Journal of Hazardous Materials 192 (2011) 1243–1249

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Combined effects of bacterial-feeding nematodes and prometryne on the soil microbial activity

Jihai Zhou [a,b], Xuechao Li [a], Ying Jiang [a], Yue Wu [a], Jiandong Chen [a], Feng Hu [a], Huixin Li [a,*]

[a] College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, Jiangsu Province 210095, China
[b] Department of Soil and Crop Sciences, Colorado State University, Fort Collins, CO 80523, USA

ARTICLE INFO

Article history:
Received 23 December 2010
Received in revised form 10 June 2011
Accepted 14 June 2011
Available online 25 June 2011

Keywords:
Bacterial-feeding nematodes
Indigenous microbes
Degradation
Prometryne
Soil microbial activity

ABSTRACT

Microcosm experiments were carried out to study the effects of bacterial-feeding nematodes and indigenous microbes and their interactions on the degradation of prometryne and soil microbial activity in contaminated soil. The results showed that soil indigenous microbes could degrade prometryne up to 59.6–67.9%; bacterial-feeding nematodes accelerated the degradation of prometryne in contaminated soil, and prometryne degradation was raised by 8.36–10.69%. Soil microbial biomass C (C_{mic}), basal soil respiration (BSR), and respiratory quotient (qCO_2) increased in the beginning of the experiment and decreased in the later stage of the experiment. Nematodes grew and reproduced quite fast, and did increase the growth of soil microbes and enhance soil microbial activity in prometryne contaminated soil during the incubation period.

1. Introduction

Most agricultural soil in Asia and Europe are treated with herbicides at least once a year [1]. There is increasing concern that herbicides not only affect the target organisms (weeds) but also the microbial community present in soil [2–5], and that these non-target effects may degrade the performance of important soil functions. It is generally accepted that xenobiotic chemicals in natural environments are degraded by multiple microbial species [6–8], so bioaugmentation is an important process for the removal of pesticides [9,10].

Prometryne [2,4-bis (isopropylamino)-6-(methylthio)-s-triazine], a selective herbicide of the s-triazine chemical family, has been extensively used as a pre- or post-emergence controller of annual grasses and broadleaf weeds in modern agriculture. Based on the classification scheme [11], the *Koc* value of prometryne is within 311–614, which indicates that prometryne is expected to have moderate to low mobility in soil and may be adsorbed to solids and suspended sediments in water. Prometryne is a ubiquitous environmental pollutant in water and soil, and it is frequently detected in groundwater, surface water, and even breast milk [12,13]. It is banned in Europe, but still widely used in China. Therefore, there is an urgent need to remediate prometryne contaminated soil and find out how greatly prometryne affects the microbial community.

As is known, nematodes are the most abundant metazoans in soil, with densities of 7.6×10^5 m^{-2} in a desert to 2.9×10^7 m^{-2} in a mixed deciduous forest [14]. Generally, 20–50% of nematodes present in soil are bacterial-feeders, and the percentage reaches 90–99% at sites of high microbial activity [15,16]. Although the number of soil nematodes is large, their direct contribution to organic matter mineralization has been estimated to be negligible, probably less than 1% of the total soil respiration [17]. However, nematodes have been found to play an important role in the decomposition of soil organic matter and the release of nutrients through their interaction with soil microbes [18], because microbivorous nematodes have been shown to stimulate microbial growth and turnover [18,19], thus increasing the turnover of soil organic matter [15,20]. Consequently, it was recognized that these circumstances may also be applicable to agroecosystems [21].

Therefore, we hypothesized bacterial-feeding nematodes can also stimulate the microbial growth and activity and change the community of indigenous microbes in contaminated soil so that the contaminants could be degraded much faster. The objectives of this study were to investigate potential effects of bacterial-feeding nematodes on the degradation of prometryne in soil, as well as the responses of soil microbial activity to bacterial-feeding nematodes and prometryne.

* Corresponding author. Tel.: +86 25 84395374; fax: +86 25 84395210.
E-mail addresses: 2007203014@njau.edu.cn (J. Zhou), huixinli@njau.edu.cn (H. Li).

0304-3894/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2011.06.035

2. Materials and methods

2.1. Experiment design

The soil used in this experiment was a sandy-loam alluvial soil collected from Banqiao town, Nanjing City, China. The soil was composed of 56.5% sand, 26.1% silt, and 17.4% clay, and contained 10.87 g kg^{-1} of organic C, 0.89 g kg^{-1} of total N, 5.42 g kg^{-1} NH_4^+-N, and 27.17 g kg^{-1} NO_3^--N and the pH (H_2O) was 6.43. Prior to usage, fresh soil was passed through a 2 mm mesh to remove stones, macrofauna and discernible plant materials. Some portions of the sieved soil were heated using ^{60}Co ray (by Jiangsu Academy of Agricultural Science, China) to kill microbes and nematodes. Soil dry weight was measured by drying soil at 105 °C overnight. WHC was measured on soil samples water-saturated in a funnel and left to stand overnight.

Ninety-six (96) sub-samples of equivalent to 110 g oven dry soil were added to 250 mL glass flasks. Twelve flasks each were used for the two controls (Control 1 and Control 2) and six treatments (Table 1). The soil was first mixed and adjusted to 60% of the maximum water holding capacity (WHC) by being added sterilized distilled water, then contaminated by prometryne (purity: 97.3%; Anhui Huaxing Chemical Industry Co., Ltd., China) with different concentrations and inoculated nematodes under sterile conditions (according to the experimental design, Table 1). After the addition of prometryne dissolved in acetone, the same amount of acetone was also added into Control 2, and the soil was aired in a fume cupboard for 3 h to let acetone volatilize completely. The flasks were sealed and stored in the incubator in the dark for 30 days at 22 ± 2 °C. Twice a week, sterilized distilled water was added to the flasks to keep the soil moisture at 60% of WHC. After 0, 8, 18 and 30 days, three flasks were selected randomly from each treatment and control, and acted as triplicates. The soil was analyzed for the concentration of prometryne, the number of nematodes, soil microbial biomass C and basal soil respiration.

