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Body size is a sensitive trait-based indicator of soil nematode community response to fertilization in rice and wheat agroecosystems

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A B S T R A C T

Nematode body size is a trait that could be responsive to environmental changes, such as agricultural management practices, and adopted as a standard trait-based indicator in soil community analysis. Our study investigated how body size in the nematode community responded to fertilization in a double-cropping system with paddy rice and upland wheat. Four fertilizer treatments were examined: an unfertilized control (CK), chemical fertilizer (CF), manure plus chemical fertilizer (MCF) and manure plus straw plus chemical fertilizer (MSCF). The community-weighted mean (CWM) of body size was the trait-based indicator used for nematode community analysis. A trend of increasing body size in fertilized plots was observed for most genera, with a relatively small increase in the size of small-bodied bacterivores and fungivores and a relatively large increase in the size of large-bodied omnivores. Fertilized plots had significantly greater CWM of body size than the CK treatment, although total nematode abundance increased significantly in the MSCF treatment only. Discriminant and multiple regression analyses showed that CWM of body size was positively correlated with the soil organic C, total N, available P and available K concentrations, which responded to fertilizer inputs. In contrast, soil fertility was weakly related to total body size in the wheat phase and the following abundance-based indicators: Margalef's richness index, Shannon's diversity index, summed maturity index ($\sum$MI) and enrichment index (EI) in both phases. Since fertilization resulted in larger body size but no other change in the nematode community (i.e. diversity and abundance were generally unaffected by fertilization), this implies that nematodes have a plastic growth habit that does not necessarily result in greater reproduction or fitness of offspring. We suggest that CWM of body size is a reliable trait-based indicator of the soil nematode community response to fertilization, but this requires further testing across a wider range of fertilized agroecosystems.

1. Introduction

The community structure of soil organisms provides insight into relationships between community composition, environmental change and ecosystem functions. Abundance-based indicators, e.g. total abundance, Margalef's richness index, Shannon's diversity index, Pielou's evenness index and nematode channel ratio, provide valuable information about community succession and are often used to predict and interpret the changes in soil community structure due to environmental change (Ferris et al., 1996; Li et al., 2007; Villenave et al., 2010; Zhang et al., 2012). Bongers (1990) ranked nematodes in five colonizer-persister (c-p) groups (c-p from 1 to 5) and developed the maturity index (MI) to better assess the free-living nematodes response to environmental disturbance. This approach is based on nematode reproductive strategies that correspond to their phylogeny, feeding habit and body size. The MI can be presented as the summed maturity index ($\sum$MI), which including both free-living and plant-parasitic nematodes (Yeates, 1994). Nematodes in agroecosystems are often described based on several feeding groups: bacterivores, fungivores, herbivores, omnivores and predators (Yeates et al., 1993). By integrating these feeding groups and c-p guilds, Ferris et al. (2001) developed the enrichment index (EI) as indicator for productive and relatively small-bodied nematodes, and the structure index (SI) based on the

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expected longevity of large-bodied nematodes. The EI and SI are suitable response metrics for nematode community change in response to environmental change (Ferris et al., 2004; Briar et al., 2007; Li et al., 2007; Liang et al., 2009).

Fertilization of agroecosystems constitutes an environmental change for nematodes, mainly because fertilizers increase trophic resources and alter habitat through their effect on soil structure. Total nematode abundance increases when organic fertilizers or inorganic nitrogen (N) fertilizers are applied (Li et al., 2007; Liang et al., 2009; Jiang et al., 2013). The EI value of the nematode community responds to the greater food supply and more favorable soil nutrient condition after fertilization because EI measures the number of opportunistic bacterial and fungal feeders that respond quickly to carbon (C) and N inputs (Ferris and Matute, 2003; Forge et al., 2008; DuPont et al., 2009). However, abundance-based indicators may not be sufficiently sensitive to detect environmental change due to fertilization. Multiple genera with similar functional traits are in the same c-p ranks, which can lead to similar results for EI and SI analyses (Ferris, 2010). Besides, the allocation of c-p scale to five rankings is too limited to indicate the variation in body size, and sometimes does not match well with body size, e.g. nematodes belonging to the c-p 1 class are larger than nematodes belonging to the intermediate classes (families with c-p 2, 3, or 4) (Vonk et al., 2013). These shortcomings of abundance-based indicators led Ferris (2010) to propose the use of trait-based indicators such as body mass and body size for soil nematode community analysis. It is not difficult to measure the body size of soil organisms (Wardle, 2002; Mulder et al., 2009; Mills and Adl, 2011; Lindo et al., 2012; Zhao et al., 2015) and it should be possible to use body size as a standard measurement of functional diversity in soil systems (Mulder et al., 2009; Turnbull et al., 2014; George and Lindo, 2015).

