Effects of experimental nitrogen and/or phosphorus additions on soil nematode communities in a secondary tropical forest

Jie Zhao\textsuperscript{a,b,1}, Faming Wang\textsuperscript{a,1}, Jian Li\textsuperscript{a,c,1}, Bi Zou\textsuperscript{a}, Xiaoli Wang\textsuperscript{a,c}, Zhian Li\textsuperscript{a,*}, Shenglei Fu\textsuperscript{a,**}\textsuperscript{1}

\textsuperscript{a}Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, PR China
\textsuperscript{b}Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha 410125, PR China
\textsuperscript{c}University of Chinese Academy of Sciences, Beijing 100049, PR China

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\textbf{Abstract}

In tropical forest ecosystems, highly weathered soils are often considered as relatively nitrogen-rich but phosphorus-poor. Nutrient availability greatly regulates ecosystem processes and functions of tropical forests. However, little is known about how nitrogen and/or phosphorus additions affect the conditions of soil food web which is an important component of belowground ecosystems. In the present study, soil nematode communities were monitored and served as indicators of soil food web conditions under experimental nitrogen and phosphorus additions in a secondary forest in tropical China. The principal response curves of soil nematode community structure revealed same tendency of changes under nitrogen and/or phosphorus additions compared with control, in terms of nematode functional guild compositions: apparent successions from communities dominated by He\textsubscript{3} and Ba\textsubscript{1} to communities dominated by Ba\textsubscript{2} and Fu\textsubscript{2} occurred after nitrogen and/or phosphorus additions. The diversity of soil nematode genera was not sensitive to either nitrogen or phosphorus addition. Phosphorus addition significantly suppressed total nematode density, density of omnivore-predators, and four nematode faunal indices (i.e. MI\textsubscript{25}, EI, SI, and SFI), but increased two faunal indices (i.e. CI and BaI). However, nitrogen addition did not induce remarkable changes of these variables in the present study. Our results suggest that nitrogen and/or phosphorus additions suppress soil nematodes in tropical secondary forests, which was inconsistent with our expectation that nitrogen addition was detrimental to and phosphorus addition was conducive to soil nematodes in this nitrogen-rich but phosphorus-poor soil. Furthermore, the effects of phosphorus addition are more powerful than the effects of nitrogen addition. Moreover, phosphorus addition degrades the structure and trophic links of the soil food web, reduces the carbon utilization of soil nematodes, and leads to a more fungal dominated decomposition pathway. The alterations of soil food web conditions might result in altered ecosystem functioning. Our findings could provide a better understanding of the responses of soil food web to nitrogen and phosphorus additions in nitrogen-rich but phosphorus-poor soils.

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\section{Introduction}

Tropical forests possess great biodiversity (Myers et al., 2000; Sala et al., 2000), contain up to 40\% of global terrestrial biomass carbon (Dixon and Brown, 1994; Cleveland and Townsend, 2006) and 30–50\% of terrestrial productivity (Grace et al., 2001). Therefore, they are considered as one of the most important ecosystems on earth. Their highly weathered soils are often considered as relatively nitrogen-rich but phosphorus-poor. Soil phosphorus level plays a key role on ecosystem functions such as net ecosystem production (NEP) and litter decomposition in those ecosystems (Vitousek, 1984; Hobbie and Vitousek, 2000; Wardle et al., 2004; Cleveland et al., 2006; Kaspari et al., 2008). In addition, global atmospheric nitrogen deposition is accelerating through time due to anthropogenic activities such as fossil fuel burning and agriculture.
fertilization (Lu et al. 2010a; Fang et al. 2011). Chronic elevated nitrogen depositions impact many aboveground and belowground ecosystem functions such as NEP, biodiversity or richness, and global carbon cycling (Vitousek, 1984; Alison et al., 2000; Nadelhoffer, 2000; Matson et al., 2002; Lu et al., 2010a, 2010b).