2.2. Bacterial-feeding nematode

The nematode was extracted from a contaminated soil sampled from Anhui Huaxing Chemical Industry Co., Ltd. (Anhui, China). It was identified as *Cephalobus* Bastian [22], a bacterial-feeding nematode. The nematodes were reared in the lab for 4 weeks on nematode growth medium agar plates (NGM) seeded with prometryne and *E. coli* strain OP50 as a food source [23]. The nematodes were transferred after 4 weeks from the NGM plates with 10 mg L^{-1} prometryne to ones with 20 mg L^{-1}, 40 mg L^{-1} or 80 mg L^{-1} prometryne.

Before the nematodes were inoculated into the soil, they were treated by surface disinfection with a mixture of 1.0 g L^{-1} streptomycin sulfate and 0.02 g L^{-1} cyclonheximide for 20 min and then centrifuged (3000 × *g*) for 3 min, after which the supernatant was discarded. The nematodes were then washed 5–6 times with sterile water to minimize the interference from bacteria during the transfer into the soil [24].

2.3. Soil characteristics and preparation for nematode-free soil

Nematode-free soil was prepared with freeze-thawing cycles method [25], in order to maintain soil microbial activity and microbial community structure. The process is as follows: first, adjust soil moisture to 60% of WHC, then incubate soil at 22 °C (the optimal temperature for growth of nematodes) for 7 days, after that kill nematodes by freezing the soil at −26 °C for 48 h. Next, incubate soil at 22 °C for 7 days in order to incubate nematode eggs that have not been killed during the freezing period. Then, nematodes were extracted from 100 g soil when the incubation was over using the sugar centrifugation method, as is described in Freckman and Virginia [26]. Repeat this freeze-thawing cycle for several times, until no nematodes were extracted from soil.

2.4. Measurements

2.4.1. Extraction and determination of prometryne in soil

Extraction and determination of prometryne in soil were done using the method of cloud point extraction and HPLC developed by Zhou et al. [27]. A 2.00 ± 0.02 g soil sample was sonicated in the presence of 10 mL of the mixture of 0.5% Triton X-114 solution (Sigma, USA) at 20 °C for 30 min. The liquid phase was transferred into a centrifugal vial through a paper filter. The residual was re-extracted with another 10 mL of the above mixture, and the supernatant was isolated and mixed with the fraction obtained from the first extraction, and then incubated in the thermostatic bath by adding 6.0 g of NaCl at 50 °C for 30 min. The phase separation was then accelerated by centrifugation at 4000 rpm for 5 min. After the water phase was removed, a surfactant-rich phase stuck to the bottom of the tube was obtained. Prometryne and most of the surfactant were removed from the surfactant-rich phase by precipitation with 200 μL of methanol–water (90:10, v/v), vortex-mixed and centrifuged at 16,000 rpm for 5 min. The upper layer solution (20 μL) was injected into the HPLC system for analysis.

2.4.2. Nematode extraction

Nematodes were extracted from 30 g fresh soil with a modified Baermann method using trays instead of funnels [28]. That is, mount trays with wire-mesh basket on basins, and then place about 30 g soil on top of a two-layered tissue paper (arranged in crisscross manner) on top of the wire screen. Spread soil subsample evenly on the tissue, and fill basins with water so that water level is about 1 mm above wire-mesh. Do not let water and soil lose contact during extraction period (add water as needed). The nematodes move through the tissue and the screen into the water in the basins, and they get settled at the bottom of the basins by gravity. After 48 h of extraction at room temperature (22–25 °C), the supernatant was decanted onto a 30 μm sieve, from which the nematodes were collected by backwashing into a counting dish, and the number of nematodes was counted under a dissecting microscope.

2.4.3. Microbial biomass C

Soil microbial biomass is regarded as a sensitive indicator of environmental changes [29]. Microbial biomass C was estimated with the chloroform fumigation extraction method [30]. Two portions equivalent to 25 g oven dry soil were taken from each soil sample. One portion was fumigated for 24 h at 25 °C with ethanol free $CHCl_3$. After fumigant removal, the soil was extracted with 100 mL 0.5 M K_2SO_4 by horizontal shaking for 1 h at 200 rpm and then filtered. The other non-fumigated portion was extracted simultaneously at the time when fumigation commenced. Organic C in the extracts was measured using the dichromate oxidation method. Microbial biomass C was calculated as follows: microbial biomass C = EC/k_{EC}, where EC = (organic C extracted from fumigated soils) − (organic C extracted from non-fumigated soils) and k_{EC} = 0.38 [30].

2.4.4. Basal soil respiration (BSR) and the respiratory quotient (qCO_2)

Soil respiration is one of the most frequently used parameters for quantifying microbial activities in soil [31] and has been used most frequently for the assessment of the side effects of chemicals such as pesticides and heavy metals [32–34]. Basal soil respiration (BSR) was determined as the rate of evolution of CO_2 from soil according to Anderson [35]. In brief, 20 g of soil sub-samples were placed into a sealed wide-mouth 750 mL glass-jar and CO_2 was trapped

Table 1
The treatments of the experiment.