The body size of five nematode functional groups ranges from 23×10^3 to 5120×10^3 μm^3, with more than 200-fold difference in the biovolume of the largest and smallest soil nematodes (Yeates, 1988). Smaller individuals (e.g. bacterivores and fungivores) are important for decomposition and nutrient recycling from microbial biomass, whereas larger individuals (e.g. omnivores and predators) function as top-down controllers of the nematode community by suppressing herbivores and modifying the abundance of intermediate nematode, both of which affect nutrient dynamics (Norkko et al., 2013). According to the conventional “size-advantage” hypothesis (Ghiselin, 1969; Berglund, 1990), individual nematodes or nematode functional groups that grow to a larger body size are expected to gain an advantage in competition for limited resources; as well, they may have the potential to become more influential in soil nutrient cycling.

An increase in body size of most nematode families is expected when agroecosystems receive nutrient inputs, such as from dung or urine patches (Mills and Adl, 2011). However, the response of nematode body size to fertilizer nutrients is not well described. Organic fertilizers supply C for microbial growth, while the N, phosphorus (P) and potassium (K) contained in organic and inorganic fertilizers enhance microbial growth directly and indirectly, by promoting crop growth. Fertilization should promote nematode growth and body size increase because greater microbial and crop biomass in fertilized agroecosystems provides more trophic resources for nematode communities. The organic C obtained from organic fertilizer, microbial biomass and crop tissues should be of particular importance for determining gains in nematode body size since nematode tissues are approximately 50% C (dry weight basis) and have a C: N ratio of 8–12 (Coleman et al., 1977; Wang et al., 2002). Resources containing organic C are a growth factor for the model organism *Caenorhabditis elegans* (Lu et al., 1979; Scotti et al., 2001), which is further evidence of the relationship between C nutrition and body size. Thus, we expect that nematode body size will be more strongly correlated with soil organic C than with other soil nutrients (total N, available P and available K) that increase as a result of fertilizer application. Still, how nematode body size responds to nutrient inputs from fertilizer could depend on the growing season and timing of fertilizer applications.

Finally, we must consider how to describe body size in nematode communities due to the wide disparity in size across diverse genera. The total body size of the community is readily measured, but a few large omnivores and predators will influence the value more than many small bacterivores and fungivores. Thus, we introduce the community-weighted mean (CWM) of body size as a new and unbiased trait-based indicator. This is due to the fact that it normalizes the mean body size according to the abundance of nematodes in a particular genus. This weighting method is common in plant community analysis (Garnier et al., 2004; Díaz et al., 2007; Lavorel et al., 2008; Suding et al., 2008; Niu et al., 2014) but has not yet been applied to soil communities.

The objective of this study was to compare the trait-based indicator CWM of body size with total body size and abundance-based indicators, the total nematode abundance, Margalef's richness index, Shannon's diversity index, $\sum$MI and EI, for their ability to detect changes in the nematode community due to fertilization. We hypothesize the CWM of body size will be more sensitive than abundance-based indicators to changes in soil nutrient concentrations following fertilization. A secondary objective was to determine whether the trait-based and abundance-based indicators responded consistently to fertilization in a double-cropping system of paddy rice and upland wheat, which have distinctive soil moisture regimes that could provoke shifts in the nematode community structure.

2. Materials and methods

2.1. Site and experimental design

The study was conducted in Jintan city, Jiangsu Province, China (31°39′N, 119°28′E, 3 m a.s.l). This region has a subtropical monsoon climate, with a mean annual temperature of 15.3 °C and a mean annual precipitation of 1063.6 mm. The experimental site was an agricultural field that supported double-cropping of winter wheat (*Triticum aestivum* L.) and summer rice (*Oryza sativa* L.) for the past 65 years. The soil is classified as Fe-leachic-gleyic-stagnic Anthrosol with a clay loam texture (250 g clay kg^{-1} and 300 g sand kg^{-1}). Before initiating the experiment, soil analyses found 13.5 g organic C kg^{-1}, 1.6 g total N kg^{-1}, 18.0 mg available P kg^{-1}, 56.4 mg available K kg^{-1} with pH of 7.3.

