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ORIGINAL PAPER

Bacterivore nematodes stimulate soil gross N transformation rates depending on their species

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Abstract We conducted a microcosm experiment with soil being sterilized, reinoculated with native microbial community and subsequently manipulated the bacterivorous nematodes, including three treatments: without (CK) or with introducing one species of the two bacterivores characterized with different body size but similar c-p (colonizer-persister) value (*Rhabditis intermedia* and *Protorhabditis oxyuroides*, accounted for 6 and 59% of bacterivores in initially undisturbed soil, respectively). We monitored the N_2O and CO_2 emissions, soil properties, and especially quantified gross N transformation rates using ^{15}N tracing technique after the 50 days incubation. No significant differences were observed on soil NH_4^+ and NO_3^- concentrations between the CK and two bacterivores, but this was not the case for gross N transformation rates. In comparison to CK, *R. intermedia* did not affect soil N transformation rates, while *P. oxyuroides* significantly increased the rates of mineralization of organic N to NH_4^+, oxidation of NH_4^+ to NO_3^-, immobilization of NO_3^- to organic N and dissimilatory NO_3^- reduction to NH_4^+. Furthermore, the mean residence time of NH_4^+ and NO_3^- pool was greatly lowered by *P. oxyuroides*, suggesting it stimulated soil N turnover. Such stimulatory effect was unrelated to the changes in abundance of bacteria and ammonia-oxidizing bacteria (AOB). In contrast to CK, only *P. oxyuroides* significantly promoted soil N_2O and CO_2 emissions. Noticeably, bacterivores increased the mineralization of recalcitrant organic N but decreased soil $\delta^{13}C_{\text{-TOC}}$ and $\delta^{15}N_{\text{-TN}}$ values, in particular for *P. oxyuroides*. Combining trait-based approach and isotope-based analysis showed high potential in moving forward to a mechanistic understanding of bacterivore-mediated N cycling.

Keywords Bacterivores (nematodes) · ^{15}N tracing · N transformation · N_2O emission · AOB

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Introduction

As the most abundant and diverse taxon of fauna, soil nematodes, especially bacterivores, occupy key trophic positions in soil food webs that enable them to interact with microbes, other soil fauna, and plants (Yeates 2003; De Mesel et al. 2004; Trap et al. 2016), and thus can affect various ecological process in terrestrial ecosystems (Ferris et al. 1998; Postma-Blaauw et al. 2005; Trap et al. 2016). Considering the importance of nitrogen (N) in agriculture, environment, and ecology

(Booth et al. 2005; Zhang et al. 2013), there is increasing concern about the functional role of bacterivorous nematode in soil N cycling (Ingham et al. 1985; Coleman et al. 2004; Neher et al. 2012). Through feeding the saprophytic and pathogenic bacteria (Neher 2010), bacterivores can significantly influence soil N availability and dynamic (Paterson et al. 2011; Kuiper et al. 2013). More than two decades ago, Griffiths (1994) synthesized the literatures from a range of ecosystems and reported that the fauna, mainly from the activities of microbiovores (nematodes and protozoa that is feeding on microorganisms), contributed approximately 30% of total net N mineralization. Later studies have confirmed that bacterivores play an important role in soil N mineralization and nitrification (Xiao et al. 2010; Trap et al. 2016). For example, Venette and Ferris (1997) and Ferris et al. (1998) found that the excess N assimilated during growth and reproduction of nematode when grazing bacteria can be excreted in mineral form (primarily as NH_4^+) for maintaining metabolic need, due to the relatively higher C:N ratio in bacterivores than that in bacteria diet.

In previous studies, analyzing the net changes of different soil N pools (e.g., net mineralization or net nitrification) was the most widely used method to evaluate bacterivore effects on N transformation process (De Mesel et al. 2004; Buchan et al. 2013; Gebremikael et al. 2014). If there is no differences in the net rates emerged, the contribution of bacterivores on N mineralization and nitrification was considered to be negligible (Rønn et al. 2001; Postma-Blaauw et al. 2005). The "net" method; however, may underestimate the bacterivore functional roles as such method could not differentiate the mechanism underlying the change in soil N dynamics (Murphy et al. 2003; Zhang et al. 2013; Lang et al. 2016). By contrast, investigating soil gross N transformation rates involved in the production and consumption of organic N, ammonium (NH_4^+) and nitrate (NO_3^-), can provide relatively more comprehensive picture of the separate N transformation phases (Hart et al. 1994; Müller et al. 2007; Zhang et al. 2013; Müller and Clough 2014). None of earlier studies, to our knowledge, have been conducted to assess the effect of bacterivores in light of soil gross N transformation rates. It is possibly that a no response in the net rates but difference in the individual gross N rates occurs, in case that bacterivores stimulate the turnover of inorganic N by simultaneously increasing the production and consumption of inorganic N pool.

However, the roles of bacterivores in soil N transformation processes and especially the inherent biologically driven mechanisms were neglected, despite previous studies have demonstrated that bacterivore nematodes could alter the abundance and community composition of bacteria (Djigal et al. 2004; Chen et al. 2007; Xiao et al. 2014) and ammonia oxidizers (Xiao et al. 2014; Jiang et al. 2014), such as ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), both being responsible for nitrification (Liu et al. 2017). Noticeably, bacterivore effects on gross N transformations rates may be species-specific. Bacterivores characterized by different life history strategies can forage on different food sources at various rates, even belonging to the same trophic group (Ferris et al. 1998; De Mesel et al. 2004), and would accordingly exert different effect on soil gross N transformation rate. But the underlying mechanism related to species trait of bacterivorous nematodes has not been well established. With the increasing concerns that land-use intensification reduces the complexity in the soil food webs, understanding the biological interactions among soil bacterivores, bacteria and N cycling in light of functionally distinct but taxonomically close nematodes would help us explore soil biodiversity values and functional mechanisms (Tsiafouli et al. 2015).

In this study, a fluvo-aquic soil was manipulated with or without two bacterivorous nematodes, namely *Rhabditis intermedia* and *Protorhabditis oxyuroides*, with different body size but similar c-p (colonizer-persister) value (Table 1). After 50 days incubation, soils were sampled to determine the basic properties, bacterivore abundances, gross N transformation rates using a ^{15}N tracing technique, bacterial 16S rRNA gene abundance, and archaeal and bacterial *amoA* gene abundance. The objectives of this study were (1) to test whether bacterivorous nematodes would have greater effects on soil gross N transformation rates compared to the conventionally measured net rates and (2) to test whether different bacterivorous nematodes with the same c-p would have a distinct effect on N transformation processes.

