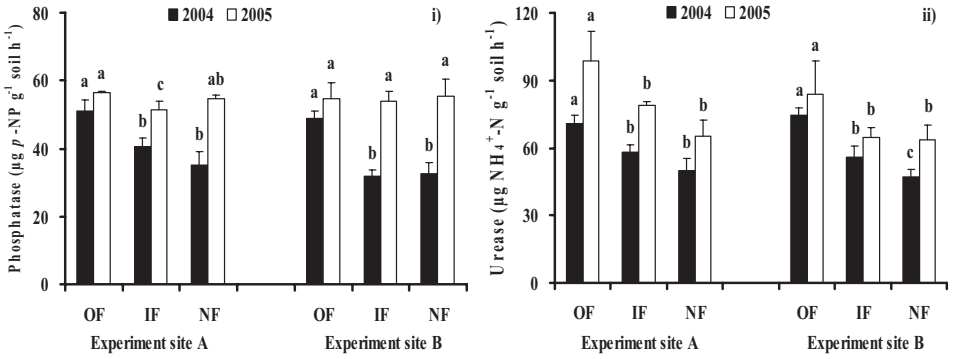

Figure 2. Soil enzyme activity under the long-term fertilizer experiment in 2004 and 2005. Different letters (a, b, c) indicate significant differences ($p < 0.05$) between treatments according to LSD multiple comparison. Bars indicate standard errors ($n = 3$). i): alkaline phosphatase activity; ii) urease activity. OF: organic compost treatment; IF: inorganic fertilizer treatment; NF: no fertilizer.

In the compost plots qCO_2 was significantly ($p < 0.001$) lower than in the chemical fertilizer and control plots for both sites in 2004, and it was significantly ($p < 0.001$) lower in the compost plots than in the control plots for both sites in 2005 (Figure 1iii). Like soil BR, the qCO_2 value was significantly ($p < 0.01$) influenced by fertilizers in 2004 and 2005, and significantly ($p < 0.01$) influenced only by experimental sites in 2005, but a significant ($p < 0.05$) interaction effect was found only in 2004.

Soil enzyme activity

The soil alkaline phosphatase activities were significantly ($p < 0.001$) higher in the compost plots than in the chemical fertilizer and control plots for both sites in 2004, and were higher in the compost and control plots than in the chemical fertilizer plots for both sites in 2005 (Figure 2i). The alkaline phosphatase activities were significantly ($p < 0.05$) influenced by fertilizers and experimental sites in 2004, but the interaction effect was not significant. However, treatments, experimental sites and their interaction effect were not significant in 2005.

The soil urease activities were significantly ($p < 0.01$) higher in the compost plots than in the chemical fertilizer and control plots for both sites in 2004 and in 2005. Soil urease activities were higher in the chemical fertilizer plots than in the control plots for both sites in 2004 and 2005 (Figure 2ii). The soil urease activities were significantly ($p < 0.001$) influenced only by fertilizers in 2004, and were significantly ($p < 0.05$) influenced by fertilizers and experimental sites in 2005, but the interaction effect was not significant in either year.

Nematode community structure

The total number of nematodes were significantly ($p < 0.001$) higher in the compost plots than in the chemical fertilizer and control plots for both sites in both years (Figure 3; supplementary tables 2 and 3 (available at journals.cambridge.org/eag)). The total number of nematodes in the control plots was higher than in the chemical

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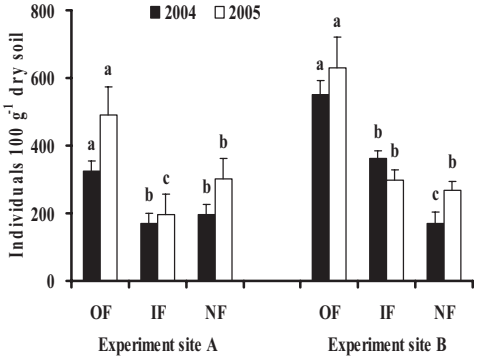