The fertilization experiment was established in November 2010. Sixteen plots were randomly allocated four fertilizer treatments into four blocks (=four replicates per treatment). Every plot was 5 m × 8 m and separated by 0.15 m concrete buffers on both sides and a 1.5 m lane between blocks. The four fertilizer treatments were: an unfertilized control (CK), chemical fertilizer (CF), manure + 50% chemical fertilizer (MCF) and manure + straw + 50% chemical fertilizer (MSCF). Each year, the CF treatment received 240 kg N ha^{-1}, 120 kg P_2O_5 ha^{-1} and 100 kg K_2O ha^{-1}, while the MCF and MSCF treatments received 120 kg N ha^{-1}, 60 kg P_2O_5 ha^{-1} and 50 kg K_2O ha^{-1}, respectively. In the MSCF treatment, pig manure containing 2.3% N, 1.3% P, 1.0% K and 45.4% organic matter with moisture content of 29.1% was applied at 400 kg pig manure ha^{-1} (wet weight basis). Straw in the MSCF treatment contained 0.63% N, 0.11% P, 0.85% K, 78.6% organic matter and 33.1% moisture when applied as 18 t rice straw ha^{-1} (wet weight basis) in the wheat phase, while wheat straw with 0.52% N, 0.11% P, 1.07% K, 82.6% organic matter and 30.7% moisture was applied at 11 t wheat straw ha^{-1} (wet weight basis) in the rice phase. Details of the NPK fertilization and organic matter inputs applied to each treatment are summarized in Table 1.

Fertilizer treatments were applied in both rice and wheat phases. The P_2O_5, K_2O, straw and pig manure were applied as basal fertilizers 3–5 d before planting summer rice in June (harvested in late October) and 3–5 d before planting winter wheat in November (harvested in late May). The urea-N fertilizer application was split into three equal amounts and applied before planting, at the tillering stage and at the panicle stage. The rice straw was returned to soil before planting wheat, while wheat straw was returned to soil before planting rice.

2.2. Soil sampling and analyzing

Soil sampling and analysis was done after 3-yr of establishing the fertilization treatments, which means that plots had received 6 repeated fertilizer applications (2 times per year) by the time samples were taken. Soil samples were collected at the ripening stage of wheat (late May) and rice (late October) in 2013. In each plot, eight soil cores (2.5 cm in diameter) were randomly collected at least 10 cm away from the taproot system from the soil plough layer (0–10 cm), and mixed together as one composite sample per plot. Samples were stored at 4 °C until soil nutrients and nematodes were analyzed.

Soil nutrient concentrations in each plot included organic C measured by the Walkley-Black procedure (Nelson and Sommers, 1982); total N measured using the semi-Kjeldahl method (Sparks et al., 1996); available P and available K concentrations were determined by the Olsen-P extraction (Watanabe and Olsen, 1965) and flame photometry methods (Knudsen et al., 1982), respectively.

2.3. Nematode abundance and body size analysis

Soil nematodes were extracted from 50.0 g field-moist soil from each plot by a modified Baermann method (Liu et al., 2008). After counting the total nematode abundance in 50.0 g soil, approximately 150 specimens were selected at random and identified to the genus level under an Olympus BX50 microscope at 400–1000× magnification according to Bongers and Vereniging (1988). We assigned all taxa to feeding groups (Yeates et al., 1993) and to the c-p classes (Bongers and Bongers, 1998). The detailed calculation of EI was following Ferris et al. (2001) and $\sum$MI following Yeates (1994). Margalef's richness index and Shannon's diversity index were calculated according to Nahar et al. (2006).

For each plot, we measured the length and greatest width of each identified individual, and then calculated nematode body size (V) of each individual based on body length (L) and diameter (D): $V = (L \times D^2)/1.7$, where 1.7 is an empirically determined constant (Ferris, 2010).

In each plot, the total body size was calculated as a sum of all identified nematode body size. The relative abundance of a given genus was calculated as a ratio of this genus abundance to total nematode abundance, and we calculated community weighted mean (CWM) of body size as mean body size weighted by its relative abundance according to Ricotta and Moretti (2011):

$$\mathrm{CWM} = \sum_{i=1}^{S} p_i x_i$$

where p_i is the relative abundance of genus i ($i = 1, 2, \ldots, S$), and x_i is the mean body size value for genus i.

2.4. Statistic analysis

We conducted the statistical analyses separately between paddy rice and upland wheat due to the disparate soil moisture regime and climatic conditions between the two crop phases. We used a linear-mixed model with residual maximum likelihood (REML) to assess the effects of fertilization on soil nutrients (organic C, total N, available P and available K) and soil nematode communities (CWM of body size, total body size, total nematode abundance, Margalef's richness index, Shannon's diversity index, $\sum$MI and EI). The model was designed as: response ~ crop phase/fertilization, random = ~1|plot. Here, "fertilization" was included as a nested fixed factor, and "plot" was taken as random factor to account for the expected spatial autocorrelation. The data were log-transformed to meet assumptions of normality and homogeneity of variance. The linear-mixed model was conducted under the *lme4* package (Bates et al., 2012).