Materials and methods

Soil, straw, and nematodes

Soils were collected from a wheat-rice rotation system from the 0–20 cm layer, in Rudong county (32°31′48″N and 121°10′64″E), Jiangsu Province. The soil was loamy soil classified as calcareous Cambisols (WRB Soil Taxonomy). After excluding large and medium-sized soil animals and root stubble, stones, and other debris, soils were sieved (2 mm) and

Table 1 The characteristic of *Rhabditis intermedia* and *Protorhabditis oxyuroides*

	Rhabditis intermedia	*Protorhabditis oxyuroides*
c-p	1	1
Body length (mm)	0.87	0.72
Body diameter (mm)	0.05	0.04
Body volume (10^{-3} mm^3)	1.71	1.26

c-p colonizer-persister (Bongers 1990)

divided into two parts. One was stored at 4 °C, and the other was air-dried and used for measuring soil basic properties, which were total organic C (TOC) 18.1 g kg^{-1}; total N (TN) 1.0 g kg^{-1}; exchangeable NH_4^+-N 0.3 mg kg^{-1}; NO_3^--N 28.8 mg kg^{-1}; and pH 7.6. The used rice straw was crushed over 100-mesh sieve and subsequently dried at 60 °C for 48 h. The straw properties were: TOC 363.1 g kg^{-1}, TN 6.7 g kg^{-1}, and C/N 55.0.

The two common bacterivorous nematodes, *Rhabditis intermedia* (6%, relative abundance of bacterivores in initial soil) and *Protorhabditis oxyuroides* (59%, relative abundance of bacterivores in initial soil) were picked out from the initial fresh soil, washed with sterile water, and cultured in the Nematode growth media (NGM) plates, feeding with *Escherichia coli OP50* (Nuttley et al. 2002).

Experimental design

The fresh soil and the powered rice straw were sterilized with 25 kGy gamma rays and then incubated at 22 °C for 3 days, and sterilized again with 25 kGy gamma rays to eliminate all organisms. After twice' sterilization, soils were incubated at 22 °C for another 3 days and then tested if there were microorganisms with LB solid medium and nematodes were extracted with a modified Baermann method (Liu et al. 2008). From the pre-experiment, no native nematodes were found after 50 days' incubation after total 50 kGy gamma rays. To reduce excessive NO_3^--N produced following sterilization by gamma rays, the soils were leached by sterile water thoroughly, and then dried at room temperature to 40% water-holding capacity (WHC) in fume-hood. Then soils (equal to 150 g of dried soils) were weighed into 500 ml glass bottle, mixed with the above sterilized rice straw powder (0.173 g), and were reinoculated with microorganism by adding 10 ml of a soil microbial suspension made from above fresh soil stored in 4 °C. The suspension was made by mixing 50 g of fresh soil in 100 ml of sterile distilled water and then gently stirring for 30 min to obtain a suspension, followed by sieving through a 65 μm mesh, 300 μm mesh and finally made to 500 ml suspension by filtration through 10 μm pore size (Wagg et al. 2014), in which no any soil nematodes were found. Then the soil-straw-microbial suspension was incubated at 25 °C for a week to stabilize the microbial activity. Also the two cultural nematodes were washed from the NGM plates and centrifuged (3000×g) for 3 min, after which the supernatant was discarded. Next the nematodes were washed with sterile water 5–6 times to minimize the interference from bacteria before transferring into the soils.

A total of three treatments with four replicates were set up: (1) sterilized soil and straw with microbial suspension (CK), (2) sterilized soil and straw with microbial suspension and *Rhabditis intermedia* (Rha), and (3) sterilized soil and straw with microbial suspension and *Protorhabditis oxyuroides* (Pro). After the bacterivores were added to respective bottles (20 ind g^{-1} dry soil), all microcosms were adjusted water content to 60% WHC. The microcosms were sealed with a sterile membrane that allows air flux but not microbes, and then microcosms were incubated in the dark at 25 °C. The emission rates of CO_2 and N_2O were quantified on day 1, 2, 3, 5, 10, 25, 40, and 50 with a gas chromatograph (Agilent 7890A; Agilent Technologies, Waldbronn, Germany). The soil moisture content was kept to 60% WHC with distilled water by weighing the microcosms every week. At the 50 days incubation, all microcosms were destructively sampled to determine the soil chemical properties, nematode abundance, bacterial 16S rRNA gene abundance, and archaeal and bacterial *amoA* gene abundance and gross N transformation rates.

Briefly, 15 g oven-dried equivalent fresh soil were weighed into a series of 100-ml Erlenmeyer flasks and a $^{15}NH_4NO_3$ (10 atom% excess) or $NH_4{}^{15}NO_3$ (10 atom% excess) solution at a rate of 100 mg N kg^{-1} (50 mg N kg^{-1} as NH_4^+ and NO_3^-, respectively) was evenly applied. Flasks were capped with plastic films to allow gas exchange, and subsequently incubated at 25 °C. Soils were extracted, with 2 M KCl, at 0.5, 24, 48, and 96 h after NH_4NO_3 solution addition. Exchangeable NH_4^+ and NO_3^- concentrations and their respective ^{15}N enrichments in the extracts were determined by a continuous-flow analyzer (Skalar, Breda, The Netherlands) and a Sercon SL Elemental Analyzer coupled to a 20–20 isotope-ratio mass spectrometer (IRMS) (Sercon Ltd., Crewe, UK), respectively (for details see Zhang et al. 2012a). The simultaneously occurring gross N transformation rates in soil were quantified by a numerical ^{15}N tracing model (Müller et al. 2007).

Nematode measurement

Nematodes were extracted from 20.0 g soil (Dry equivalent) by a modified Baermann method (using shallow pans instead of funnels to lower the soil depth) followed by sugar centrifugal flotation (Liu et al. 2008). Collected nematodes were counted using a dissecting microscope (40×), heat-killed for 2 min at 60 °C and preserved in 4% formaldehyde for identification at a higher magnification (100 and 400×) to check if all nematodes were only *Rhabditis intermedia* or *Protorhabditis oxyuroides*, which were added before.