Codes of the treatments	Soil	Prometryne concentration (mg kg^{-1} dry soil)	Nematode (individuals g^{-1} dry soil)
Control 1	Sterilized soil	10	0
Control 2	Unsterilized soil	0	0
P5	Unsterilized soil	5	0
P5N5	Unsterilized soil	5	5
P5N10	Unsterilized soil	5	10
P10	Unsterilized soil	10	0
P10N5	Unsterilized soil	10	5
P10N10	Unsterilized soil	10	10

in 0.05 M NaOH. Jars were incubated for 24 h at room temperature (22 °C). The NaOH solution was treated with excess 1.5 M $BaCl_2$ and titrated with a 0.025 M HCl. Phenolphthalein was used as the indicator of the titration end point. The quantity of CO_2-C evolved was reported as mg CO_2 g^{-1} dry soil d^{-1}.

Respiratory quotient (qCO_2) has been used as a bioindicator of environmental stress on microbial communities [31], disturbance and ecosystem development [36]. The qCO_2 was calculated according to the basal soil respiration rate and the amount of microbial biomass C using the formula of Anderson and Domsch [31]. Additionally, the study of the relationship between microbial biomass C (C_{mic}) and the respiratory quotient (qCO_2) can provide an understanding of the biological and chemical changes that occur under different agricultural practices [37]. The respiratory quotient (qCO_2) indicates how efficiently the microbial biomass is utilizing available C for biosynthesis and is considered as a sensitive indicator for estimating biological activity and substrate quality [36]. The qCO_2 is a measure of the specific metabolic activity that varies according to the composition and physiological state of the microbial biomass, the availability of nutrients and various abiotic factors. The qCO_2 results were expressed as μg CO_2–C mg^{-1} C_{mic} h^{-1}. It expresses the relation between the activity (basal respiration) and the carbon content of the microbial biomass, and allows the evaluation of the effects of external disturbances.

2.5. Statistical analysis

All statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Repeated measures analysis of variance (PROC GLM, SAS Release 9.1, SAS Institute, Cary, NC) was performed to obtain p values for the microcosm experimental design using the appropriate error terms in the model, to take into account treatments and treatment × time interaction (Table 2). Multi-comparison of least significant difference (LSD) was conducted for all measured variables between treatments or sampling times. All significant differences were at 0.05 level. The data presented were the means of triplicates ± standard errors.

3. Results and discussion

3.1. Dynamic of prometryne and nematodes in soil

During the incubation period, the prometryne concentration decreased significantly over time in all treatments (LSD test, $p < 0.05$) (Fig. 1). The prometryne concentration decreased from an initial level of ~5 mg kg^{-1} dry soil to a final level of 1.49, 1.53, and 2.02 mg kg^{-1} dry soil (that is, decreased by ca. 70.2, 69.4, and 59.6%) for P5N5, P5N10, and P5, respectively, and an initial level of ~10 mg kg^{-1} dry soil to a final level of 2.37, 2.38, and 3.21 mg kg^{-1} dry soil (that is, decreased by ca. 76.3, 76.2, and 67.9%) for P10N5, P10N10, and P10, respectively (Fig. 1). As is seen in Fig. 1, the degradation of prometryne continued until about Day 18, at which point there was a much slower rate of prometryne disappearance. No significant degradation was seen in Control 1 due to the lack of soil microbes. The rapid prometryne degradation in the treatments without nematodes indicated that indigenous microbes had huge potential for degrading prometryne. Compared with the treatments without nematodes, the degradation of prometryne in the treatments with nematodes increased by 8.36–10.69%, which suggested nematode grazing on indigenous microbes could enhance the degradation of prometryne (LSD test, $p < 0.05$). The degradation of prometryne in the treatments with higher density of nematodes was not different from that in the treatments with lower density of nematodes (LSD test, $p > 0.05$), which indicated the density-dependent regulation effect of nematode grazing was not significant in this study.

Nematodes increased 1.13–1.66 times from Day 0 through Day 8 and increased 2.48–6.97 times from Day 8 through Day 18 (Fig. 2), which was probably determined by nematode's adaptation and the recovery of the activity of nematodes in soil. There were enough food resources for supporting nematode growth and reproduction due to an increase in microbes during Day 0 through Day 8 (Fig. 3). The increase rate of nematodes at Day 18 was 8.99, 7.83, 4.96,