Figure 3. Total number of nematodes in soils under the long-term fertilizer experiment in 2004 and 2005. Different letters (a, b, c) indicate significant differences ($p < 0.05$) between treatments according to LSD multiple comparison. Bars indicate standard errors ($n = 3$). OF: organic compost treatment; IF: inorganic fertilizer treatment; NF: no fertilizer.

fertilizer plots for both years under experimental site A, whereas the total number of nematodes in the chemical fertilizer plots was higher than in the control plots for both years under experimental site B. The total number of nematodes were significantly ($p < 0.05$) influenced by fertilizers and experimental sites in 2004 and 2005, but significant ($p < 0.001$) interaction effect was found only in 2004.

Bacteria-feeding nematodes showed similar trends to those for total number of nematodes. Bacteria-feeding nematodes were significantly ($p < 0.001$) higher in the compost plots than in the chemical fertilizer and control plots for both sites in both years (Figure 4i). Bacteria-feeding nematodes in the control plots were higher than in the chemical fertilizer plots for both years at experimental site A, whereas bacteria-feeding nematodes in the chemical fertilizer plots was higher than in the control plots in 2004 at experimental site B. The relative abundance of bacteria-feeding nematodes in the compost plots were significantly ($p < 0.05$) higher than in the chemical fertilizer plots at both sites in both years. Bacteria-feeding nematodes were significantly ($p < 0.001$) influenced by fertilizers in both years, but experimental sites effect and interaction effect were not significant.

Fungi-feeding nematodes were the least abundant trophic group. Fungi-feeding nematodes were higher in the compost plots than in the chemical fertilizer plots, and were higher in the chemical fertilizer plots than in the control plots for both sites in 2005 (Figure 4ii). Fungi-feeding nematodes also showed a similar trend at experimental site B in 2004. Fungi-feeding nematodes were significantly ($p < 0.01$) influenced only by experimental site in 2004.

Plant parasitic nematodes were the most dominant trophic groups in the present study. Plant parasites were significantly ($p < 0.05$) higher in the compost plots than in the control plot for both years in experimental site B, and were higher in the chemical fertilizer plots than in the control plots (Figure 4iii). Nevertheless, the relative abundance of plant-parasites in the compost plots was lower than in the chemical fertilizer plot for both sites in both years. Plant parasites were significantly ($p < 0.05$)

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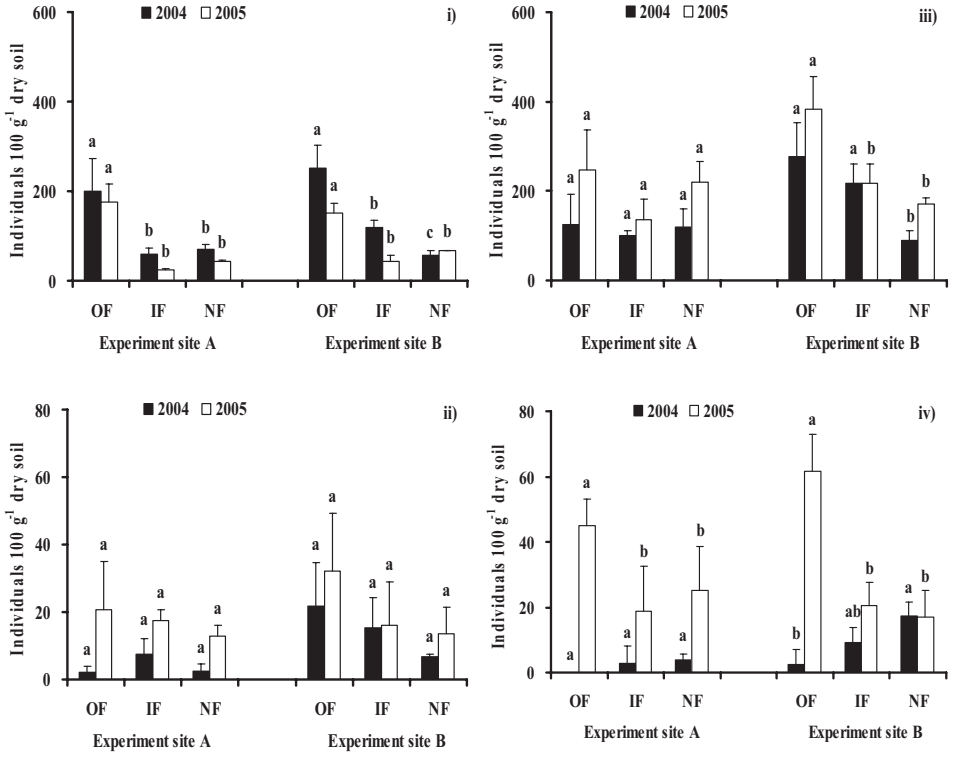