DNA extraction and quantitative PCR analysis

DNA was extracted from soil samples using a FastDNA® Spin Kit for Soil (MP Biomedicals, Cleveland, OH, USA), according to the manufacturer's instructions. Soil DNA quantity and purity were determined by a Nanodrop ND-2000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). DNA extracts were stored at −20 °C until use. The abundances of bacterial 16S rRNA, and archaeal *amoA* or bacterial *amoA* genes were quantified by quantitative PCR

method on a CFX96 Optical Real-Time Detection System (Bio-Rad, Laboratories Inc., Hercules, CA, USA) in three biological replicates and each with three technical replicates. The primer pairs 515F/907R (Angenent et al. 2005), Arch-amoAF/Arch-amoAR (Francis et al. 2005) and amoA1F/amoA2R (Rotthauwe et al. 1997) were used for bacterial 16S rRNA, and archaeal *amoA* or bacterial *amoA* genes, respectively. The reaction was performed in a 20-μl mixture containing 10 μl SYBR Premix Ex Taq (Takara, Dalian, China), 0.5 μM of each primer, and 1 μl of DNA template (7.0–23.5 ng). The Real-time PCR condition for all three genes was as follows: 95 °C for 3 min, 40 cycles of 95 °C for 20 s, 55 °C for 20 s, and 72 °C for 20 s and followed by plate reads at 83 °C. A melting-curve and standard agarose gel electrophoresis was performed to check the specificity of the amplification products. Blanks were always run with water as the template instead of soil DNA extract. The standards for qPCR were generated using plasmid DNA from representative clones containing bacterial 16SrRNA and archaeal *amoA* or bacterial *amoA* genes. A dilution series of the standard templates across seven orders of magnitude (2.31×10^2–2.31×10^8 for archaeal 16S rRNA gene, 6.30×10^2–6.30×10^8 for archaeal *amoA* gene, and 1.56×10^2–1.56×10^8 for bacterial *amoA* gene) per assay was used. Amplification efficiencies of 94.7–103.6% were obtained, with R^2 values of 0.992–1.000. In addition, a serial dilution of the DNA templates was used to assess whether the PCR was inhibited during the amplification (Wang et al. 2015).

Soil properties

Soil pH was determined at a 1:2.5 (w: v) soil: water ratio by a DMP-2 mV/pH detector (Quark Ltd., Nanjing, China). After removing carbonate using a pretreatment with H_3PO_4, TOC, $\delta^{13}C_{\_TOC}$, TN, and $\delta^{15}N_{\_TN}$ were analyzed by a Sercon SL C/N Elemental Analyzer coupled to a 20–20 isotope-ratio mass spectrometer (IRMS) (Sercon Ltd., Crewe, UK). In the ^{15}N tracing model, two conceptual SOM pools, labile organic N (N_{lab}) and recalcitrant organic N (N_{rec}), were compiled. Soil N_{lab} and N_{rec} were isolated using physical fractions method according to Meijboom et al. (1995). Briefly, 2 mm sieved soil was wet sieved over 150 μm sieve with a jet of deionized water. The obtained macroorganic matter (MOM) from 150 to 2000 μm fractions was an active, microbial available N pool, which was defined as the N_{lab} pool in the ^{15}N tracing model (Fig. S1) (Huygens et al. 2005). This fraction was dried at 60 °C for 24 h and ground for N determination. Soil N_{rec} was calculated as TN minus N_{lab}.

Soil ^{15}N measurement

Exchangeable NH_4^+ and nitrate (NO_3^-) were separated for ^{15}N measurements by distillation with magnesium oxide and Devarda's alloy (Bremner and Keeney 1966). In brief, a portion of the extract was steam-distilled with MgO to separate NH_4^+; thereafter, the sample in the flask was distilled again after addition of Devarda's alloy to separate NO_3^-. The liberated NH_3 was trapped in boric acid solution in a conical flask. The trapped N was acidified and converted to $(NH_4)_2SO_4$ using 0.02 M H_2SO_4 solution. The H_2SO_4 solution containing NH_4^+ was then evaporated to dryness at 80 °C in an oven and analyzed to determinate the ^{15}N isotopic composition of NH_4^+ and NO_3^- using a Sercon SL Elemental Analyzer coupled to a 20–20 isotope-ratio mass spectrometer (IRMS) (Sercon Ltd., Crewe, UK).

^{15}N tracing model

The simultaneously occurring gross N transformation rates were quantified based on the full process-based N cycle models previously developed by Müller et al. (2007). In this study, ten N transformation rates were considered (Fig. S1): mineralization of recalcitrant organic N to NH_4^+ (M_{Nrec}), mineralization of labile organic N to NH_4^+ (M_{Nlab}), immobilization of NH_4^+ to labile organic N (I_{NH4_Nlab}); immobilization of NH_4^+ to recalcitrant organic N (I_{NH4_Nrec}), release of adsorbed NH_4^+ (R_{NH4ads}), adsorption of NH_4^+ on cation exchange sites (A_{NH4}), oxidation of NH_4^+ to NO_3^- (O_{NH4}, autotrophic nitrification), oxidation of recalcitrant organic N to NO_3^- (O_{Nrec}, heterotrophic nitrification), immobilization of NO_3^- to recalcitrant organic N (I_{NO3}), and dissimilatory NO_3^- reduction to NH_4^+ (D_{NO3}, *DNRA*). The model simultaneously optimized the kinetic parameters of various N transformation rates by minimizing the misfit between modeled and measured concentrations and ^{15}N enrichments of NH_4^+ and NO_3^-. The average N transformation rates were calculated over the entire period and expressed in units of mg N kg^{-1} day^{-1}. For detailed information on this ^{15}N transformation model see Müller et al. (2007).

Statistical analyses

The net NO_3^- production rate was calculated as the total NO_3^- production rate minus the total NO_3^- consumption rate following the equation:

$$\text{net } NO_3^- \textit{ production rate} = \left(O_{NH4} + O_{Norg}\right) - \left(I_{NO3} + DNRA\right) \quad (1)$$

$$\textit{gross mineralization rate } \left(M_{Norg}\right) = M_{Nrec} + M_{Nlab} \quad (2)$$

$$\textit{gross nitrification rate } (N) = O_{Norg} + O_{NH4} \quad (3)$$

$$\textit{immobilization rate of } NH_4^+ \textit{ to organic } N\ (I_{NH4}) = I_{NH4\_Nlab} + I_{NH4\_Nrec} \quad (4)$$

The mean residence time (MRT) indicates the turnover rate of a certain N pool, that is, a low value represents a fast

turnover (Corre et al. 2007). The mean residence time (d) of exchangeable NH_4^+ (MRT NH_4^+) and NO_3^- (MRT NO_3^-) was calculated as:

$$\text{MRT } NH_4^+ = \frac{c(NH_4^+)}{M_{Norg}}; \text{MRT } NO_3^- = \frac{c(NO_3^-)}{N} \quad (5)$$

where c (NH_4^+) and c (NO_3^-) is the initial exchangeable NH_4^+ and NO_3^- concentration (mg N kg^{-1}) in soil after 50 days incubation, respectively; M_{Norg} and N is soil gross mineralization and nitrification rate (mg N kg^{-1} day^{-1}), respectively.