We used generalized canonical discriminant and correlation analyses (gCCA) with linear model (multiple response variable ~ crop phase/fertilization) to visualize the relationships between soil nutrients and soil nematode communities in rice and wheat phases. The gCCA was performed under the *candisc* package (Friendly et al., 2013). We performed linear regression models to analyze the relationships between soil nutrients and CWM of body size across four fertilizer treatments in rice and wheat phases. All statistics and figures were produced with R software version 3.0.1 (Team, 2013).

3. Results

3.1. Effect of fertilization on soil nematodes

The MSCF, MCF and CF treatments significantly increased CWM of body size and total body size both in rice and wheat phases compared to CK treatment ($P < 0.05$; Fig. 1 and Table 2). The MSCF treatment significantly increased CWM of body size in two phases compared to MCF and CF treatments ($P < 0.05$; Fig. 1). Total body size increased by fertilizer treatments differed in phases, with the highest increment by MSCF treatment in the rice phase and by CF treatment in wheat phase (Table 2). Total nematode abundance was significantly greater in MSCF treatment compared to CK treatment ($P = 0.002$; Table 2), but similar in the MCF, CF and CK plots in both phases ($P = 0.064$–0.395; Table 2). MSCF and MCF treatments significantly increased the EI in the rice phase compared to CK

Table 1
Annual application rates of N, P, K and organic matter from inorganic and organic fertilizers spread on rice and wheat phases of a double-cropping system. Fertilizer treatments were: CK, no fertilizer; CF, chemical fertilizer; MCF, pig manure + 50% chemical fertilizer; MSCF, pig manure + straw + 50% chemical fertilizer.

Fertilizer treatment	**Rice** (inputs in kg ha^{-1})				**Wheat** (inputs in kg ha^{-1})			
	N	P_2O_5	K_2O	Organic matter	N	P_2O_5	K_2O	Organic matter
CK	0	0	0	0	0	0	0	0
CF	240	120	100	0	240	120	100	0
MCF	127	69	53	129	127	69	53	129
MSCF	167	88	151	757	203	99	176	1072

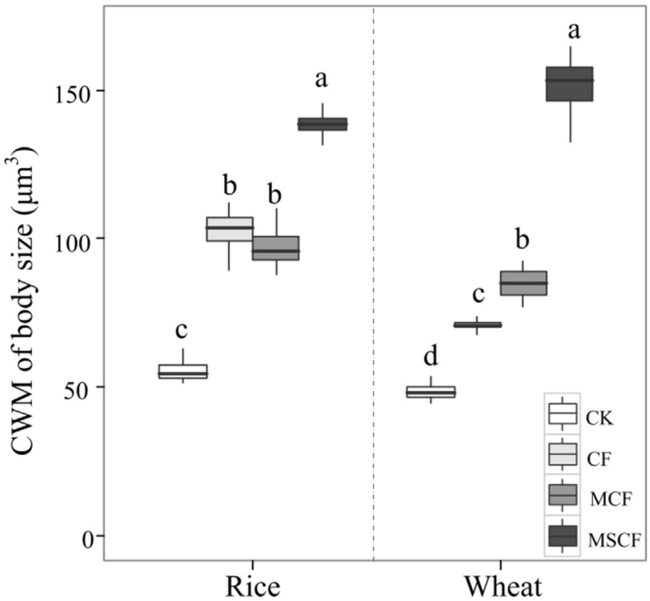

Fig. 1. Effects of fertilization treatments on CWM of body size in rice and wheat phases of a double-cropping system. Within a crop phase, different letters above the bar indicate significant differences ($P < 0.05$) among fertilizer treatments. Fertilizer treatments were: CK, no fertilizer; CF, chemical fertilizer; MCF, pig manure + 50% chemical fertilizer; MSCF, pig manure + straw + 50% chemical fertilizer.