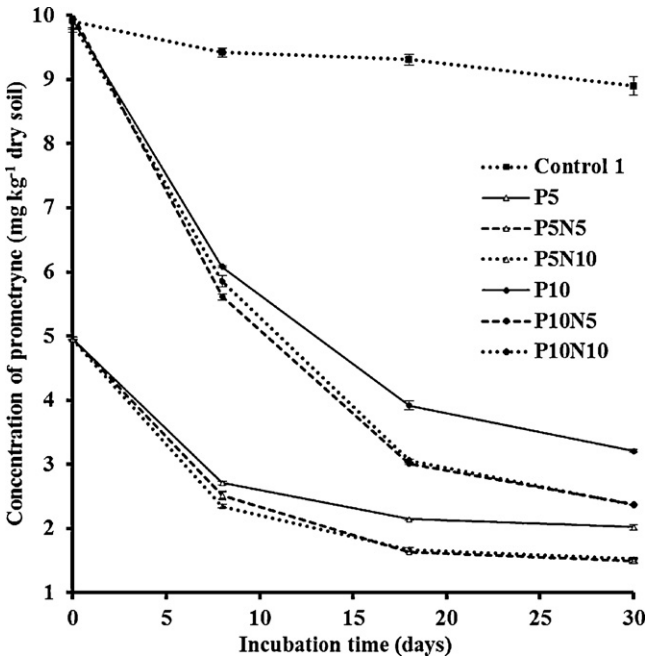

Fig. 1. The concentration of prometryne in soil for seven treatments contaminated with prometryne, Control 1, P5, P5N5, P5N10, P10, P10N5, and P10N10. Treatment: Control 1 (sterilized soil + 10 mg prometryne kg^{-1} dry soil), Control 2 (unsterilized soil), P5 (unsterilized soil + 5 mg prometryne kg^{-1} dry soil), P5N5 (unsterilized soil + 5 mg prometryne kg^{-1} dry soil + 5 individuals nematodes g^{-1} dry soil), P5N10 (unsterilized soil + 5 mg prometryne kg^{-1} dry soil + 10 individuals nematodes g^{-1} dry soil), P10 (unsterilized soil + 10 mg prometryne kg^{-1} dry soil), P10N5 (unsterilized soil + 10 mg prometryne kg^{-1} dry soil + 5 individuals nematodes g^{-1} dry soil), P10N10 (unsterilized soil + 10 mg prometryne kg^{-1} dry soil + 10 individuals nematodes g^{-1} dry soil). The data presented were the mean of triplicates ± standard errors (LSD test, $p < 0.05$). Error bars represented the standard error of the mean ($n = 3$). A few error bars were smaller than symbols.

Table 2
Analysis of variance of prometryne concentration, the number of nematodes, basal soil respiration (BSR), microbial mass C (C_{mic}), and respiratory quotient (qCO_2) with degree of freedom (df), *F* values and treatment significance levels.

Variable	Prometryne concentration		The number of nematodes		SBR		C_{mic}		qCO_2	
	df	*F*-Value	df	*F*-Value	df	*F*-Value	df	*F*-Value	df	*F*-Value
Treatment	6	4194.21****	3	230.65****	6	68.80****	6	20.95****	6	31.98****
Replicate	2	1.51	2	0.10	2	0.59	2	1.28	2	0.95
Treatment × replicate	12	1.50	6	0.76	12	1.21	12	0.66	12	1.28
Day	3	5604.83****	3	1003.78****	3	213.45****	3	308.02****	3	113.28****
Treatment × day	18	258.46****	9	71.35****	18	19.34****	18	7.97****	18	11.88****

Data based on repeated measures analysis of variance.
*Significant level at $p < 0.05$.
**Significant level at $p < 0.01$.
***Significant level at $p < 0.001$.
**** Significant level at $p < 0.0001$ (LSD test).

and 4.15 times for P10N5, P10N10, P5N10, and P5N5, respectively. It also indicated that prometryne did not inhibit the growth and reproduction of nematodes in this study. From Day 18 through Day 30, nematodes in the treatments with 5 mg kg^{-1} prometryne still increased slowly up to 1.15 and 1.18 times, while it decreased up to 0.67 and 0.75 times in the treatments with 10 mg kg^{-1} prometryne. One potential explanation for this change was that the nematode population had increased more rapidly in the treatments with 10 mg kg^{-1} prometryne, and they consumed a lot of soil microbes [34]. The increase of microbial biomass in the treatments P10N5 and P10N10 was 6.1% and 6.6%, while in the treatments P5N10 and P5N5 was 31.3% and 34.3% (Fig. 3), and the increased bacteria in the treatments P10N5 and P10N10 were probably far less than those consumed by nematodes [38]. Therefore, there were not enough food sources to support the growth and reproduction of nematodes in the treatments with 10 mg kg^{-1} prometryne after Day 18.

The biological removal of prometryne was 59.20–76.30% in this study, while Di et al. [39] found that prometryne degraded more slowly. Betancur-Galvis et al. [40] also found that activated indigenous microbial activity could enhance the pollutants degradation, so boosting the activity of indigenous microbes in physical, chemical and biological ways will be an important means of removing contaminants from soil. Biostimulation of soil with organic or inorganic fertilizers introduces additional nutrients, such as glucose, sawdust, manure, sewage sludge, compost and vermicompost into the contaminated ecosystem, increases the population of the indigenous microorganisms and consequently enhances the removal of the contaminants [41,42]. The addition of nutrients into soil also could stimulate the growth and reproduction of bacterial-feeding nematodes [43], and thus enhance soil microbial activity to remove contaminants from soil. In the future, we also can make nematodes inoculum which can be applied to the soil to make some contaminants decompose more rapidly.

3.2. Dynamic of microbial biomass C, basal soil respiration and qCO_2

The trends of the dynamic of soil microbial biomass C in all treatments were almost the same during the incubation period except in Control 2. That is, it increased by 7.6–28.7% from Day 0 through

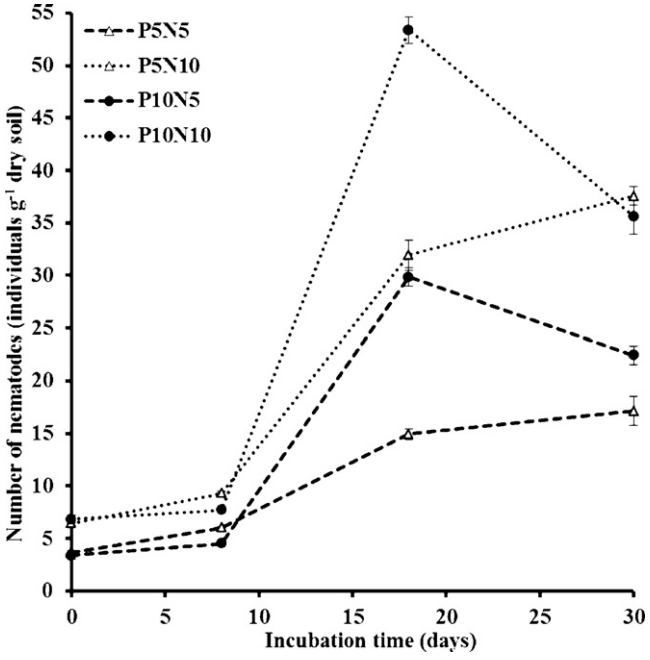

Fig. 2. Dynamic of the number of nematodes over time through the interaction between nematodes and indigenous microorganisms for four treatments with nematodes, P5N5, P5N10, P10N5, and P10N10. The data presented were the mean of triplicates ± standard errors (LSD test, $p < 0.05$). Error bars represented the standard error of the mean ($n = 3$). Abbreviations as in Fig. 1.