Nematodes are the most abundant soil mesofauna, occupy key positions at most trophic levels in the soil food web, and correlate with ecosystem processes and functions such as soil nutrient cycling, litter decomposition, net ecosystem production (NEP), and ecosystem succession (Van der Putten et al., 1993; De Deyn et al., 2003; Neher et al., 2012; Zhao et al., 2012; Zhao and Neher, 2013b). They are sensitive to various disturbances (e.g., agriculture management, pollution, land-use change) and it is well documented that soil nematodes can be useful ecological indicators (Neher, 2001; Todd et al., 2006; Zhao and Neher, 2013; Zhao et al., 2013a, 2013b). Soil nematode communities are quite susceptible to the changes in soil nitrogen and/or phosphorus levels (Todd, 1996; Sarathchandra et al., 2001; Coyne et al., 2004; Wang et al., 2006; Liang et al., 2009). Therefore, the additions of nitrogen and phosphorus may lead to alteration in soil nematode communities. Addressing the effects of nitrogen and phosphorus additions on soil nematodes can improve our insights of how nitrogen and phosphorus additions alter soil food web structure and ecosystem processes (e.g., nutrient mineralization) in tropical forest ecosystems. However, soil nematode communities are usually overlooked in studies of nitrogen and phosphorus additions, particularly, the phosphorus addition.

Only few studies have monitored the effects of simulated nitrogen deposition on soil nematode communities. The main findings of these studies are that high levels of nitrogen addition (>80 kg N ha⁻¹ yr⁻¹) suppressed soil nematode communities but low levels of nitrogen deposition (<50 kg N ha⁻¹ yr⁻¹) did not induce significant changes of soil nematode communities in both alpine tundra soils in Wyoming, USA (Lokupitiya et al., 2000) and in grassland soils in Inner Mongolia, China (Ruan et al., 2012; Wei et al., 2012; Li et al., 2013). Many previous studies monitor the effects of nitrogen fertilization on soil nematode communities in agriculture, forest and grassland ecosystems. However, there are conflicting observations of the same monitored variables even in similar ecosystems. For examples, mineral N fertilization increased total nematode abundance of a boreal forest in southern British Columbia (Ferge and Simard, 2001) but decreased that of a boreal forest in central Sweden (Sohlenius and Wasilewska, 1984), respectively. Nitrogen fertilization significantly increased the relative abundance of herbivores and decreased that of omnivores in a subtropical farm in Florida, US (Wang et al., 2006) but had no significant impact on soil nematode communities in a temperate farm in northeast China (Liang et al., 2009). Moreover, chronic nitrogen fertilization significantly increased the abundances of herbivorous and microbivorous nematodes in a talgrass prie in Kansas, US (Todd, 1996) and significantly reduced the abundance of omnivores in a grass sward in British Columbia (Ferge et al., 2005). In addition, 3-yr application of nitrogen fertilizer increased the abundances of total nematodes and herbivores and decreased the abundances of fungivores and carnivores in a pasture in New Zealand (Sarathchandra et al., 2001). However, long-term applications of different types and levels of nitrogen fertilization did not significantly influence the abundances of total nematodes or trophic groups in a Kentucky bluegrass (Poa pratensis) turf in Ohio, US (Cheng et al., 2008).

Compared with nitrogen, studies focused on the effects of mineral phosphorus fertilization on soil nematode communities are rare. The effects of phosphorus fertilizer on soil nematode community composition and structure are poorly known. Sarathchandra et al. (2001) reported that the abundances of total nematodes, bacterivores, fungivores and omnivores increased slightly (statistically insignificant) but nematode maturity index values did not change after phosphorus fertilization in pasture soils in New Zealand. Todd (1996) reported that the effects of phosphorus fertilization on soil nematodes were limited. Some other studies focused on how phosphorus fertilization affects specific nematode species. For example, Coyne et al. (2004) reports that phosphorus fertilization decreases the abundance of cyst nematode (Heterodera avenae) in soils with both upland and lowland rice in Ivory Coast of Africa. In contrast, Simon and Rovira (1985) and Price et al. (1995) report that phosphorus fertilization increased abundances of Heterodera avenae and Heterodera glycines. In the present study, we test the effects of experimental nitrogen and/or phosphorus additions on soil nematode communities in soils of secondary forests in tropical China where soils are nitrogen-rich but phosphorus-poor and face increasing deposition of atmospheric nitrogen depositions (Lu et al., 2010a; Fang et al., 2011). Therefore, we expected the additional nitrogen application might aggravate nitrogen-saturation which might be detrimental to soil nematode communities. Phosphorus application might improve the available phosphorus levels which was conducive to soil nematode communities. We hypothesize that: 1) nitrogen addition may have negative effects on the abundance, diversity, and/or community complexity of soil nematodes and 2) phosphorus addition may have positive effects on these variables of soil nematodes.