N_2O and CO_2 fluxes were calculated according to the following equation (Liu et al. 2017):

$$F = \rho \times V \times \Delta C/\Delta t \times 273/(273 + T)/m \quad (6)$$

where F is the N_2O and CO_2 fluxes (μg N kg^{-1} day^{-1}, mg C kg^{-1} day^{-1}), ρ is the density of N_2O and CO_2 under the standard state (kg m^{-3}), V is the volume of the head space (m^3), $\Delta C/\Delta t$ is the change in gas concentration (c) per unit of time (t) (ppb N_2O-N day^{-1}, ppm CO_2-C day^{-1}), T is the ambient air temperature (°C) and m is soil dried weight (kg).

$$\textit{Cumulative } N_2O \textit{ or } CO_2\textit{production} = \sum_{i=1}^{n} \frac{(F_i + F_{i+1})}{2} \times (t_{i+1} - t_i) \quad (7)$$

where F is the N_2O or CO_2 flux, i is the ith measurement, the term (t_{i+1}−t_i) is the number of days between two measurements, and n is the total number of the measurements.

Due to the high number of iterations of the ^{15}N tracing model, statistical tests are inappropriate for the comparison of parameter results. We analyzed parameter results based on the comparisons of standard deviations and the 95% confidence intervals to distinguish three cases: (a) standard deviations overlap, the parameters are not different, (b) standard deviations do not overlap but 95% confidence intervals overlap, parameters are not significantly different but show a clear tendency to be different, and (c) 95% confidence intervals do not overlap, parameters are significantly different (Müller et al. 2009). Differences in soil properties, cumulative emissions of N_2O and CO_2, nematode abundances, bacterial 16S rRNA gene abundances, and archaeal and bacterial *amoA* gene abundances among three bacterivore treatments were estimated using analysis of variance (ANOVA) at $P < 0.05$ level. All data were analyzed using SAS 9.1 (SAS Institute, Cary, NC, USA).

Results

Soil chemical properties

After 50 days incubation, no significant differences in C/N and TOC, TN, exchangeable NH_4^+ and NO_3^- concentrations of soil were observed between treatments with and without bacterivores (Table 2), but bacterivores greatly decreased soil $\delta^{13}C_{\_TOC}$ and $\delta^{15}N_{\_TN}$ values, with the lowest in *P. oxyuroides* treatment than CK and *R. intermedia* ($P < 0.05$). Soil inorganic N was dominated by NO_3^- pool among three treatments, and the ratio of NO_3^- to NH_4^+ ranged from 12.9 (CK) to 18.4 (*P. oxyuroides*). A decreased trend in soil pH in the presence of bacterivores than CK was observed.

Soil N_2O and CO_2 emissions

The N_2O emission rates in soils of three treatments increased at the beginning of incubation, and reached the peak on day 20, with higher values (244 μg N kg^{-1} day^{-1}) for *P. oxyuroides* than *R. intermedia* (208 μg N kg^{-1} day^{-1}), and CK (167 μg N kg^{-1} day^{-1}) ($P < 0.05$) (Fig. 1a). Thereafter, the N_2O emission decreased rapidly, and showed smaller changes after 40 days incubation. During the entire incubation, the cumulative N_2O production was significantly higher for *P. oxyuroides* (4158 μg N kg^{-1}) than *R. intermedia* (3916 μg N kg^{-1}) and CK (3629 μg N kg^{-1}) ($P < 0.05$), but there was no difference between *R. intermedia* and CK ($P > 0.05$) (Fig. 1b).

The pattern of CO_2 evolution from soil was similar to that of N_2O fluxes (Fig. 2a). CO_2 emissions increased immediately, reaching a maximum for three treatments on day 25 (from 338 mg C kg^{-1} day^{-1} for CK to 412 mg C kg^{-1} day^{-1} for *P. oxyuroides*), and declined rapidly thereafter. Compared to

Table 2 Soil physical and chemical properties among three bacterivore treatments after 50 days incubation. (average ± SD, $n = 4$)

Soil properties	CK	Rha	Pro
TOC (g C kg^{-1})	13.6 ± 0.31a	13.3 ± 0.19a	13.5 ± 0.05a
$\delta^{13}C_{\_TOC}$ (‰)	−22.3 ± 0.38a	−24.8 ± 0.29b	−26.0 ± 0.35c
TN (g N kg^{-1})	1.50 ± 0.02a	1.48 ± 0.04a	1.53 ± 0.05a
$\delta^{15}N_{\_TN}$ (‰)	5.32 ± 0.40a	3.76 ± 0.32a	3.03 ± 0.27b
C/N	8.90 ± 0.13a	8.95 ± 0.08a	8.82 ± 0.07a
NH_4^+ (mg N kg^{-1})	4.53 ± 1.12a	3.40 ± 0.85a	3.19 ± 0.85a
NO_3^- (mg N kg^{-1})	58.6 ± 1.08a	59.1 ± 1.52a	58.8 ± 0.91a
pH	7.63 ± 0.02a	7.52 ± 0.08b	7.53 ± 0.04b

Different small letters in the same row indicated significant difference among different treatments at 0.05 level

CK sterilized soil and reinoculated by native microbial community without bacterivore; *Rha* sterilized soil and reinoculated by native microbial community with *Rhabditis intermedia*; *Pro* sterilized soil and reinoculated by native microbial community with *Protorhabditis oxyuroides*