Figure 4. Nematode trophic group in soils under the long-term fertilizer experiment in 2004 and 2005. Different letters (a, b, c) indicate significant differences ($p < 0.05$) between treatments according to LSD multiple comparison. Bars indicate standard errors ($n = 3$). i) bacteria-feeding nematodes; ii) fungi-feeding nematodes; iii) plant-parasitic nematodes; iv) omnivore-predator nematodes. OF: organic compost treatment; IF: inorganic fertilizer treatment; NF: no fertilizer.

influenced by fertilizers and interaction effect in both years, but were significantly ($p < 0.01$) influenced by experimental sites only in 2004.

The omnivores-predators were higher in the compost plots than in the chemical fertilizer and control plots for both sites in 2005 (Figure 4iv). The omnivores-predators were significantly ($p < 0.01$) influenced by fertilizers and experimental sites in 2004, but were significantly ($p < 0.001$) influenced only by experimental sites in 2005.

Nematode community indices

The fungi-feeders/bacteria-feeders ratio (F/B) values ranged from 0.01 to 0.72 (Figure 5i). The F/B was significantly ($p < 0.05$) higher in the chemical fertilizer plots than in the compost and control plots in 2005. The F/B value was significantly ($p < 0.05$) influenced by experimental site in 2004, but was significantly ($p < 0.05$) influenced by fertilizers in 2005. The trophic group (T) values ranged from 1.80 to 2.51 (Figure 5ii). The T value was higher in the compost plots than in the chemical fertilizer and control plots in 2005. The T value was significantly ($p < 0.05$) influenced by experimental sites in 2004, and was significantly ($p < 0.05$) affected by fertilizers