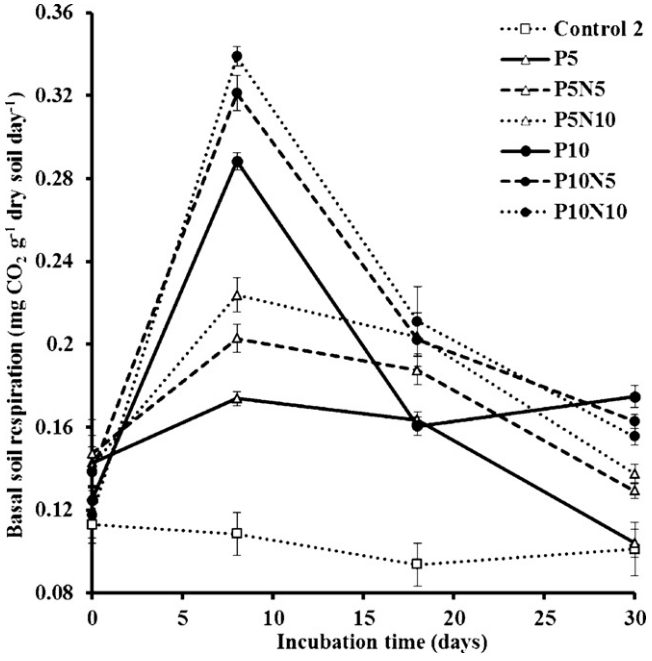

Fig. 3. The temporal variation of basal soil respiration during the incubation period for seven treatments with indigenous microorganisms, Control 2, P5, P5N5, P5N10, P10, P10N5, and P10N10. The data presented were the mean of triplicates ± standard errors (LSD test, $p < 0.05$). Error bars represented the standard error of the mean ($n = 3$). Abbreviations as in Fig. 1.

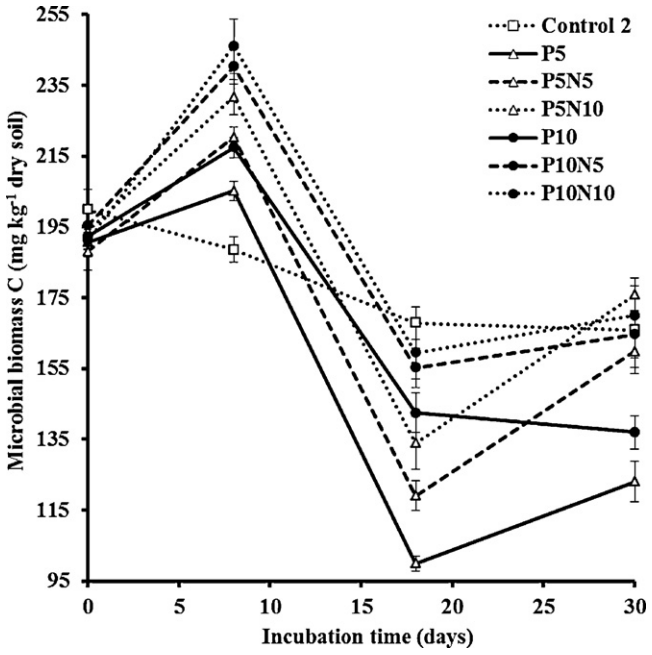

Fig. 4. Dynamic of microbial biomass C over time for seven treatments with indigenous microorganisms, Control 2, P5, P5N5, P5N10, P10, P10N5, and P10N10. The data presented were the mean of triplicates ± standard errors (LSD test, $p < 0.05$). Error bars represented the standard error of the mean ($n = 3$). Abbreviations as in Fig. 1.

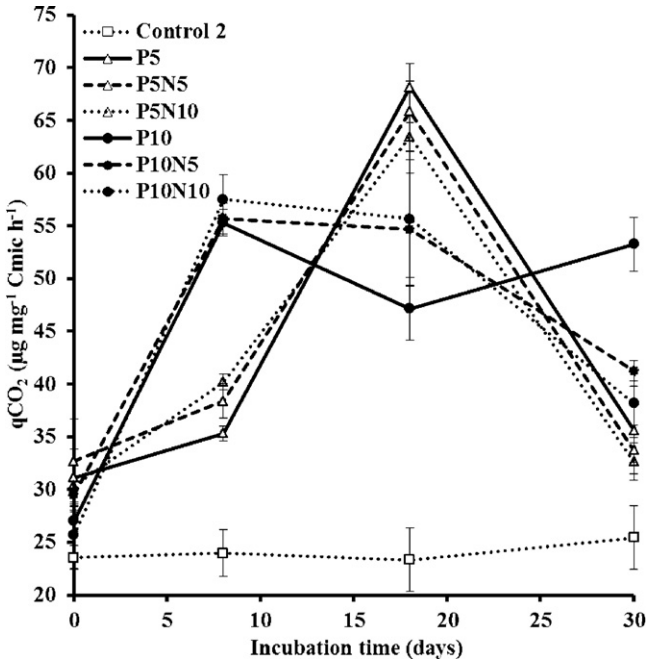

Fig. 5. The temporal changes in respiratory quotient (qCO_2) during the incubation period for seven treatments with indigenous microorganisms, Control 2, P5, P5N5, P5N10, P10, P10N5, and P10N10. The data presented were the mean of triplicates ± standard errors (LSD test, $p < 0.05$). Error bars represented the standard error of the mean ($n = 3$). Abbreviations as in Fig. 1.