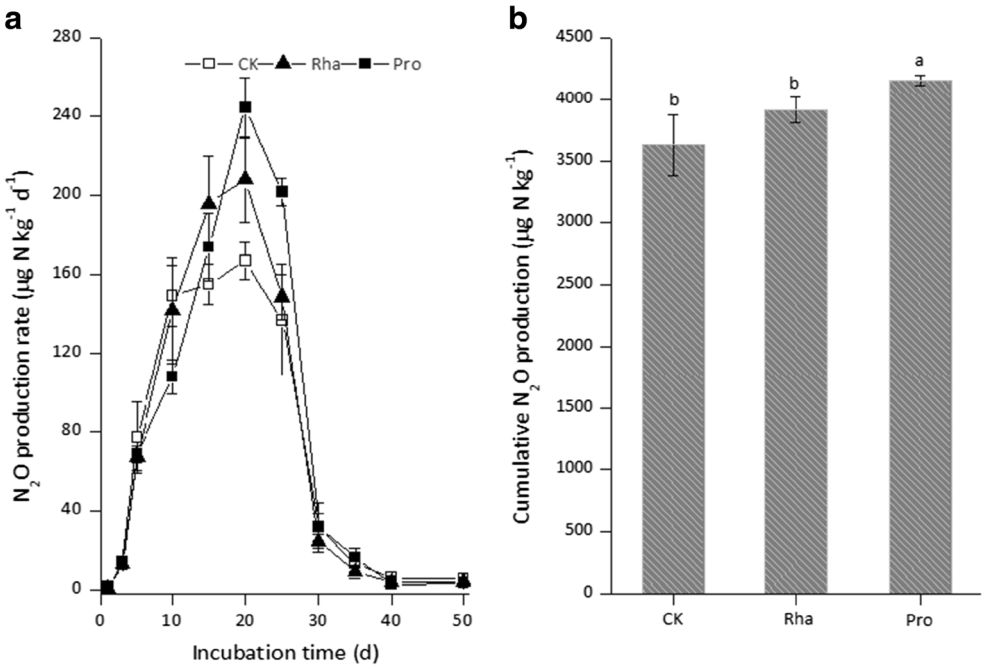

Fig. 1 The average emission rate (**a**) and cumulative production (**b**) of N_2O in soils inoculated by bacterivores during 50 day incubation. The different letters indicate significant difference among all treatments at $P < 0.05$. CK, sterilized soil and reinoculated by native microbial community without bacterivore; Rha, sterilized soil and reinoculated by native microbial community with *R. intermedia*; Pro, sterilized soil and reinoculated by native microbial community with *P. oxyuroides*

CK (6.91 g C kg^{-1} day^{-1}), *R. intermedia* (7.55 g C kg^{-1} day^{-1}) did not influence cumulative CO_2 production, while *P. oxyuroides* significantly increased CO_2 production to 7.87 g C kg^{-1} day^{-1} ($P < 0.05$) (Fig. 2b).

Gross N transformation rates

The measured ^{15}N atom% and concentrations of soil exchange NH_4^+ and NO_3^- in three treatments differentially labeled with $^{15}NH_4NO_3$ and $NH_4{}^{15}NO_3$ corresponded well with the modeled data obtained from the ^{15}N tracing model (Fig. S2 and Fig. S3), indicated that soil N transformation rates could be well modeled out using the ^{15}N tracing model (Müller et al. 2007).

Only *P. oxyuroides* (5.57 mg N kg^{-1} day^{-1}) increased significantly M_{Norg} (mineralization of organic N to NH_4^+) in comparison to CK (4.15 mg N kg^{-1} day^{-1}) ($P < 0.05$), while *R. intermedia* (4.70 mg N kg^{-1} day^{-1}) was not significant

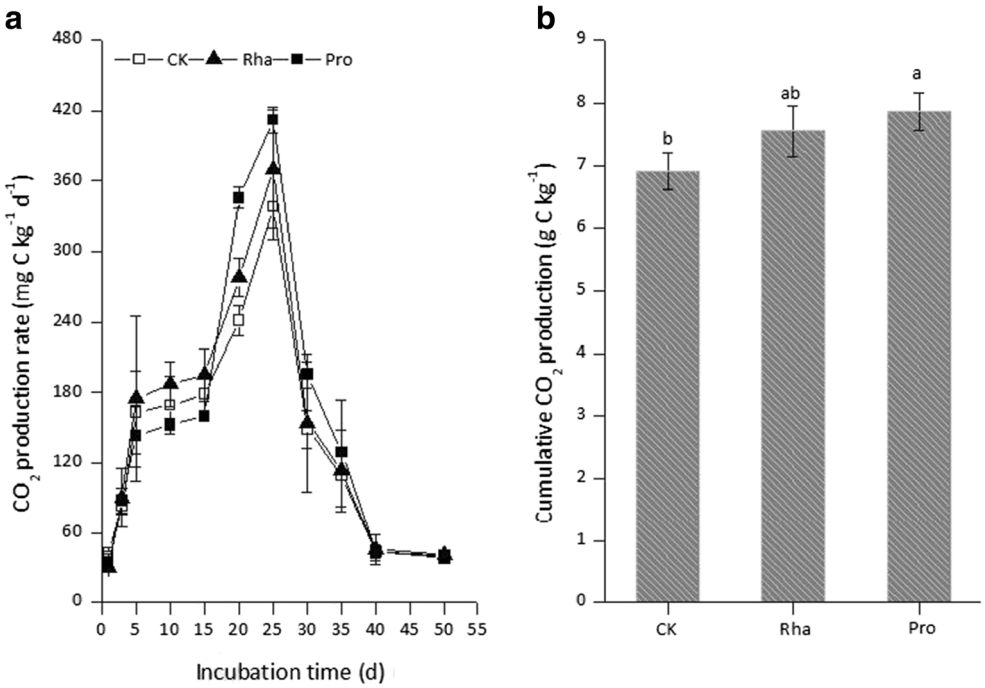

Fig. 2 The average emission rate (**a**) and cumulative production (**b**) of CO_2 in soils inoculated by bacterivores s during 50 days incubation. CK, sterilized soil and reinoculated by native microbial community without bacterivore; Rha, sterilized soil and reinoculated by native microbial community with *R. intermedia*; Pro, sterilized soil and reinoculated by native microbial community with *P. oxyuroides*

different to CK (Fig. 3). The mineralization rate of labile organic N (M_{Nlab}) ranged from 2.87 (CK) to 2.94 mg N kg^{-1} day^{-1} (*P. oxyuroides*), which contributed to approximately 52.8–69.0% of M_{Norg}. Compared to CK, bacterivores did not alter M_{Nlab} rate but significantly increased the mineralization rate of recalcitrant organic N (M_{Nrec}) ($P < 0.05$), with the highest value for *P. oxyuroides* (Fig. 4). There was no significant difference in the microbial NH_4^+ immobilization (I_{NH4}) rate between CK (4.53 mg N kg^{-1} day^{-1}) and *R. intermedia* (4.39 mg N kg^{-1} day^{-1}), but this rate greatly increased to 5.52 mg N kg^{-1} day^{-1} for *P. oxyuroides* ($P < 0.05$). The MRT of NH_4^+ pools for CK was 1.08 days, which was significantly higher than those for *R. intermedia* (0.72 days) and *P. oxyuroides* (0.57 days) ($P < 0.05$); the latter two treatments were also statistically different ($P < 0.05$, Fig. 5a).