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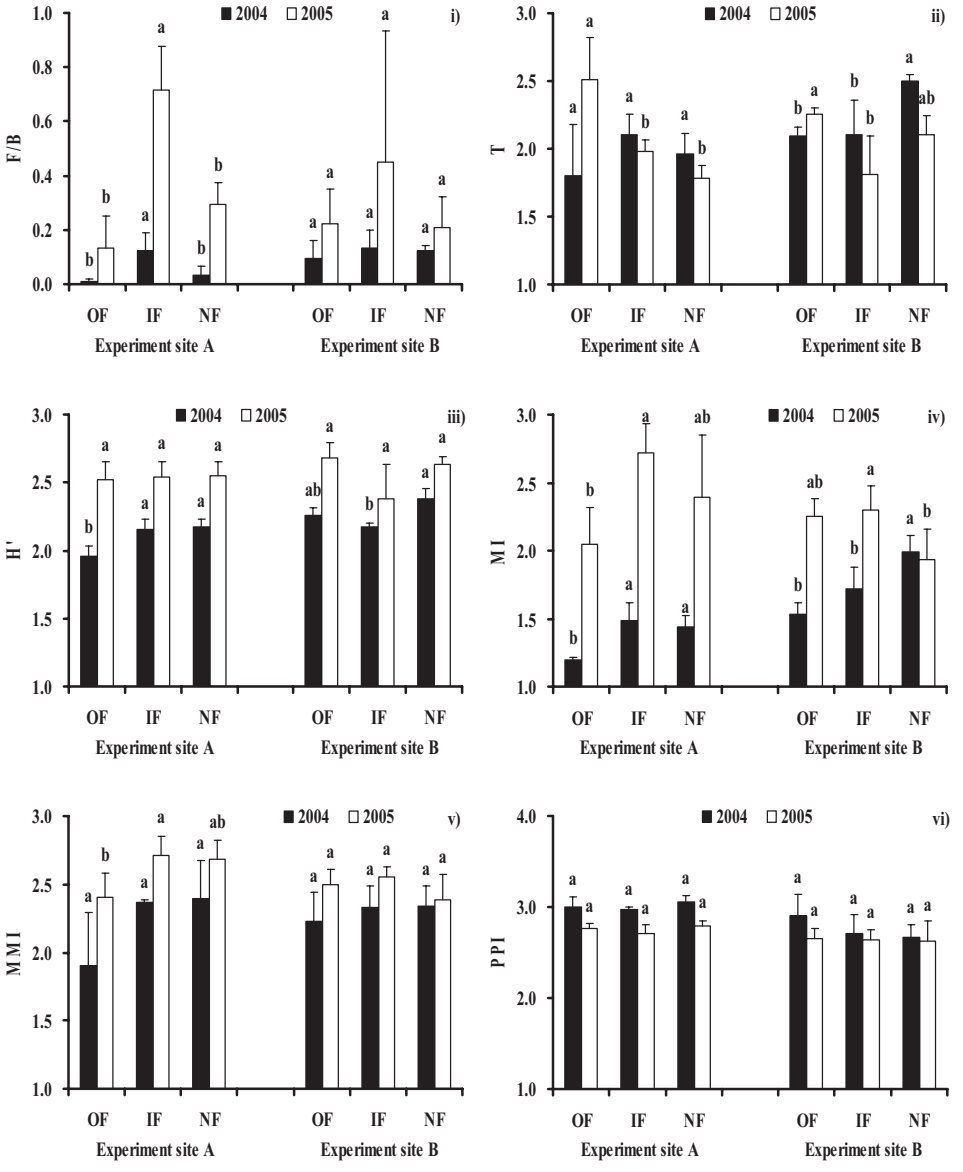

Figure 5. Community indices of soil nematodes under the long-term fertilizer experiment in 2004 and 2005. Different letters (a, b, c) indicate significant differences ($p < 0.05$) between treatments according to LSD multiple comparison. Bars indicate standard errors ($n = 3$). i) fungi-feeders/bacteria-feeders ratio (F/B); ii) trophic diversity (T); iii) Shannon index (H'); iv) maturity index (MI); v) modified maturity index (MMI); vi) plant-parasite index (PPI). OF: organic compost treatment; IF: inorganic fertilizer treatment; NF: no fertilizer.

and interaction effect in 2005. The Shannon index (H') values ranged from 1.96 to 2.68 (Figure 5iii). The H' values were significantly influenced ($p < 0.01$) by fertilizers and experimental sites, and interaction effect in 2004, but no significant effect was found in 2005.

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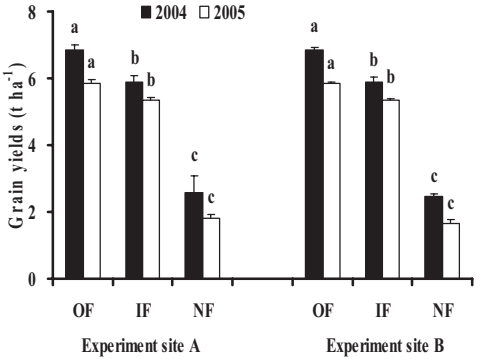

Figure 6. Grain yields of wheat under the long-term fertilizer experiment in 2004 and 2005. Different letters (a, b, c) indicate significant differences ($p < 0.05$) between treatments according to LSD multiple comparison. Bars indicate standard errors ($n = 3$). OF: organic compost treatment; IF: inorganic fertilizer treatment; NF: no fertilizer.