Day 8 due to the activation on the growth and activity of certain microbes in the beginning of prometryne contamination and the inoculation of nematodes; it decreased by 34.5–51.3% from Day 8 through Day 18, which indicated the activation by prometryne to the growth of some microbes was weakened due to the fact that prometryne was degraded (Fig. 1) and also certain microbes was consumed by increased nematodes (Fig. 2) [38]; it increased by 6.1–34.3% in all treatments except the treatment USP10 which decreased by 3.9% after Day 18, probably because some other soil microbes reproduced after prometryne was degraded (Fig. 3). We also found that microbial biomass C in the treatments with nematodes was much more than that in the treatments without nematodes (LSD test, $p < 0.05$) (Fig. 3), which indicated that nematode grazing could boost the growth of certain soil microbes [44,45].

The results (Fig. 4) also showed that basal soil respiration was enhanced by 21.7–189.7% in all treatments except in Control 2 from Day 0 through Day 8, then gradually weakened to the level reached at the beginning of the incubation period from Day 8 through Day 30. That was probably because prometryne could activate the activity of certain microbes in the beginning of prometryne contamination and the activation of prometryne to microbial growth and activity weakened in the later stage of prometryne contamination. Basal soil respiration in the treatments with prometryne concentration at 10 mg kg^{-1} was 3.4–68.3% stronger than that in the treatments with prometryne concentration at 10 mg kg^{-1} (LSD test, $p < 0.05$). That was because prometryne of higher concentration could be easily utilized by certain microbes and produced some more CO_2. Basal soil respiration in the treatments with nematodes was 11.5–32.7% stronger than that in the treatments without nematodes, which suggested that nematode grazing enhanced the microbial activity (LSD test, $p < 0.05$).

What occurred most probably after the addition of prometryne and nematodes, was reflected by high values of the qCO_2 (Fig. 5). Microbes, in order to survive in a hostile environment, may develop defence mechanisms by increasing their respiration per unit of biomass [31]. The qCO_2 is often considered to be an index of microbial stress in soil [31,36]. The qCO_2 was 14.9–192.1% higher in the treatments with nematodes and/or with prometryne than that in Control 2. The qCO_2 in those treatments with similar prometryne concentrations were grouped together, regardless of initial nematode population size. At Day 8, the qCO_2 in those treatments with prometryne concentration at 5 mg kg^{-1}, were 17–56.5% lower than those with 10 mg kg^{-1} (LSD test, $p < 0.05$). The qCO_2 in the treatments with nematodes was 0.8–13.8% higher than that in those without nematodes at Day 8 (LSD test, $p < 0.05$) (Fig. 5). It suggested that soil microbes were more metabolically active in the treatments with nematodes and/or with prometryne than that in the natural soil. It also probably indicated that soil microbial populations were changing and shifting to younger and more active individuals due to nematode grazing. The values of qCO_2, however, tended to fall with time (Fig. 5) and reached constant values, probably due to the protective and buffering capacity of the soil [46] and the degradation of prometryne in soil.

Microbial biomass C, basal soil respiration and qCO_2 in all treatments changed obviously, that is, an increase in the beginning and then a decrease during the incubation period (Figs. 3–5), which indicated prometryne could affect the growth and activity of soil microbes. After nematodes were inoculated into soil, microbial biomass C increased by 7.4–43% (Fig. 3), basal soil respiration was enhanced by 11.3–32% (Fig. 4) and qCO_2 increased by 1–17% (Fig. 5) compared with the treatments without nematodes. This suggested that nematode grazing stimulated both microbial growth and activity (LSD test, $p < 0.05$) [20,47–50]. The changes of nematode densities in prometryne contaminated soil did not bring any significant effects on microbial biomass C, basal soil respiration and qCO_2 (LSD test, $p > 0.05$), so the density-dependent regulation effect of nematode grazing was not notable in our study. Further research is needed to verify whether these results were caused by small differences in prometryne concentrations and nematode densities.

4. Conclusions

Our study demonstrated that bacterial-feeding nematodes and prometryne could affect soil microbial growth and activity. Indigenous microbes can degrade prometryne in contaminated soil, so boosting the activity of indigenous microbes in physical, chemical and biological ways will actually be an important means of removing contaminants. Nematodes grazing on some microbes could boost and change soil microbial activity and growth, and thus enhance the degradation of prometryne in contaminated soil.