Nitrate production was dominated by autotrophic nitrification (O_{NH4}) in studied soils where the heterotrophic nitrification rate (O_{Nrec}) was almost zero (Fig. 3). In contrast to CK (8.18 and 0.32 mg N kg^{-1} day^{-1}), *R. intermedia* did not significantly affect soil O_{NH4} and dissimilatory NO_3^- reduction to NH_4^+ (*DNRA*), but *P. oxyuroides* greatly increased those rates to 10.3 and 0.69 mg N kg^{-1} day^{-1}, respectively ($P < 0.05$). Soil immobilization rate of NO_3^- to recalcitrant organic N rate (I_{NO3}) ranged from 0.08 mg N kg^{-1} day^{-1} for CK and *P. oxyuroides* to 0.28 mg N kg^{-1} day^{-1} for *R. intermedia*, which were significantly lower than I_{NH4} ($P < 0.01$). The highest net NO_3^- production rate was found for *P. oxyuroides* (9.58 mg N kg^{-1} day^{-1}) than *R. intermedia* (7.54 mg N kg^{-1} day^{-1}) and CK (7.78 mg N kg^{-1} day^{-1}) ($P < 0.05$). Compared to CK (7.16 days), only *P. oxyuroides* significantly decreased the MRT of NO_3^- pools to 5.68 days ($P < 0.05$, Fig. 5b).

Abundances of bacterivore, bacteria, AOA, and AOB in soils

After 50 days incubation, no nematode was detected for CK. Compared to the initial inoculum number of bacterivore (20 ind g^{-1} dry soil), the abundance of *R. intermedia* declined

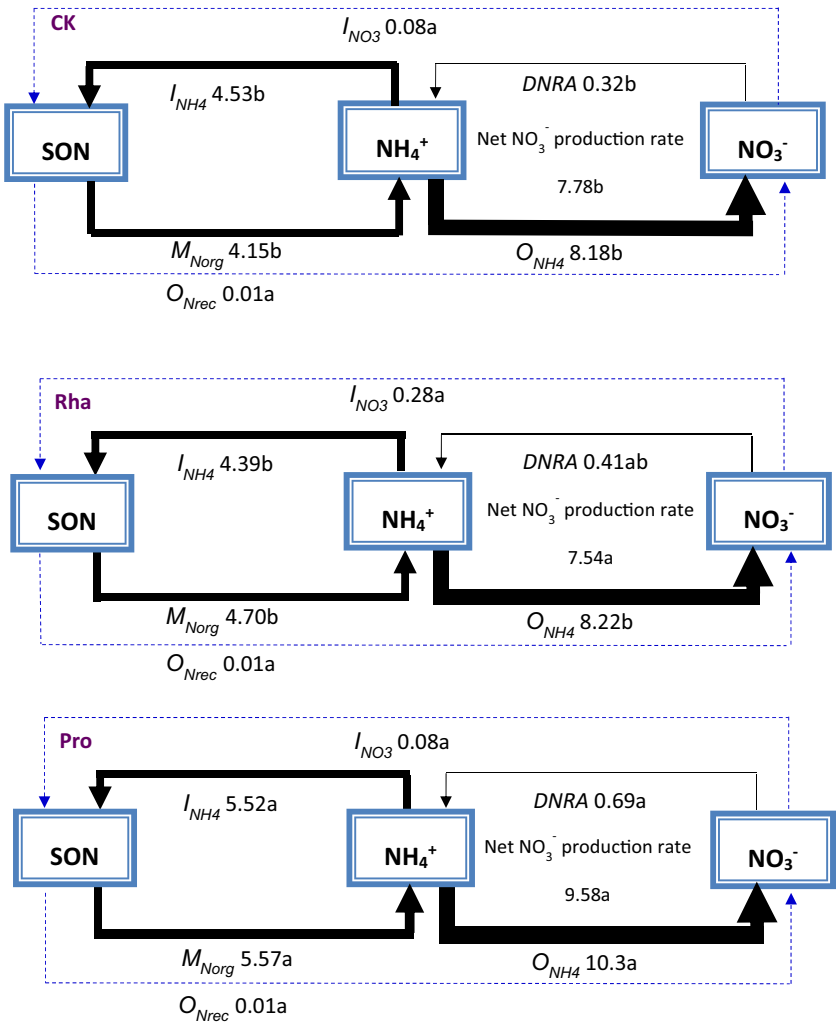

Fig. 3 Effect of bacterivores on soil N transformation rates (mg N kg^{-1} day^{-1}) after 50 days incubation ($n = 4$, ± SD). The arrow width *s* represents the size of the corresponding gross N transformation rate. For the same N transformation rate in different treatments, different letters indicate significant differences among three treatments at $P = 0.05$. M_{Norg} is mineralization of organic N to NH_4^+, I_{NH4} is immobilization of NH_4^+ to organic N, O_{NH4} is oxidation of NH_4^+ to NO_3^-, O_{Nrec} is oxidation of recalcitrant organic N to NO_3^-, I_{NO3} is immobilization of NO_3^- to recalcitrant organic N, and *DNRA* is dissimilatory NO_3^- reduction to NH_4^+. Different small letters for the same N transformation rate indicated significant difference among different treatments at 0.05 level

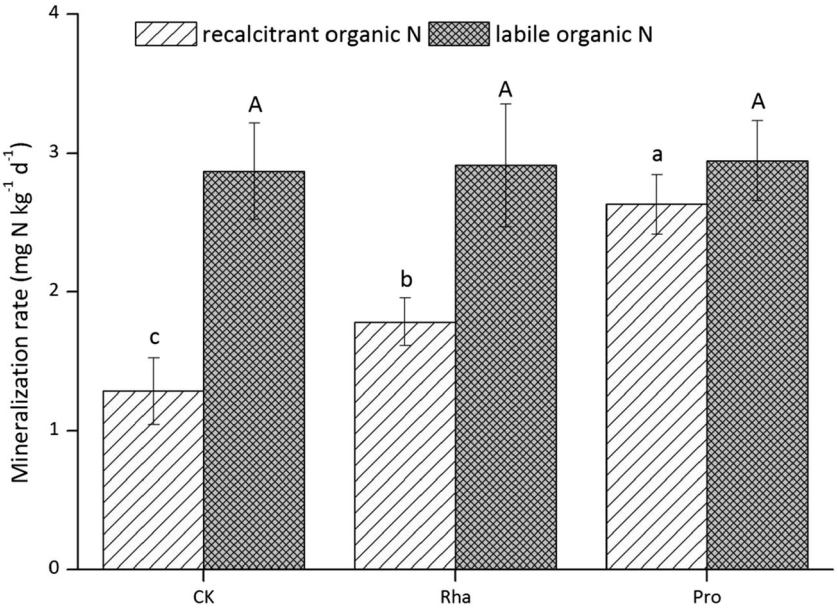

Fig. 4 Effect of bacterivores on the mineralization of recalcitrant organic N and labile organic N after 50 days incubation. Different letters for the mineralization of recalcitrant organic N (lower case) or labile organic N (upper case) indicated significant difference among different treatments at 0.05 level

to 2.97 ind g^{-1} dry soil, while the abundance of *P. oxyuroides* was significantly increased to 49.4 ind g^{-1} dry soil (Table 3).