2. Materials and methods

2.1. Study site

This study was conducted at the Xiaoliang Tropical Coastal Ecosystem Research Station (110°54'E, 21°27'N), Chinese Academy of Sciences (CAS), Guangdong Province, China. The climate is tropical monsoon with a distinct wet (from April to September) and dry season (from October to March). The mean annual temperature is 23 °C and the annual precipitation is 1400–1700 mm, respectively. The soil is lateritic, formed from highly weathered granite (Table 1).

Our experiment was conducted in a secondary mixed forest. The forest started as Eucalyptus exserra plantation in 1959, then 312 species were introduced between 1964 and 1975 (Ding et al., 1992; Ren et al., 2007). Now, the most common tree species are: Castanopsis fissa, Cinnamomum camphora, Caralia brachiata, Aphanamixis polystachya, Ternstroemia pseudoeverticillata, Acacia auriculiformis, Casisia siamea, Albizia procer, Albizia odoratissima, Leucaena leucocephala, Aquilaria sinensis and Chukrasia tabularis.

2.2. Experimental design

A nitrogen and phosphorus addition experiment was designed as a randomized complete block (n = 5) and established in August

Table 1

<table>
<thead>
<tr>
<th>Soil properties</th>
<th>Controls (CK)</th>
<th>+N</th>
<th>+P</th>
<th>+NP</th>
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</thead>
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<tr>
<td>Moisture (%)</td>
<td>25.0 ± 1.3</td>
<td>24.5 ± 1.2</td>
<td>24.1 ± 1.1</td>
<td>25.0 ± 1.4</td>
</tr>
<tr>
<td>SOC (g/kg)</td>
<td>39.8 ± 0.7</td>
<td>33.8 ± 0.9</td>
<td>32.3 ± 0.7</td>
<td>33.4 ± 0.8</td>
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<tr>
<td>TN (g/kg)</td>
<td>2.60 ± 0.15</td>
<td>2.23 ± 0.21</td>
<td>2.54 ± 0.10</td>
<td>2.57 ± 0.19</td>
</tr>
<tr>
<td>TP (g/kg)</td>
<td>0.38 ± 0.04</td>
<td>0.36 ± 0.02</td>
<td>0.40 ± 0.02</td>
<td>0.41 ± 0.03</td>
</tr>
</tbody>
</table>

SOC, soil organic carbon; TN, soil total nitrogen; and TP, soil total phosphorus.
2009. Each block was located in a site more than 50 m apart in the forest. Within each block, four 10×10 m plots were established and each plot was surrounded by a 2-m-wide buffer strip in each site. The treatments nitrogen addition (+N), phosphorus addition (+P), nitrogen addition with phosphorus addition (+NP), and control (CK, no addition of mineral nutrients) were assigned randomly to the four plots within each block. Both nitrogen and phosphorus were applied at 100 kg ha\(^{-1}\) yr\(^{-1}\). Briefly, 476.6 g NH\(_4\)NO\(_3\) (equal to 166.6 g N) and/or 808 g Na\(_2\)HPO\(_4\) (equal to 166.6 g P) were dissolved in 30 L tap water and, then, applied to the corresponding plots near soil surface using a backpack sprayer in every two months from 2009 to 2011, respectively. Thirty liters of tap water was applied to control plots in each treatment event. The amount of nitrogen and phosphorus additions correspond to those studies of experimental nitrogen (Lu et al., 2010a; Zhao et al., 2013c) and phosphorus (Liu et al., 2012) additions in neighboring area in Guangdong province, PR China, which was lower than the amount of nitrogen and phosphorus fertilizers that used in tropical rain forest in southwest Costa Rica (Cleveland and Townsend, 2006).

2.3. Soil sampling and analysis

Soil was sampled in September 2009 before the first mineral nutrient addition treatment application (background sampling), September 2010 (after one-year treatment), and September 2011 (after two-year treatment), respectively. Soil cores (5 cm diameter) were taken at 0–10 cm depth from five randomly selected locations in each plot in each block. Six cores from the same plot were combined to form one composite sample. The litter above each sample area was removed before the cores were collected.

Soil pH was determined in 1:2.5 (w/v) soil:water solutions, and soil water content (SWC %, g of water per 100 g dry soil) was measured by oven-drying for 48 h at 105 °C. Soil organic C (SOC, g/kg dried soil) was determined by the loss-on-ignition technique by ashing in a muffle furnace at 600 °C for 6 h. Total N (TN, g/kg dried soil) was measured with a ultraviolet spectrophotometer using the procedure of molybdenum antimony blue colorimetry, ammonium nitrogen was determined colorimetrically by the salicylate-nitroprusside method on a Flow-Injection Autoanalyzer (FIA, Lachat Instruments, USA), nitrate nitrogen was determined colorimetrically by the vanadium-nitroprusside method on a Flow-Injection Autoanalyzer (FIA, Lachat Instruments, USA), and available P (mg/kg dried soil) was determined by the loss-on-ignition technique by extraction with the solution of Bray-1 (0.03 M NH\(_4\)F) and measured colorimetrically (Liu, 1996).