The MI values ranged from 1.19 to 2.72 (Figure 5iv). The MI values were lower in the compost plots than in the chemical fertilizer and control plots in 2004. The MI values were significantly ($p < 0.01$) influenced by fertilizers and experimental sites in 2004, but no significant effect was found in 2005. The MMI values ranged from 1.91 to 2.71 (Figure 5v). The MMI values were lower in the compost plots than in the chemical fertilizer and control plots for both years in experimental site A. The PPI values ranged from 2.63 to 3.05 (Figure 5vi). The PPI values were significantly ($p < 0.01$) influenced only by experimental sites in 2004.

Crop yields

Grain yields of wheat were significantly ($p < 0.001$) higher in the compost plots than in the chemical fertilizer plots, and were significantly ($p < 0.001$) higher in the chemical fertilizer plots than in the control plots for both sites and years (Figure 6). Grain yields of wheat were significantly ($p < 0.001$) affected by fertilizers, but not by experimental sites and interaction effect.

DISCUSSION

Soil physicochemical properties

Soil physicochemical properties were significantly influenced by fertilizers, consistent with the study of Singh *et al.* (2004), who reported that soil total organic C, and available K contents were significantly increased with the application of organic manure. Nevertheless, soil total organic C and total N contents in the chemical fertilizer plots were found to be slightly increased compared to the controls, which is in contrast with the result of Mandal *et al.* (2007), who found that total organic C and total N contents were significantly higher in chemical fertilizer plots than in controls. Gami *et al.* (2001) reported available P contents were increased with the long-term application of manure and chemical fertilizers, which is similar to our result. The bulk density and

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pH values of saline-alkaline soil have shown to decrease with application of organic manure (Lee *et al.*, 2009).

Soil biological properties

The soil MBC content showed a significant increase in the compost plots compared to the chemical fertilizer and control plots at both experimental sites and sampling dates. Increased microbial biomass content after application of organic manure was also reported by Elfstrand *et al.* (2007), and the most important factor was the amount of carbon from organic manure input into soils because carbon was often the limiting factor for soil microbial propagation (Gunapala and Scow, 1998). Application of compost increased readily available C and N sources leading to stimulation of the soil microbial community. The increased MBC levels after application of chemical fertilizer, which is in accordance with the study of Mandal *et al.* (2007), may be due to high levels of chemical fertilizer increasing plant root biomass and crop residue and improving soil organic carbon, and thus enhancing the size of microbial community (Gong *et al.*, 2009; Gu *et al.*, 2009).

Microbial biomass alone did not provide information on microbial activity. So, soil respiration was measured to assess soil microbial activity; soil respiration is also considered to reflect the availability of carbon for microbial maintenance (Masto *et al.*, 2006). The increase of basal respiration after application of organic manure was compatible with the study by Masto *et al.* (2006), who considered that high respiration activity in organic manure plots was associated with higher organic carbon levels, which had built up with continuous additions of organic manure.

The compost plots had the lowest qCO_2 values compared to the other plots at both sites and sampling dates, which confirmed previous observations that qCO_2 is lower in soil treated with organic manure than soil treated with chemical fertilizer (Böhme *et al.*, 2005). The highest qCO_2 was found in the control plots at both sites, and it may be assumed that in these plots microbial populations living under carbon resource stress (Nsabimana *et al.*, 2004).

Soil enzyme activities

Phosphatases are important enzymes, because they provide P for plant uptake by releasing PO_4^- from immobile organic P (Amador *et al.*, 1997). The increase of soil alkaline phosphatase activities after application of organic manure is in agreement with other studies (Böhme *et al.*, 2005). Soil urease activities were increased after application of organic manure, which was similar to the result reported by Bandick and Dick (1999). Soil urease activities closely correlated with available N (Bandick and Dick, 1999). The long-term effect of organic amendments on enzyme activities is probably a combined effect of a higher degree of stabilization of enzymes on humic substances, and an augmentation of microbial biomass with increased soil C content (Elfstrand *et al.*, 2007). Furthermore, as well as being the substrate for microbial activity, soil organic matter plays an important role in protecting soil enzymes since they become immobilized in a three-dimensional network of clay and humus complexes (Tabatabai, 1994).