There were no significant differences in the copy numbers of bacterial 16S rRNA genes among three treatments ranging from 1.26×10^{11} copies g^{-1} dry soil for CK to 1.41×10^{11} copies g^{-1} dry soil for *R. intermedia* ($P < 0.05$, Table 3). The higher AOB population size was detected for CK (1.25×10^7 *amoA* gene copies g^{-1} dry soil) than *R. intermedia* (9.00×10^6 *amoA* gene copies g^{-1} dry soil) and *P. oxyuroides* (7.83×10^6 *amoA* gene copies g^{-1} dry soil) ($P < 0.05$). Oppositely, AOA population sizes for *R. intermedia* (1.70×10^7 *amoA* gene copies g^{-1} dry soil) and *P. oxyuroides* (1.44×10^7 *amoA* gene copies g^{-1} dry soil) treatments were greatly higher than that for CK (6.12×10^6 *amoA* gene copies g^{-1} dry soil) ($P < 0.05$). This resulted in higher AOA to AOB ratio for *R. intermedia* (1.89) and *P. oxyuroides* (1.83) than CK (0.49) ($P < 0.05$).

Discussion

After 50 days incubation, there were no differences in soil NH_4^+ or NO_3^- concentrations between in the presence or absence of bacterivores; however, this was not the case for soil gross N transformation rates. In this study, O_{Nrec} and I_{NO3} rates were very low and not affected by bacterivores. Bacterivores influenced the rates of M_{Norg}, I_{NH4}, O_{NH4}, and *DNRA*, but the effect varied between *R. intermedia* and *P. oxyuroides*. In comparison to CK, only *P. oxyuroides*

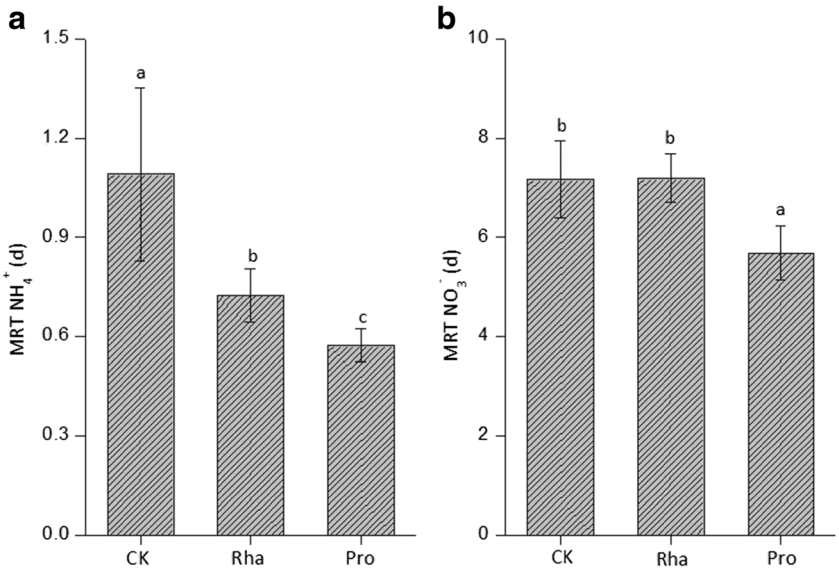

Fig. 5 Effect of bacterivores on the mean residence time of soil NH_4^+ pool (MRT NH_4^+, **a**) and NO_3^- pool (MRT NO_3^-, **b**) after 50 days incubation. Different letters for MRT NH_4^+ or MRT NO_3^- indicated significant difference among different treatments at 0.05 level

Table 3 Bacterial-feeding nematodes numbers, bacterial 16S rRNA gene abundance, and archaeal and bacterial *amoA* gene abundance after 50 days incubation. (avg ± SD, $n = 4$)

	CK	Rha	Pro
Nematode numbers (ind g^{-1} dry soil)	0 ± 0c	2.97 ± 1.10b	49.4 ± 8.20a
Bacterial 16S rRNA gene abundance $\times 10^{11}$ gene copies g^{-1} dry soil	1.26 ± 0.17a	1.41 ± 0.24a	1.39 ± 0.16a
AOB abundance $\times 10^6$ *amoA* gene copies g^{-1} dry soil	12.5 ± 1.73a	9.00 ± 0.86b	7.83 ± 1.28b
AOA abundance $\times 10^6$ *amoA* gene copies g^{-1} dry soil	6.12 ± 0.51b	17.0 ± 2.66a	14.4 ± 3.17a

Different small letters in the same row indicated significant difference among different treatments at 0.05 level

CK sterilized soil and reinoculated by native microbial community without bacterivore; *Rha* sterilized soil and reinoculated by native microbial community with *Rhabditis intermedia*; *Pro* sterilized soil and reinoculated by native microbial community with *Protorhabditis oxyuroides*

increased M_{Norg}, *DNRA*, O_{NH4}, and I_{NH4} rates, while *R. intermedia* did not affect these rates. Due to the simultaneously increased production and consumption rates of NH_4^+ and NO_3^- pools, *P. oxyuroides* lowered the mean residence times of NH_4^+ (MRT NH_4^+) and NO_3^- (MRT NO_3^-), suggesting that *P. oxyuroides* could promote soil inorganic N turnover. These results support our hypothesis 1 that bacterivores have greater effects on soil gross N transformation rates, even no significant difference in the net change of inorganic N is observed between in the presence and absence of bacterivores.

We also found the effects of bacterivores varied with bacterivore species even with the same c-p value (c-p 1) for the two nematodes, supporting the hypothesis 2. In contrast to *P. oxyuroides*, the body sizes (e.g., length, width, and weight) of *R. intermedia* were bigger and it has relatively slow movement and growth rate (personal observation). Besides the factor of species identity, *R. intermedia* cannot inhabit the soil over time probably due to unfavorable food resource and environment. Consequently its abundance decreased from initially 20 ind g^{-1} dry soil to finally 2.97 ind g^{-1} dry soil at the end of 50 days incubation, significantly lower than *P. oxyuroides* abundance (49.4 ind g^{-1} dry soil). This generally conforms with the pattern of the studied nematode abundance of initial soil before incubation experiment (i.e., the dominant *P. oxyuroides* make up 59% and *R. intermedia* 6% of bacterivore nematodes). Although this decrease in number of *R. intermedia* would definitely weaken the effect on soil bacteria involved in N transformation (Postma-Blaauw et al. 2005; Gebremikael et al. 2014), it indeed reflected the possible situation in the real world where different species trait were closely linked with distinct density and functions. To the best of our knowledge, the present study is the first to quantify the role of bacterivores, particularly provides evidence that the traits-dependent functional performance of different nematode species within the same c-p category, in soil N transformation processes involved in the production and consumption of inorganic N.