Nematodes were extracted from 50 g of moist soil using the Baermann funnel method (Barker et al., 1985). After fixation in 4% formalin solution, nematodes were counted with a DIC microscope (ECLIPSE 801, Nikon) (Zhao et al., 2011, 2014a), and the first 100 individuals encountered were identified to trophic group (bacterivores, fungivores, herbivores, omnivores, predators) and functional guild (Bongers, 1990; Yeates et al., 1993a; Bongers and Bongers, 1998; Okada et al., 2005). The functional guild is defined by the nematode’s trophic behavior and by its ecological life strategy as a colonizer or persister (Bongers, 1990; Ferris et al., 2001). All nematodes were identified to trophic group and functional guild when the sample contained fewer than 100 individuals.

2.4. Data analysis

The diversity of the nematode community was determined by the Shannon–Wiener diversity index (\(H'\)), the Pielou evenness index (\(J\)), the Margalef richness index (\(SR\)), and the Simpson dominance index (\(\lambda\)) (Neher and Darby, 2009).

Shannon – Wiener diversity index, \(H’ = - \sum_{i=1}^{S} \frac{P_i}{N} \ln \frac{P_i}{N} \)  
(1)

Pielou evenness index, \(J = \frac{H’}{H’_{\text{max}}} \)  
(2)

\(H’_{\text{max}} = \ln S\)  
(3)

Margalef richness index, \(SR = \frac{S - 1}{\ln N}\)  
(4)

Simpson dominance index, \(\lambda = \sum P_i^2 \)  
(5)

in which \(P_i\) is the proportion of the individuals of “ith” group in the community, ‘\(S\)’ is the total number of nematode genera in the community, and ‘\(N\)’ is the total number of nematodes in the community.

The nematode data were also used to calculate two maturity indices (i.e. MI and MI25) (Bongers, 1990). These indices are calculated by a colonizer–persister (cp) scale that range from a colonizer (cp value = 1, r-strategy) to a persister (cp value = 5, K-strategy). Small and large values of these indices represent highly disturbed and highly stable soil ecosystems, respectively (Bongers, 1990). These indices were calculated as follows:

\(\text{MI} = \sum v(i) cp^{1-5} \times f(i) cp^{1-5} \)  
(6)

\(\text{MI25} = \sum v(i) cp^{2-5} \times f(i) cp^{2-5} \)  
(7)

Where \(v(i)\) is the cp value of free-living taxa and \(f(i)\) is the proportion of that taxa of the total number of free-living nematodes in a sample; and the superscript \(cp^{1-5}\) and \(cp^{2-5}\) indicate the nematodes in cp1–5 and cp2–5 guilds are involved in the calculations, respectively (Bongers, 1990; Neher, 2001). In addition, the enrichment index (EI) and structure index (SI) values were computed for each sample, as described by Ferris et al. (2001). The EI value is calculated from the relative weighted abundance of opportunistic bacterial- and fungal-feeding nematodes that respond rapidly to input of food resources; a high EI value indicates an environment that has been enriched. The SI value is calculated from the relative weighted abundance of disturbance-sensitive guilds, a high SI value indicates a complex and stable food web. EI and SI were calculated as follows:

\(\text{EI} = 100 \times \left(\frac{e}{e + b}\right)\)  
(8)

\(\text{SI} = 100 \times \left(\frac{s}{s + b}\right)\)  
(9)

Where ‘\(b\)’ is the basal food web component (Ba2, Fu2), ‘\(e\)’ is the enrichment component (Ba1, Fu1) and ‘\(s\)’ is the structure component (Ba3–Ba5, Fu3–Fu5, Om3–Om5, Pr2–Pr5). Ba, Fu, Om, and Pr refer to trophic groups (bacterivores, fungivores, omnivores, and predators, respectively) and the associated subscripts refer to particular guilds. Moreover, two metabolic footprint indices that, the enrichment footprint index (EFI) and structure footprint index (SFI), were computed for each sample, as described by Ferris (2010). EFI and SFI values indicate the magnitudes of the carbon utilization (i.e. carbon used for production and respiration) of the enrichment component (Ba1, Fu1) and structure component (Ba2–Ba5, Fu2–Fu5, Om2–Om5, Pr2–Pr5) of soil nematode communities, respectively (Ferris, 2010). The metabolic footprint index for each of the \(i\) taxa were calculated as follows:
\[ FI = \sum \left( N(i) \times \left( 0.1 \times \left( W(i) / v(i) \right) + 0.273 \times \left( W(i)^{0.75} \right) \right) \right) \]  