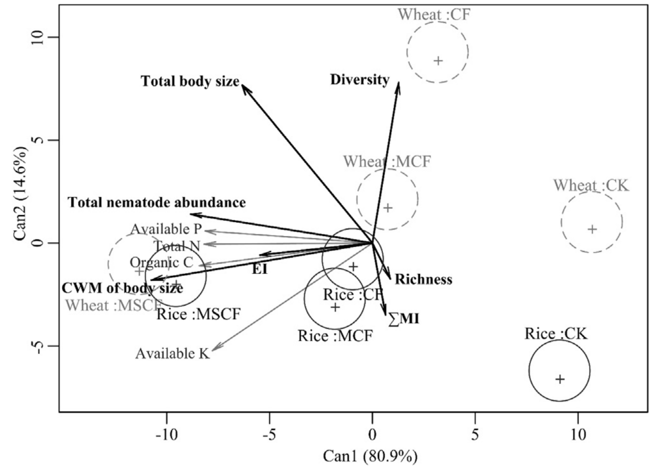

Fig. 2. Generalized canonical discriminant and analysis (gCCA) showing relationships among soil nutrients, trait-based and abundance-based indicators of soil nematode communities. The trait-based indicator is the community-weighted mean (CWM) of body size. Abundance-based indicators are the total nematode abundance, Margalef's richness index, Shannon's diversity index, summed maturity index (∑MI) and the enrichment index (EI). The circles (solid lines for the rice phase, dotted lines for the wheat phase) represent the 95% confidence interval around the site-treatment mean. Fertilizer treatments were: CK, no fertilizer; CF, chemical fertilizer; MCF, pig manure + 50% chemical fertilizer; MSCF, pig manure + straw + 50% chemical fertilizer.

treatment ($P = 0.01–0.04$; Table 2), but there was no difference in the EI among fertilizer treatments in the wheat phase ($P = 0.241–0.818$; Table 2). No significant difference in richness, diversity and ∑MI was found between fertilized and CK plots, the one exception being higher diversity but lower ∑MI in CF than CK plots in the rice phase ($P < 0.05$; Table 2).

3.2. The relationship between soil nematodes and soil nutrients

Discriminant and further regression analyses showed a high correlation between CWM of body size and soil nutrients (Fig. 2), with a better correlation observed in the wheat phase ($R^2 = 0.50–0.78$) than the rice phase ($R^2 = 0.36–0.43$) (Fig. 3). CWM of body size had highest correlation with organic C in the wheat phase ($R^2 = 0.78$, $P < 0.001$, Fig. 3A) and available P in the rice phase ($R^2 = 0.43$, $P = 0.006$, Fig. 3C). Total body size had higher correlation with available P in both phases ($R^2 = 0.46–0.53$, $P < 0.003$, Table S2), but no correlation with total N in the rice phase ($R^2 = 0.11$, $P = 0.11$, Table S2). Total nematode abundance had a high correlation with organic C (rice, $R^2 = 0.60$, $P < 0.001$; wheat, $R^2 = 0.52$, $P = 0.001$), as well showed better correlation with other nutrients in both phases (Table S2). The correlation between EI and soil nutrients was generally weak in the rice phase ($R^2 = 0.14–0.41$, $P = 0.005–0.08$, Table S2). There were no correlations in richness, diversity, or ∑MI observed with soil nutrients in both phases ($R^2 = 0.001–0.15$, $P = 0.07–0.95$, Fig. 2 and Table S2), indicating these indices respond weakly to changes in soil nutrient concentrations following fertilization.

4. Discussion

4.1. Nutrient inputs from fertilizer increase nematode body size

Overall, body size increased in most genera present in fertilized plots compared to the CK plots, especially large-bodied omnivores

Table 2
Results of linear-mixed modeling for the effect of fertilization (nested in each crop phase) on soil nutrients and soil nematodes. Abbreviated terms and their definition are: Richness, Margalef's richness index; Diversity, Shannon's diversity index; ∑MI, summed maturity index; EI, enrichment index. Fertilizer treatments were: CK, no fertilizer. CF, chemical fertilizer. MCF, pig manure + 50% chemical fertilizer. MSCF, pig manure + straw + 50% chemical fertilizer. Values are the slope ± standard error and an asterisk (*) indicates a slope that is significantly different from X_0 (CK plots) at $P < 0.05$. The slope values were derived from the results of mixed model, which indicate the strength of the fertilization effect gauged by the slope of the relationship. AIC: the Akaike's An Information Criteria, BIC: the Schwarz's Bayesian Information Criteria. Degrees of freedom = 21.