Acknowledgements

We thank Dr. X.J. Huang for comments on the manuscript. We thank two anonymous reviewers whose constructive comments have improved this manuscript greatly. Financially supported by the National Key Basic Research Program of China (No. 2007CB109301) and the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

References

[1] B. Engelen, K. Meinken, F. von Wintzintgerode, H. Heuer, H.P. Malkomes, H. Backhaus, Monitoring impact of a pesticide treatment on bacterial soil communities by metabolic and genetic fingerprinting in addition to conventional testing procedures, Appl. Environ. Microbiol. 64 (1998) 2814–2821.
[2] M.D. Busse, A.W. Ratcliff, C.J. Shestak, R.F. Powers, Glyphosate toxicity and the effects of long-term vegetation control on soil microbial communities, Soil Biol. Biochem. 33 (2001) 1777–1789.
[3] R.E. Macur, J.T. Wheeler, M.D. Burr, W.P. Inskeep, Impacts of 2,4-D application on soil microbial community structure and on populations associated with 2,4-D degradation, Microbiol. Res. 162 (2007) 37–45.
[4] I. Mijangos, J.M. Becerril, I. Albizu, L. Epelde, C. Garbisu, Effects of glyphosate on rhizosphere soil microbial communities under two different plant compositions by cultivation-dependent and -independent methodologies, Soil Biol. Biochem. 41 (2009) 505–513.
[5] Y. Zielezny, J. Groeneweg, H. Vereecken, W. Tappe, Impact of sulfadiazine and chlorotetracycline on soil bacterial community structure and respiratory activity, Soil Biol. Biochem. 38 (2006) 2372–2380.
[6] W. Luo, Y. Zhao, H. Ding, X. Lin, H. Zheng, Co-metabolic degradation of bensulfuron-methyl in laboratory conditions, J. Hazard. Mater. 158 (2008) 208–214.
[7] L. Qiao, J. Wang, Microbial degradation of pyridine by *Paracoccus* sp. isolated from contaminated soil, J. Hazard. Mater. 176 (2010) 220–225.
[8] S. Sarkar, S. Seenivasan, R.P.S. Asir, Biodegradation of propargite by *Pseudomonas putida*, isolated from tea rhizosphere, J. Hazard. Mater. 174 (2010) 295–298.
[9] M. Cea, M. Jorquera, O. Rubilar, H. Langer, G. Tortella, M.C. Diez, Bioremediation of soil contaminated with pentachlorophenol by *Anthracophyllum discolor* and its effect on soil microbial community, J. Hazard. Mater. 181 (2010) 315–323.
[10] M. Kumar, L. Philip, Bioremediation of endosulfan contaminated soil and water—optimization of operating conditions in laboratory scale reactors, J. Hazard. Mater. B136 (2006) 354–364.
[11] R.L. Swann, D.A. Laskowski, P.J. McCall, K.V. Kuy, H.J. Dishburger, A rapid method for the estimation of the environmental parameters octanol/water partition coefficient, soil sorption constant, water to air ratio, and water solubility, Residue Rev. 85 (1983) 17–28.
[12] T.A Albanis, T.G. Danis, M.K. Kourgia, Transportation of pesticides in estuaries of Axios, Loudias and Aliakmon rivers (Thermaikos Gulf), Sci. Total Environ. 156 (1994) 11–22.
[13] E. Papadopoulou-Mourkidou, D.G. Karpouzas, J. Patsias, A. Kotopoulou, A. Milothridou, K. Kintzikoglou, P. Vlachou, The potential of pesticides to contaminate the groundwater resources of the Axios river basin. Part II. Monitoring study in the south part of the basin, Sci. Total Environ. 321 (2004) 147–164.
[14] E.C. Bernard, Soil nematode biodiversity, Biol. Fertil. Soils 14 (1992) 99–103.
[15] B.S. Griffiths, K. Ritz, R.E. Wheatley, Nematodes as indicators of enhanced microbiological activity in a Scottish organic farming system, Soil Use Manage. 10 (1994) 20–24.
[16] H.X. Li, I. Kazuyuki, M. Johji, Effects of temperature on population growth and N mineralization of soil bacteria and a bacterial-feeding nematode, Microbes Environ. 16 (2001) 141–146.
[17] B. Sohlenius, Abundance, biomass and contribution to energy flow by soil nematodes in terrestrial ecosystems, Oikos 34 (1980) 186–194.
[18] R.E Ingham, J.A. Trofymow, E.R. Ingham, D.C. Coleman, Interactions of bacteria, fungi and their nematode grazers: effects on nutrient cycling and plant growth, Ecol. Monogr. 55 (1985) 119–140.
[19] R.D. Bardgett, R. Cook, G.W. Yeates, C.S. Denton, The influence of nematodes on below-ground processes in grassland ecosystems, Plant Soil 212 (1999) 22–33.
[20] J. Alphei, M. Bonkowski, S. Scheu, Protozoa, Nematoda and Lumbricidae in the rhizosphere of *Hordelymus europaers* (*Poaceae*): faunal interactions, response of microorganisms and effects on plant growth, Oecologia 106 (1996) 111–126.
[21] R.W. Parmelee, D.G. Alston, Nematode trophic structure in conventional and no-tillage agroecosystems, J. Nematol. 18 (1986) 403–407.
[22] T. Bongers, De Nematoden van Nederland, KNNV-bibliotheek-uitgave 46, Pirola, Schoorl, 1994.
[23] S. Brenner, The genetics of *Caenorhabditis elegans*, Genetics 77 (1974) 71–94.
[24] H. Xiao, B. Griffiths, X. Chen, M. Liu, J. Jiao, F. Hu, H. Li, Influence of bacterial-feeding nematodes on nitrification and the ammonia-oxidizing bacteria (AOB) community composition, Appl. Soil Ecol. 45 (2010) 131–137.