Where the \( N(i) \) is the number of individuals in each of the \( i \) taxon; \( W(i) \) is the body weight of taxon \( i \); and \( v(i) \) is the cp value of taxon \( i \). The \( i \) taxa involved in the calculation of EFl and SFI only include the enrichment component and structure component, respectively. The values of \( W(i) \) are according to Ferris (2010). Additionally, a soil nematode channel index (CI) (Ferris et al., 2001) was calculated for each sample to evaluate soil decomposition pathways of soil food web and a bacterivore index (Bal) (Ferris and Matute, 2003) was calculated to indicate rates of succession from enrichment-opportunist (Ba1) to general-opportunist (Ba2) bacterivore nematodes. CI and Bal were calculated as follows:

\[ \text{CI} = 100 \times 0.8 \times N_{2} / (3.2 \times B_{1} + 0.8 \times N_{2}) \]  

(11)

\[ \text{Bal} = 100 \times 0.8 \times B_{2} / (3.2 \times B_{1} + 0.8 \times B_{2}) \]  

(12)

The experiment was set up as a two-factor (2^2) design. Repeated-measure COANOVA was employed to determine the effects of two main factors (N and P) and their interaction (N*P) through the whole experimental period with the background data (first sampling data) as co-variables. One-way ANOVA was used to compare effects of the four treatments (CK, +N, +P and +NP) on soil nematode variables in each sampling event. Statistical significance was determined at \( p < 0.05 \). ANOVAs were performed using SPSS 16.0 (SPSS Inc., Chicago, IL). The principal response curves (PRC) method was used to determine the temporal trends of soil nematode community composition (represented by functional guilds) for each treatment using CANOCO 4.5 (Ithaca, NY, USA). PRC is based on redundancy analysis (RDA). The result is a diagram showing the first Principal Component of the variance explained by treatment on the x-axis along the sampling periods on the y-axis. The control treatment is treated as a zero baseline (the horizontal line). The treatment effect is represented by the deviation of each fluctuating line (i.e. +N, +P and +NP) from the zero baseline through time. Both environmental class variables (treatments) and covariables (sampling times) were coded as nominal 0 or 1 variables (Leps and Šmilauer, 2003; Zhao and Neher, 2013a). Monte Carlo permutation tests were applied to compute statistical significance (\( n = 499 \)).

3. Results

Experimental phosphorus addition significantly increased the soil available phosphorus content (Fig. 1A). There was no significant interaction effect of phosphorus addition and nitrogen addition on the content of soil available phosphorus. Experimental nitrogen addition apparently increased both of the soil ammonium and nitrate nitrogen contents during the study (Fig. 1B and C). There was a significant interaction effect on soil ammonium nitrogen content (Fig. 1B). Neither phosphorus addition nor nitrogen addition had significant influence on soil pH (Fig. 1D).