	Rice				Wheat			
	AIC/BIC	CF	MCF	MSCF	AIC/BIC	CF	MCF	MSCF
Soil nutrients								
Organic C	58.7/61.6	0.5 ± 0.9	1.5 ± 0.9	3.8 ± 0.9*	56.0/58.9	1.6 ± 0.8	4.0 ± 0.8*	6.9 ± 0.8*
Total N	−30.2/−27.3	0.02 ± 0.02	0.07 ± 0.02*	0.12 ± 0.02*	−20.7/−17.8	0.01 ± 0.03	0.05 ± 0.03	0.18 ± 0.03*
Available P	95.1/98.0	11.7 ± 4.3*	19.6 ± 4.3*	20.7 ± 4.3*	75.0/77.9	13.6 ± 1.4*	18.2 ± 3.5*	22.1 ± 3.5*
Available K	84.1/87.0	1.8 ± 2.7	6.5 ± 2.7*	11.6 ± 2.7*	86.6/89.5	7.0 ± 3.0*	5.8 ± 3.0	20.1 ± 3.0*
Soil nematodes								
CWM of body size	−8.3/−5.4	0.6 ± 0.06*	0.6 ± 0.06*	0.9 ± 0.06*	−11.5/−8.6	0.38 ± 0.05*	0.53 ± 0.05*	1.13 ± 0.05*
Total body size	0.5/3.4	0.8 ± 0.07*	0.7 ± 0.07*	1.1 ± 0.07*	4.4/7.3	0.90 ± 0.08*	0.91 ± 0.08*	0.93 ± 0.08*
Total abundance	23.4/26.3	0.26 ± 0.22	0.46 ± 0.22	1.01 ± 0.22*	18.3/21.2	0.21 ± 0.18	0.16 ± 0.18	0.78 ± 0.18*
Richness	0.07/3.0	−0.1 ± 0.08	−0.09 ± 0.08	0.04 ± 0.08	8.3/11.2	−0.02 ± 0.1	−0.005 ± 0.1	−0.12 ± 0.1
Diversity	−6.8/−3.9	0.13 ± 0.06*	−0.04 ± 0.06	0.10 ± 0.06	3.2/6.1	0.11 ± 0.09	0.05 ± 0.09	−0.08 ± 0.09
∑MI	−24.0/−21.1	−0.08 ± 0.03*	−0.04 ± 0.03	−0.01 ± 0.03	−18.9/−16.0	0.04 ± 0.03	0.04 ± 0.03	−0.02 ± 0.03
EI	93.8/96.7	8.4 ± 4.1	9.6 ± 4.1*	12.6 ± 4.1*	93.9/96.8	−3.4 ± 3.7	0.9 ± 3.7	4.7 ± 3.7

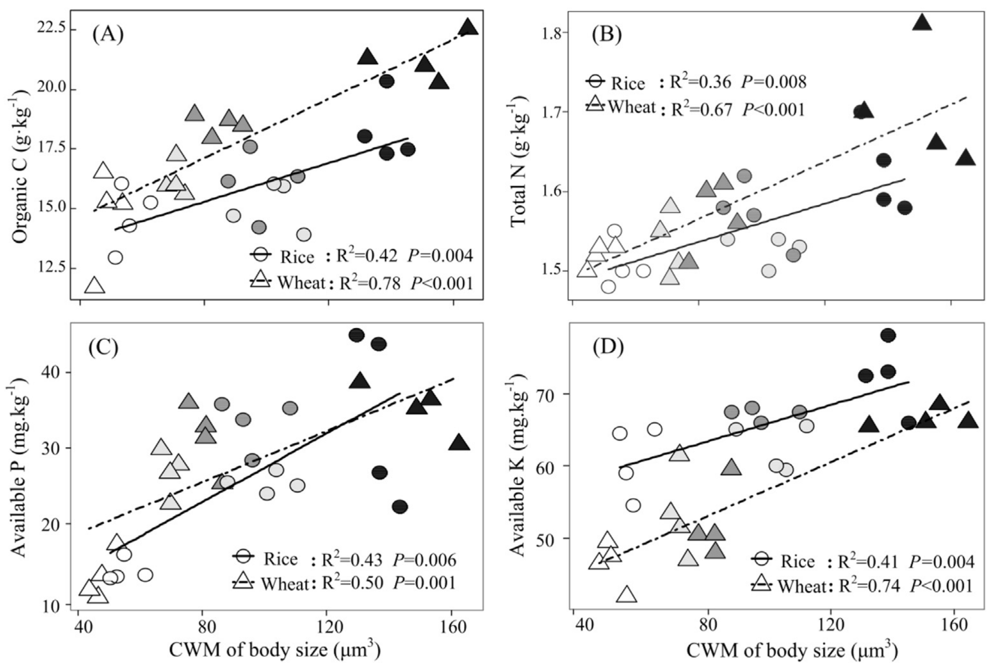

Fig. 3. Relationships between soil nutrients the community-weighted mean (CWM) of body size in the rice and wheat phases of a double-cropping system. R-squared (R^2) and *P*-values were estimated from a linear regression model, and the best-fit line (—, rice or –, wheat) is shown on the graph. Fertilizer treatments were: CK, no fertilizer (○, rice or △, wheat); CF, chemical fertilizer (○, rice or △, wheat); MCF, pig manure + 50% chemical fertilizer (●, rice or ▲, wheat); MSCF, pig manure + straw + 50% chemical fertilizer (●, rice or ▲, wheat).