Noticeably, a relatively higher net NO_3^- production rate was observed in soil reinoculated by *P. oxyuroides* than by *R. intermedia* and CK, indicating that the relatively much more NO_3^- could be produced. However, this was not in agreement with soil apparent NO_3^- concentration which showed no difference among treatments after 50 days incubation. Except for the enhanced consumption of produced NO_3^-, NH_4^+ concentration is also important factor affecting NO_3^- production in soil. Undoubtedly, increased I_{NH4} in the presence of *P. oxyuroides* could lower NO_3^- production through competing with O_{NH4} for NH_4^+ pool, even O_{NH4} was kept at the relatively high level. In addition, the increased loss of NO_3^- in form of nitrogenous gas emissions (e.g., NO, N_2O, and N_2) by bacterivores may be another mechanism responsible for no difference in NO_3^- concentration among three treatments (Kuiper et al. 2013; Hiltpold et al. 2016). Indeed, *P. oxyuroides* increased the cumulative N_2O emission by 14.6% than CK. Under aerobic condition, nitrification process was able to produce more NO than N_2O (Zhu et al. 2013), meaning NO emission might be also an important component for inorganic N loss in this study. To accurately quantify the N loss caused by bacterivores, all components of nitrogenous gases should be determined, but knowledge about the crucial roles of bacterivores in nitrogenous gas emissions is very limited and only few studies have considered this aspect, frequently focusing on the greenhouse gas (i.e., N_2O) (Kuiper et al. 2013).

After 50 days incubation, the concentrations and natural abundances (i.e., $\delta^{13}C$ and $\delta^{15}N$) of total organic C and total N before measuring gross N transformation rate showed different response to bacterivores. The bacterivores did not affect total organic C and total N concentrations, but decreased $\delta^{13}C_{-TOC}$ and $\delta^{15}N_{-TN}$ values, which may be attributed to the altered microbial utilization of C and N sources with different $\delta^{13}C$ and $\delta^{15}N$ values driven by bacterivores (Anderson et al. 1981; Moens et al. 2002). In this study, the mineralization rate of labile organic N to NH_4^+ (2.87–2.94 mg N kg^{-1} day^{-1}) was similar among three treatments, but bacterivores increased the mineralization rate of recalcitrant organic N to NH_4^+ (*R. intermedia*, 1.78 mg N kg^{-1} day^{-1}; *P. oxyuroides*, 2.63 mg N kg^{-1} day^{-1}) in comparison to CK (1.29 mg N kg^{-1} day^{-1}). Characterized by slow turnover and big pool size, recalcitrant organic matter had greater $\delta^{15}N$ and $\delta^{13}C$ values due to humification and the loss of the lighter ^{12}C via respiration (Kramer et al. 2003; Rusalimova and Barsukov 2006). Therefore, once bacterivores accelerated the decomposition of recalcitrant organic matter via promoting soil microbial activity or

modulating microbial community composition, soil $\delta^{13}C_{\text{-TOC}}$ and $\delta^{15}N_{\text{-TN}}$ values would decline. Selective utilization of organic fractions by bacterivores and their interaction with microbes has been documented before (Griffiths 1994; Moens et al. 2002; Irshad et al. 2011), but the relative mechanisms whereby organic matter sources are utilized remains poorly understood.

The present study showed no significant effect of bacterivorous nematodes on soil bacterial abundance, which is contrary to previous studies (Ferris et al. 1998; Griffiths 1994; Xiao et al. 2014). Fu et al. (2005) considered bacteria-to-nematode ratio (i.e., density effect) to greatly influence the bacteria growth and number. The relatively low bacterivore grazing intensity by *R. intermedia* might not effectively change bacteria numbers, while the high grazing intensity caused by *P. oxyuroides* might offset the stimulatory effects on bacteria numbers in our study, despite it was well-known that bacterivore grazing usually increased microbial abundance and activity (Chen et al. 2007; Trap et al. 2016). As the specific functional group of microbial community, the dominate ammonia oxidizers being responsible for autotrophic nitrification were AOB rather than AOA in neutral to high pH habitat (Zhang et al. 2012b). Thus, the effect of bacterivores on autotrophic nitrification might be primarily through the alteration in AOB in the studied soil. In contrast to the increase in autotrophic nitrification; however, the decrease in AOB abundance but increase in AOA abundance was observed in the presence of bacterivores, suggesting AOA may contribute to more in nitrification process under the grazing pressure of bacterivore. Previous studies found the changes in community composition of bacteria, AOA and AOB caused by bacterivores could affect nitrification process in soil (Xiao et al. 2010; Jiang et al. 2014). But there is a lack of direct evidence for this mechanism, highlighting more work on the interactions between bacterivore and targeted microbial groups, a critical but neglected area.

Conclusion and outlook

The two bacterivorous nematodes characterized different body size but belonging to same c-*p* value did not affect soil inorganic N concentrations, but showed different roles in N transformation process. *P. oxyuroides* simultaneously increased the production (i.e., M_{Norg}, *DNRA*) and consumption (i.e., O_{NH4}, I_{NH4}) rates of NH_4^+ as well as the increased production (i.e., O_{NH4}) and consumption (i.e., *DNRA*) rates of NO_3^-, which enhanced the turnover rate of NH_4^+ and NO_3^- pools, leading to a negligible response in the net inorganic N rates. In contrast, *R. intermedia* did not affect all these N transformation rates, consistent with its fewer numbers and larger body size as well as poor survival rate in the studied soil. Interestingly, bacterivores, particularly for *P. oxyuroides*, significantly increased the mineralization rate of recalcitrant organic N but decreased $\delta^{13}C_{\text{-TOC}}$ and $\delta^{15}N_{\text{-TN}}$ values, implicating that bacterivores might stimulate selective use of recalcitrant organic N through the effects on composition and activity of soil microbial communities. Noticeably, *P. oxyuroides* also significantly stimulated soil N_2O and CO_2 emissions, which should draw more attention in the future studies in terms of greenhouse gas emissions.

We acknowledge the current experimental design with limited number of species, few quantitative traits measured and particularly missing the nematode density manipulation, is not capable of disentangling the effects of interdependent variables such as species identify and the final abundance (and therefore the growth rate). With the increasing interest of the roles of soil food web in mediating biogeochemical cycling, a trait-based approach that exhaustively considers the functional traits of soil invertebrates would be promising (Moretti et al. 2017). It should be noted that future work that integrating realistic condition such as soil food web, and environment heterogeneity, plant growth and functional traits across multiple biological and spatio-temporal scales, would pave the way to mechanistic understanding the N cycling in terrestrial ecosystem (Nannipieri et al. 2003; Nielsen et al. 2015).

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