There were altogether 78 nematode genera collected (Table 2). The most common nematode genera included: Acrobeloides, Pris- matolaimus, Apherelchoides, Protohabditis, Ditylenchus, Diploscapter, Xiphinema, Rhabditonema, Filelchus, Lelenchus, Clarkus, and Wilsonema. Bacterivores were the most abundant trophic group followed by fungivores and herbivores (Fig. 2). There were remarkable negative effects of phosphorus addition on total nematode density (\( F = 7.602, p = 0.012 \)) (Fig. 2A) and density of omnivore-predators (\( F = 4.812, p = 0.040 \)) (Fig. 2E) during the study. Phosphorus addition tended to decrease the density of fungivores (\( F = 3.288, p = 0.085 \)) (Fig. 2C). Neither nitrogen addition nor interaction of nitrogen addition and phosphorus addition affected total nematode density, bacterivore density, fungivore density, herbivore density, and omnivore-predator density (Fig. 2). Diversity (mean ± S.E.) of soil nematode communities under each treatment in each sampling event is shown in Table 3. Repeated-measure COANOVA did not show significant main effect and interaction effect of nitrogen addition and phosphorus addition on Shannon–Wiener index (\( H' \)), Pielou evenness index (J), Margalef richness (\( S_R \)), and Simpson dominance index (\( I_s \)). Phosphorus addition suppressed the nematode enrichment index (EI) (\( F = 5.711, p = 0.027 \)) (Fig. 3A), the nematode structure index (SI) (\( F = 8.413, p = 0.009 \)) (Fig. 3C) and the nematode structure footprint index (SFI) (\( F = 8.015, p = 0.010 \)) (Fig. 3D) during
the study. Phosphorus addition did not significantly affect the nematode enrichment footprint index (EFI); and there were no apparent effect of nitrogen addition and interaction effect of nitrogen addition and phosphorus addition on EI, SI, EFI and SFI during the study (Fig. 3). Phosphorus addition suppressed MI25 (F = 7.208, p = 0.014), but had no significant effect on MI (Fig. 4A and B). There were no apparent effect of nitrogen addition and interaction effect of nitrogen addition and phosphorus addition on MI and MI25 (Fig. 4A and B). Moreover, phosphorus addition increased the nematode channel index (CI) (F = 5.203, p = 0.034) and the bacterivore index (Bal) (F = 5.476, p = 0.030) (Fig. 4C and D). There were no apparent effect of nitrogen addition and interaction effect of nitrogen addition and phosphorus addition on either CI or Bal (Fig. 4C and D).

Although the background soil nematode community composition within these plots were somewhat different from each other, the temporal dynamics of soil nematode community compositions within +N, +P and +NP plots were similar (Fig. 5). Specifically, the effect of phosphorus addition was slightly more powerful on soil nematode community composition than the effect of nitrogen addition, which was revealed by that the variation of PRC of +P treatment was larger compared with the variation of PRC of +N treatment (Fig. 5). In addition, the nematode community composition showed apparent successions from communities dominated by He3 and Ba1 to communities dominated by Ba2 and Fu2 after nitrogen and phosphorus additions (Fig. 5).

4. Discussion

4.1. Effects of nitrogen addition on soil nematode communities

The nematode community, in terms of trophic group composition, calculated indices of community diversity and structure, was not sensitive to the nitrogen addition in this study. Previous studies reported that experimental nitrogen addition, when exceeding 80 kg N ha⁻¹ yr⁻¹, suppressed soil nematode communities in both alpine tundra soils in Wyoming, USA (Lokupitiya et al., 2000) and in grassland soils in Inner Mongolia, China (Wei et al., 2012; Li et al., 2013), which in general were considered as nitrogen-poor ecosystems. However, little is known about how experimental nitrogen addition affects soil nematode communities in tropical forests which are relatively nitrogen-rich. Many previous studies reported that soil nematode trophic groups responded differently to nitrogen fertilizations. Consistent with the present study, there was a study that reported nitrogen addition had no effects on total nematode or each trophic group densities in turf soils in Ohio, US (Cheng et al., 2008). In addition, many previous studies reported that nitrogen addition did not affect one or more indices (Saratchandhra et al., 2001; Forge et al., 2005; Wang et al., 2006; Cheng et al., 2008; Liang et al., 2009).

Although ANOVAs did not detect significant effects of nitrogen addition on the trophic composition and faunal indices of soil nematode community structure; nematode functional guild composition, which revealed by multivariate analysis (PRC), was significantly altered after two-year nitrogen addition compared with control treatment. During the study, the importance of Ba1 and He2, as well as the importance of Ba3, Ca4, Fu3, Fu4, reduced gradually; however, the importance of Ba2 and Fu2, as well as the importance of Om2, increased gradually. These results indicated nematode genera in the same trophic groups (e.g., bacterivore and fungivore) or similar cp-guilds (e.g., cp3–5) responded idiosyncratically to nitrogen addition. Therefore, the idiosyncratic responses of different genera in a soil nematode trophic group and/or cp-guild might lead to that the nematode trophic group composition and community indices were not affected by the nitrogen addition. In addition, Ba2 and Fu2 are basal components of soil nematode communities, and the

### Table 2

<table>
<thead>
<tr>
<th>Bacterivorous</th>
<th>Fungivorous</th>
<th>Herbivorous</th>
<th>Carnivorous</th>
<th>Omnivorous</th>
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<td>Genus name</td>
<td>cp-value</td>
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<td>cp-value</td>
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<td>Aphanus</td>
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**Note:** The cp-value indicates the proportion of nematode genera in the same trophic groups (e.g., bacterivore and fungivore) or similar cp-guilds (e.g., cp3–5) that responded idiosyncratically to nitrogen addition.
communities (Ferris et al., 2001). The increased weights of \( Ba_2 \) and \( Fu_2 \) might demonstrate that the succession of the soil food web shifted towards a relatively simple system (a basal condition) under nitrogen addition treatments.