in the family Dorylaimidae such as *Aporcelaimus* and *Dorylaimus* (Table S1). Nematode body size was increased more by MCF treatment than by CF treatment, with further gains in body size in the MSCF treatment, which had straw added. Straw inputs had high content of organic C, which were highly correlated with the CWM of body size and total body size, and which is a key growth factor for the model nematode *C. elegans* (Scotti et al., 2001). The MSCF treatment increased the body size of most individuals, including small-bodied nematode *Filenchus*, which increased from 22 μm^3 to 40 μm^3 and *Dorylaimus* omnivores that increased from 1496 μm^3 to 10,039 μm^3 (Table S1). Our results are similar to Mills and Adl (2011), who observed a significant increase in the Dorylaimidae body size when they added more dung and urine patches in a grazed pasture.

Since fertilization enhances soil fertility regardless of the crop phase, we found that increasing soil nutrient concentrations were linked to greater total body size and the CWM of body size. We hypothesized that nematode body size will be more strongly correlated with soil organic C than with other soil nutrients (total N, available P and available K) that increase as a result of fertilizer application. This was true for the CWM of body size in the wheat phase, which was more strongly correlated with soil organic C than other soil nutrients based on R^2 values from the regression analysis. Nematodes require C for metabolic functions, tissue and egg production, thus C is often the most important resource for population growth and activity (Coleman et al., 1977; Ferris, 2010). Since organic materials contain approximately 50% C, this could explain why many studies report an increase in total nematode abundance with the addition of organic materials (Nahar et al., 2006; Liang et al., 2009; Treonis et al., 2010). However, there were stronger correlations of available P with the CWM of body size in the rice phase and total body size in both the phases. Soil available P is a chemically extractable pool containing inorganic P and is related to crop P uptake. If soil available P is bioavailable to nematodes, it could be used to construct essential biological molecules including DNA, RNA, ATP and phospholipids (Gillooly et al., 2005). Vonk et al. (2013) reported that nematode abundance was highly correlated with soil total P in clay soil type. Similarly, the clay loam soils at our experimental site have high P fixation capacity, such that microbial populations and crop production are highly responsive to P fertilizer inputs (Luo et al., 2007; Zhao et al., 2014), which suggests that bioavailable P concentrations are naturally low and thus all biological organisms – including nematodes – respond positively to greater P fertilizer inputs.

4.2. Trait-based indicators vs. abundance-based indicators

While trait-based indicators of nematode communities responded positively to fertilization, they also showed differential responses to crop phases with distinctive soil moisture regimes. Trait-based indicators (CWM of body size and total body size) were better correlated with soil nutrients in wheat phase ($R^2 = 0.44–0.78$) than the rice phase ($R^2 = 0.36–0.47$). The flooding condition of paddy rice induced relatively small variations in soil nutrients and soil nematode communities among treatments. As a result, the relationships between these variables were weaker than the relationships between soil nutrients and nematode communities in the wheat phase (Fig. 3).

We expected trait-based indicators to be more sensitive than abundance-based indicators to changes in soil nutrient concentrations following fertilization. This hypothesis was supported by the fact that body size, particularly when expressed as the CWM of body size, was more strongly correlated with soil nutrients than the abundance-based indicators (i.e. total nematode abundance, richness, diversity, ∑MI and EI). The CWM of body size could be considered an unbiased trait-based indicator (Newbold et al., 2012), because it could normalize the data when fertilization altered the number of nematodes in a particular genus. For instance, the CF and MCF treatments resulted in a small increase in body size of small-bodied nematodes (dominant genera *Eucephalobus*, *Filenchus* and *Rhabdolaimus* increased by 6–23%, 72–78% and 71–75%, respectively; Table S1) and a large increase in large-bodied nematodes (*Aporcelaimus* increased by 130–135% and *Dorylaimus* increased by 66–372%; Table S1). In such case, the disproportionate increase in body size of smaller vs. larger nematodes, relative to their abundance, needs to be considered. The CWM of body size is recommended as a potential trait-based indicator that can account for changes in the traits and abundance of a genus.