[25] X.Y. Chen, H.X. Li, F. Hu, M.Q. Liu, Effect of bacterivorous nematode on soil microbial biomass and microbiocoenosis, Acta Ecol. Sin. 24 (2004) 2825–2831.
[26] D.W. Freckman, R.A. Virginia, Extraction of nematodes from Dry Valley Antarctic soils, Polar Biol. 13 (1993) 483–487.
[27] J. Zhou, J. Chen, Y. Cheng, D. Li, F. Hu, H. Li, Determination of prometryne in water and soil by HPLC–UV using cloud-point extraction, Talanta 79 (2009) 189–193.
[28] W.L. Goodfriend, W.L. Olsen, R.J. Frye, Soil microfloral and microfaunal response to *Salicornia bigelovii* planting density and soil residue amendment, Plant Soil 223 (2000) 23–32.
[29] A. Agnelli, J. Ascher, G. Corti, M.T. Ceccherini, P. Nannipieri, G. Pietramellara, Distribution of microbial communities in a forest soil profile investigated by microbial biomass, soil respiration and DGGE of total and extracellular DNA, Soil Biol. Biochem. 36 (2004) 859–868.
[30] E.D. Vance, P.C. Brookes, D.S. Jenkinson, An extraction method for measuring soil microbial biomass C, Soil Biol. Biochem. 19 (1987) 703–707.
[31] T.H. Anderson, K.H. Domsch, The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on microbial biomass of the soil, Soil Biol. Biochem. 25 (1993) 393–395.
[32] A.S.F. Araujo, R.T.R. Monteiro, Microbial biomass and activity in a Brazilian soil amended with untreated and composted textile sludge, Chemosphere 64 (2006) 1028–1032.
[33] O. Crouzet, I. Batisson, P. Besse-Hoggan, F. Bonnemoy, C. Bardot, F. Poly, J. Bohatier, C. Mallet, Response of soil microbial communities to the herbicide mesotrione: a dose–effect microcosm approach, Soil Biol. Biochem. 42 (2010) 193–202.
[34] O.I. Nwachukwu, I.D. Pulford, Microbial respiration as an indication of metal toxicity in contaminated organic materials and soil, J. Hazard. Mater. 185 (2011) 1140–1147.
[35] J.P.E. Anderson, Soil respiration, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods of Soil Analysis. Part 2, 2nd ed., American Society of Agronomy and Soil Science Society of America, Madison, WI, 1982, pp. 837–871.
[36] D.A. Wardle, A. Ghani, A critique of the microbial metabolic quotient (qCO_2) as bioindicator of disturbance and ecosystem development, Soil Biol. Biochem. 27 (1995) 1601–1610.
[37] T.H. Anderson, K.H. Domsch, Application of ecophysiological quotients (qCO_2 and qD) on microbial biomasses from soils of different cropping histories, Soil Biol. Biochem. 22 (1990) 251–255.
[38] W.J. Landesman, A.M. Treonis, J. Dighton, Effects of a one-year rainfall manipulation on soil nematode abundances and community composition, Pedobiologia 54 (2011) 87–91.
[39] H.J. Di, L.A.G. Aylmore, R.S. Kookana, Degradation rates of eight pesticides in surface and subsurface soils under laboratory and field conditions, Soil Sci. 163 (1998) 404–411.
[40] L.A. Betancur-Galvis, D. Alvarez-Bernal, A.C. Ramos-Valdivia, L. Dendooven, Bioremediation of polycyclic aromatic hydrocarbon-contaminated saline–alkaline soils of the former Lake Texcoco, Chemosphere 62 (2006) 1749–1760.
[41] M. Hafidi, S. Amir, A. Jouraiphy, P. Winterton, M. El Gharous, G. Merlina, J.C. Revel, Fate of polycyclic aromatic hydrocarbons during composting of activated sewage sludge with green waste, Biores. Technol. 99 (2008) 8819–8823.
[42] W. Namkoong, E.Y. Hwang, J.S. Park, J.Y. Choi, Bioremediation of diesel-contaminated soil with composting, Environ. Pollut. 119 (2002) 23–31.
[43] X. Mao, F. Hu, B. Griffiths, H. Li, Bacterial-feeding nematodes enhance root growth of tomato seedlings, Soil Biol. Biochem. 38 (2006) 1615–1622.
[44] H.W. Hunt, D.C. Coleman, E.R. Ingham, R.E. Ingham, E.T. Elliott, J.C. Moore, S.L. Rose, C.P.P. Reid, C.R. Morley, The detrital food web in a short grass prairie, Biol. Fertil. Soil 3 (1987) 57–68.
[45] H.A. Verhoef, L. Brussaard, Decomposition and nitrogen mineralization in natural and agroecosystems: the contribution of soil animals, Biogeochemistry 11 (1990) 175–211.
[46] J.A. Pascual, C. Garcia, T. Hernandez, M. Ayuso, Changes in the microbial activity of an arid soil amended with urban organic wastes, Biol. Fertil. Soils 24 (1997) 429–434.

[47] S.L. Fu, H. Ferris, D. Brown, R. Plant, Does the positive feedback effect of nematodes on the biomass and activity of their bacteria prey vary with nematode species and population size? Soil Biol. Biochem. 37 (2005) 1979–1987.
[48] C. Blanc, M. Sy, D. Djigal, A. Brauman, P. Normand, C. Villenave, Nutrition on bacteria by bacterial-feeding nematodes and consequences on the structure of soil bacterial community, Eur. J. Soil Biol. 42 (2006) 70–78.
[49] D. Djigal, A. Brauman, T.A. Diop, J.L. Chotte, C. Villenave, Influence of bacterial-feeding nematodes (*Cephalobidae*) on soil microbial communities during maize growth, Soil Biol. Biochem. 36 (2004) 323–331.
[50] R. Rønn, A.E. McCaig, B.S. Griffiths, J.I. Prosser, Impact of protozoan grazing on bacterial community structure in soil microcosms, Appl. Environ. Microbiol. 68 (2002) 6094–6105.