Nitrogen addition usually can facilitate abundance and community complexity (as indicated SI) of soil nematodes directly or indirectly by bottom-up control via plant growth or microbial activity (Yeates et al., 1993b; Vestergård, 2004; Liang et al., 2009).

![Fig. 2. Densities of total nematodes (A), bacterivores (B), fungivores (C), herbivores (D), and omnivore-predators (E) under Control (CK), N fertilization (+N), P fertilization (+P), and NP fertilization (+NP) in each sampling event (2009, 2010 and 2011). Bars indicate standard errors of means. Non-significant (\( p > 0.05 \)) main and interaction effects were not provided.]

![Table 3](image)
Additionally, nitrogen addition commonly reduces soil pH, and causes ammonium and aluminum toxicity or introduces sufficient salt to harm soil biota (Russell et al., 2006; Liang et al., 2009; Wei et al., 2012). The trade-off between positive and negative effects determined the responses of soil nematodes to nitrogen application (Zhao et al., 2014b). In the current study, experimental nitrogen addition significantly enhanced the contents of soil ammonium and nitrate nitrogen, which potentially had negative impacts on soil nematodes. In addition, studies in neighboring area of the present study have demonstrated that the diversity or cover of the understory vegetation significantly reduced and the aluminum mobility changed in nitrogen addition plots than that in control plots in both secondary and old-growth tropical forests which are nitrogen-saturated (Lu et al., 2010a, 2010b). Therefore, the negative changes of both plant diversity or cover and soil properties after nitrogen addition might result in shifts in soil nematode communities.

4.2. Effects of phosphorus addition on soil nematode communities

Studies focused on the effects of mineral P addition on soil nematode communities are rare. The effects of phosphorus...
addition on soil nematode community composition and structure are not clear. In the present study, phosphorus addition did not significantly affect ML. Similarly, Sarathchandra et al. (2001) found that the MI was not significantly affected by phosphorus addition in pasture soils in New Zealand. However, phosphorus addition significantly reduced MI25 in the current study. The main difference between those two maturity indices is that cp1 (or Ba1) free-living nematodes are involved in the calculation of MI rather than MI25. Therefore, the alterations of Ba1 nematode abundance might result in the idiosyncratic responses of MI and MI25 to phosphorus addition during the study. In addition, phosphorus addition significantly reduced omnivore–predator density (K-selected) and SI in our study, which indicated the soil food web had fewer trophic links after phosphorus addition. Fewer links or connectances might lead to decline in functional resilience of food webs to disturbance (Wardle et al., 1995; Ferris et al., 2001). In the present study, the relative weight of fungal mediated decomposition pathway was significantly increased after phosphorus addition, which indicated by the significant increase in nematode channel index (CI) (Fig. 4C) and the abundance of Fu2 guild (Fig. 5) after phosphorus applications. This finding was consistent with the results of a previous study that evaluated soil decomposition pathways using soil microbial communities in secondary subtropical mix-forests in southern China near to the present sites (Liu et al., 2012). Additionally, phosphorus addition induced remarkable reduction of the carbon utilization of structure component of soil nematodes, which is indicated by the significant increase in nematode structure footprint index (SFI) (Fig. 3D) after phosphorus applications. Therefore, the alterations of soil food web complexity, soil decomposition pathways, and carbon utilization of soil organisms after phosphorus applications may result in the changes of ecosystem functioning and processes.