Abundance-based indicators like the EI were sensitive to increases in trophic resources and soil nutrient conditions in other studies when bacterial-feeding nematodes of c-p 1 guilds (Ba_1) responded rapidly to organic substrate inputs and dominated the community (Ferris and Matute, 2003; Forge et al., 2008; DuPont et al., 2009). In our study, EI responded positively to fertilization, but this was significant in the MCF and MSCF treatments of the rice phase and weakly correlated to soil nutrient concentrations in both phases. It may also be a function of the time of soil sampling since our samples were collected at crop harvest, 4–5 months after fertilization. This is consistent with Bongers and Ferris (1999), who reported that most Ba_1 were replaced by bacterial-feeding nematodes of c-p 2 guilds (Ba_2) at the end of growing season after nutrients were removed by crop uptake, and Liang et al. (2009), who found greater abundance of Ba_2 and fungal-feeding nematodes of c-p 2 guilds (Fu_2) due to manure addition at the ripening stage than in other growing stages. On the other hand, because EI is calculated based on the abundance of Ba_1, Ba_2 and Fu_2, it underestimates the contribution of large-bodied herbivores, omnivores and predators to nematode community structure. The trait-based indicator CWM of body size could account for the greater abundance in these nematode trophic groups and their increasing body size in response to fertilization, even when sampling was delayed for 4–5 months after fertilizer application.

Although considered all the nematodes including small-bodied and large-bodied ones, the $\sum$MI approach was not sensitive in indicating a disturbed soil environment following fertilization. The $\sum$MI has similar problems as EI to classify species in five c-p groups, for the growth rate of c-p from 1 to 5 does not match well with the growth rate of body size from smallest *Filenchus* (33 μm^3) to largest *Dorylaimus* (6069 μm^3). The insignificant change in richness and diversity observed in this study suggest that these abundance-based community indices were relatively unaffected by fertilization. Although nematode populations will be changed after fertilizer inputs, probably because the extra nutrient input results in more trophic resources, most of the genera identified in the study were always present because the local pedogenesis and species pool have strong effect on nematode community assembly (Lessard et al., 2012; Laliberté et al., 2013). Similar results in $\sum$MI, richness and diversity have been found in response to clear-cutting and ash amendment by George and Lindo (2015).

There is an emerging idea that trait-based approaches hold promise to understand how nematode communities change following disturbance. Vonk et al. (2013) proposed that MI, EI and SI could be considered trait-based indicators of nematode communities due to the fact they are derived from feeding habits or by combining life strategy and feeding habits. In their study on the effect of agricultural management and soil types on nematode communities, Vonk et al. (2013) reported that total nematode abundance reflects more variance of abiotic and biotic information than trait-based indices did; MI could point to differences in management intensity between ecosystems but explained only a small fraction of the variance of soil parameters. These findings were consistent with our observation that EI was poorly related to soil nutrient concentrations, compared to nematode abundance. On the other hand, George and Lindo (2015) compared two trait-based indicators, the $\sum$MI and the body size spectra (plotted body size against abundance on a log–log scale) to determine how wood ash application and clear-cut forest harvesting affected the soil nematode community. Results indicated that body size spectra was more sensitive to changes in nematode community structure due to forestry management practices than the $\sum$MI or nematode abundance. To some extent, the body size spectra appears analogous to our proposed new trait-based indicator, the CWM of body size. Both indices present body size distribution weighted against the abundance of nematodes. Additionally, both indices ignore taxonomic information. Measuring the CWM of body size does not require identification each nematode to the genus level and can be calculated based on hierarchical body size weighted by its relative abundance. The mathematical and experimental tests showing that both taxon-explicit and taxon-free methods represent the same trait (body size) distribution and approximately equal CWM in plant community analysis was described by Lavorel et al. (2008).

5. Conclusion

We found that nematode body size responds positively to fertilization, and it is a sensitive trait-based indicator of soil nematode community response to fertilization in rice and wheat agroecosystems. The trait-based indicators like CWM of body size are more reliable in measuring the soil nematode community response to fertilization than abundance-based indicators (i.e. total nematode abundance richness, diversity, $\sum$MI and EI), as they can differentiate nematode responses to soil nutrients in crop phases with distinctive soil moisture regimes. The ecological implications of this finding for nematode trophic groups, in particular the pathogenic and biocontrol nematode genera in agroecosystems, merits further study. Our study provides an indication that trait-based indicator like the CWM of body size, could serve as a potential measure of nematode community shifts in response to environmental change (especially nutrient addition) in soil systems. This is a fruitful area for further research in agroecosystems that rely on fertilizer inputs to sustain crop production.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.05.027.

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