Soil nematode community

The total number of soil free-living nematodes in the present study (ranging from 172 to 629 individuals per 100 g dry soils) was similar to the study by DuPont *et al.* (2009), who reported that total nematode density ranged from 435 to 617 individuals per 100 g dry soils in organic agriculture systems of the USA. But our observations were lower than those found following long-term application of fertilizer agriculture soil reported by Liang *et al.* (2009), where the values ranged from 225 to 899 individuals per 100 g dry soils. This indicates a difference between different agriculture ecosystems. Increased nematode density after application of organic manure was also reported by Villenave *et al.* (2003) and Forge *et al.* (2005); the reason was that the increase of nematode populations could be linked directly to higher food resources associated with the input of organic manure (Bulluck *et al.*, 2002).

The plant-parasitic nematodes were the most dominant trophic group in the present study, similar to the findings of Ou *et al.* (2005) in maize fields, but incompatible with the study of DuPont *et al.* (2009), who found that bacteria-feeding nematodes were the dominant trophic group in organic agriculture systems. The bacterial feeders were higher in the compost plot than in the other plots, which revealed that application of organic manure could increase soil microbial community, and confirmed previous results (Bittman *et al.*, 2005). We found that the number of omnivores-predators was increased due to application of organic compost in 2005. Increased omnivores-predators may contribute to enhancement of nutrient mineralization, via the feeding of predatory nematodes, as nutrients passed from microbes to bacteria-feeding nematodes are released at the predator trophic levels (Yeates and Wardle, 1996), as well as through predation on other nematodes including plant-parasitic nematodes (Khan and Kim 2007).

The number of nematode genera in the present study was 43, higher than the result (37) obtained by Li *et al.* (2009) in a rice-wheat cropping system. Nematode genus richness could reflect diversity of soil habitat (Ekschmitt *et al.*, 2001). The number of nematode genera was greater in the compost plots than in the chemical fertilizer and control plots, which reflected a greater diversity of soil habitat in the compost plots.

Ecological indexes are known to be useful in elucidating differences between treatments. The F/B ratio, which reflects the status of the decomposition pathway in detrital food webs and the structure of the microflora community, was found to differentiate between the two components of fungi and bacteria (Ruess, 2003). F/B values were lower in the compost plots than in the chemical fertilizer plots, which indicated that there were more microbial communities in the compost plots (Freckman and Ettema, 1993). The trophic diversity index (T) demonstrates the diversity of the functional groups within the nematode community and affords greater weight to common taxa and a higher index indicates greater diversity (Heip *et al.*, 1988). However, we only observed the higher T values in the compost plots, compared to the other plots, in 2005. The maturity index (MI) is a measurement based on the composition of the nematode community and could reflect the degree of stability of the soil ecosystem (Bongers, 1990). The modified maturity index (ΣMI) includes the

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plant-parasitic nematode population in order to better reflect the ecosystem development (Yeates, 1994). The MI values obtained (1.19–2.72) are comparable to values in agriculture systems in the USA (2.19–2.32) (Neher *et al.*, 2005). The MMI values obtained (1.91 to 2.71) are comparable to values found in tomato fields (1.9–2.9) (Bulluck *et al.*, 2002). Bongers (1999) demonstrated that MI values decreased with increasing fertility, whereas we only observed the lower MI values in the compost plots in comparison to the other plots for two sites in 2004.

CONCLUSION

This study has shown that soil physicochemical parameters, microbial biomass, biological activities, and nematode communities were influenced by long-term application of fertilizers. In general, soil total organic carbon, dissolved organic carbon, total N, alkaline-hydrolysable N, available P, and available K, microbial biomass, basal respiration, soil enzyme activities, total number of nematodes and bacterial-feeding nematodes were significantly higher in the compost plots than in the chemical fertilizer and control plots at two experimental sites for two sampling dates, whereas soil bulk density and pH values were significantly lower. As a result, we conclude that integrated factors, such as physical-chemical, biochemical and biological parameters should be taken into account as soil quality indicators.

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Supplementary data available at journals.cambridge.org/eag.

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