In contrast to our hypothesis, we found negative effects of phosphorus addition on soil nematode community. The most likely reason is that phosphorus fertilization improves the soil available phosphorus condition and results in the decrease of resources input from plants to soil organisms in the short-term. Plants efficiently and economically allocate resource to nutrient acquisition (Treseder and Vitousek, 2001). When ecosystem is phosphorus-poor, plants allocate substantive resources (include extracellular phosphatase) to soil organisms that decompose litter and release phosphorus to soil which can be used by plants. In conditions of phosphorus addition, plants can directly obtain enough available phosphorus from soil; thus, plants will reduce the resources input to soil organisms. In fact, experimental phosphorus addition significantly increased the soil available phosphorus content during the study. Therefore, the decrease of soil nematode density might be due to the resource/flood limitation after phosphorus addition. Phosphorus fertilization reduced the water soluble organic carbon in a low land tropical rain forest in Costa Rica (Cleveland and Townsend, 2006) and reduced the phosphatase activity and mycorrhizal colonization in Hawaii rain forests (Treseder and Vitousek, 2001), which might support this view. Phosphorus addition also inhibited the mycorrhizal symbiosis in a tallgrass prairie in Kansas (Bentivenga and Hetrick, 1992) and suppressed the mycorrhizal formation of two poplar clones in South Germany (Baum and Makeschin, 2000). Consistent with these results, we found phosphorus addition tended to reduce the density of fungivorous nematodes in this study. Another likely reason is that the additional mineral phosphorus input to soil leads to salt toxicity that harms soil nematodes. In the present study, the amount of applied phosphorus is 100 kg ha$^{-1}$ yr$^{-1}$, which might be sufficient to introduce salt toxicity to soil nematodes. And as noted above, the content of soil available phosphorus was indeed increased after phosphorus application. In addition, phosphorus addition in proper levels can improve plant growth, however, excess phosphorus addition has no further positive effects on plant growth. For example, Sarathchandra et al. (2001) reported that dry matter production significantly increased from 0 to 50 kg ha$^{-1}$ yr$^{-1}$ P rate, but no further increases were observed at 100 kg ha$^{-1}$ yr$^{-1}$ P rate. Therefore, the negative effect of phosphorus addition might be more powerful than its positive effect on the abundance and community complexity of soil nematodes, which would have resulted in a net negative effect (Zhao et al., 2014b).

4.3. Interaction effects of nitrogen and phosphorus addition on soil nematode communities

Nitrogen and phosphorus are the most common elements in limiting primary productivity in most terrestrial ecosystems. In agricultural practices and experimental studies, nitrogen and phosphorus fertilizers (chemical fertilizer usually with other nutrient elements) are usually used together. In the present study, although no interaction effect of nitrogen and phosphorus additions on the monitored variables of nematode community was observed, the −NP treatment tend to have the most powerful effects on all tested variables among the four treatments. In addition, PRC showed a tendency for the effect of −N and −P combined to be greater than the effect of −P or −N alone. Therefore, the effect of −NP treatment on soil nematode community might be a superposition effect of −N treatment and −P treatment. Unfortunately, we did not find any studies that quantitatively compared the effects between NP addition and N or P addition on soil nematodes in field. Previous studies reported that nematode abundances were higher under N application than under NP application in the rhizospheres of barley plants in pot experiments (Vestergård et al., 2004; Bjørnland et al., 2006), which was inconsistent with our finding. However, the responses of soil nematode abundance to nutrient applications were mediated by bottom-up control through resource (or food) quality and/or quantity changes in those pot experiments (Bjørnland et al., 2006). Furthermore, previous field studies focused on the effects of NP addition on soil nematode communities have revealed conflicting results. For examples, NP addition had no effects on nematode
trophic groups in grassland (Todd, 1996; Forge et al., 2005); however, NP addition significantly reduced the herbivorous nematode density in the soybean phase of a soybean–wheat–corn rotation in northeast China (Pan et al., 2010). In addition, chemical fertilizers (N, P and K) significantly increased total nematode density and bacterivorous nematode density of 2-year study in a Japanese soybean field (Okada and Harada, 2007), but decreased total nematode density in a 20–25 year old stand of Scots pine located in central Sweden (Sohlenius and Wasilewska, 1984). Moreover, chemical fertilizers (N, P and S) did not influence the total nematode density, bacterivore density, fungivore density and omnivore density, but significantly reduced predator density in mature forests of British Columbia (Forge and Simard, 2001).

5. Conclusion

In summary, our results suggest that experimental nitrogen and/or phosphorus additions significantly affect soil food web and soil decomposition pathways in tropical secondary forests. Particularly, the effects of nitrogen and/or phosphorus additions on soil nematode communities were negative and the effects of phosphorus addition are more powerful than the effects of nitrogen addition in relatively nitrogen-rich but phosphorus-poor ecosystems. Nitrogen and/or phosphorus additions degrade the structure of and trophic links within the soil food web, reduced the carbon utilization of soil nematodes, and degrade the fungal mediated decomposition pathway more important, which may affect other forest ecosystem processes and functions (e.g., carbon sequestration, nutrient mineralization, ecosystem succession, and NEP). In addition, our findings could provide a better understanding of the responses of soil food web to nitrogen and phosphorus additions in nitrogen-rich but phosphorus-poor soils.

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