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Influence of invasive plants on nematode communities under simulated CO_2 enrichment

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ABSTRACT

Relationships between exotic plant invasion and environmental change including CO_2 increase have been studied, but little is known about effects of invasive plants and global change on belowground faunal communities. Nematodes are abundant and trophically diverse soil biota, and are susceptible to environmental changes. Alteration of their community composition can illustrate changes in belowground ecosystems. In this study, we examined responses of nematode communities to exotic invasive plants and native plants under current and increased CO_2. We grew individual plants of two invasive and two native species under ambient and elevated CO_2 under controlled conditions. Soil nematode abundance, the proportion of nematode trophic groups and their ecological indices were measured to determine their responses to invasive and native plants under different CO_2 conditions. Canonical correspondence analysis (CCA) showed that elevated CO_2 and invasiveness significantly affected nematode communities. Elevated CO_2 significantly increased bacteria-feeding nematode proportions under two invasive plants, and decreased their proportion under the native *Eupatorium heterophyllum*. Elevated CO_2 decreased the proportion of plant-feeding nematodes under the invasive plant *Chromolaena odorata*, and increased the proportion of plant-feeding nematodes under *E. heterophyllum*, indicating that elevated CO_2 could benefit *C. odorata* by reduced belowground herbivory compared to this native plant species. Ecological indices showed that invasives had higher nematode diversity than natives. Under elevated CO_2, the value of structural index (SI) was greater than 50, while the value of enrichment index (EI) was <50 with two invasive plants. SI and EI were <50 under two native plants, indicating that these invasive plants developed more stable belowground ecosystems than native species, and that this could promote their colonization under CO_2 increase. Overall, our results suggested that elevated CO_2 can favor particular invasive plants over native species, and increase successful invasion by these plant species.

1. Introduction

Exotic plant invasions and global environmental changes such as carbon dioxide (CO_2) increase both may affect global biodiversity, and thus attract substantial research attention [1]. Previous studies show that ongoing global changes could alter impacts of invasive plants on native vegetation [2–4]. However, there is limited knowledge about the relationships of invasive plants with belowground faunal communities under climate change. Soil fauna such as protozoa and nematodes have strong effects on soil structure and processes, and soil community composition and functions are closely linked with plant communities [5,6]. Nematodes are of particular interest because they are abundant and trophically diverse in soils [7], and have been used as sensitive indicators of environmental changes [8]. With increasing atmospheric CO_2 concentration, the study of nematode communities in response to invasive plants under elevated CO_2 can illustrate potential effects of exotic plant invasions on soil ecosystem functioning.

Several studies have demonstrated that, in contrast to native plants, invasives have higher net primary production and more plant biomass [9,10] because of higher photosynthetic capacity and resource-capture efficiency [11,12]. Thus, invasive plants can benefit more from atmospheric CO_2 concentration increases.

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Greater accumulation of C in invasive plants also implies that C input to soils as root turnover and root exudates may be greater under invasive than native plants [9]. Altered C input from invasive and native plants might affect soil nematode communities either directly (root turnover affecting herbivory) or indirectly (root exudates affecting microorganisms and with subsequent change in bacterial feeding nematode communities) [13,14]. Based on these ideas, we hypothesized that populations and diversity of nematodes would be higher in soil under invasive plants, due to increased resource inputs to the soil. Thus, elevated CO_2 may increase the responses of nematodes to invasive plants. On the other hand, invasive plant root exudates often contains more secondary compounds than exudates from native plants [15,16]. Such compounds can constrain plant-feeding nematodes, thereby reducing populations of this trophic group and altering nematode communities [17]. Therefore elevated CO_2 may have stronger negative effects on nematodes when invasive plants are present. In general, positive or negative impact of elevated CO_2 on nematodes may depend on plant species because their root exudations (quantity and quality) are influenced by atmospheric CO_2 concentrations [18,19].

In this study, we selected *Ageratina adenophora* and *Chromolaena odorata* as invasive plant species because they are aggressive invasive perennial forbs in Yunnan Province, southwest China [11,20]. Native *Eupatorium japonicum* and *Eupatorium heterophyllum* were selected in this study because they are related to the two invasive species. These four plant species were used to investigate the response of the nematode community (reflected by nematode abundances, trophic group proportions and ecological indices) to invasive and native plants under CO_2 enrichment. In this study, four nematode ecological indices (Shannon–Weaver diversity index, maturity index, enrichment index, structural index) were used. They are important and useful indices and have been applied to monitor environmental changes [21,22]. Our objective was to explore potential belowground ecological mechanisms affecting invasion success by exotic plants under current and future conditions.

2. Materials and methods

2.1. Experimental design

The study was conducted at Ai Lao Mountain; a long-term ecological experimental station of the Chinese Academy of Sciences in Yunnan Province, China (24.32° N, 101.01° E). We individually planted four species (a single plant in each pot) under ambient and elevated CO_2 in chambers automatically controlling CO_2 concentrations (Fig. S1). These experimental treatments were exposed to two levels of CO_2: ambient (360 μmol mol^{-1}) and elevated (700 μmol mol^{-1}). Four plant species, invasive *A. adenophora* and *C. odorata*, and native *E. heterophyllum* and *E. japonicum* were planted in pots.

Soil used in this experiment was a sandy loam (brown soil), containing 53.5% sand, 24.6% silt, and 21.9% clay. The soil had 30.84 g kg^{-1} of organic C, 2.51 g kg^{-1} of total *N*, 19.46 mg kg^{-1} NH_4^+, and 3.13 mg kg^{-1} NO_3^-, 198 mg kg^{-1} available *P*, and 135 mg kg^{-1} available K. The pH (H_2O) was 6.8 at the beginning of the experiment. Initial nematode communities also were identified (Table 1).

Plant seeds were broadcast evenly in humus soil mixed with vermiculite in pots. Then, the seeds were covered with a layer of fine soil, and were watered sufficiently. All the containers were placed under 50% relative light intensity in an incubator. When the seedlings had grown to 8 cm height, similarly sized and healthy ones were selected and transplanted into pots with diameters of 30 cm. After two weeks of incubation, all samples were moved to the controlled chambers and exposed to different CO_2 concentrations throughout the experiment as described. The plants were watered as necessary to keep the soil moist. There were four plant species (two invasive and two native) and two treatments (ambient and elevated CO_2). Each treatment had five replicates; thus overall there were 40 pots. Pots were randomly arranged in the chambers and repositioned in their chamber every month.

Table 1
Soil nematode communities at the beginning of the experiment.

	Nematode abundance (individual g^{-1} dry soil)	Proportion (%) of nematode trophic groups
TNEM	15.68 ± 1.30	
Ba	8.16 ± 0.40	52.20 ± 3.21
Fu	0.90 ± 0.61	5.56 ± 3.37
Pl	5.84 ± 0.36	37.34 ± 1.68
Om&Ca	0.70 ± 0.15	4.42 ± 0.62

Note: TNEM, total nematode numbers; Ba, bacterial-feeding nematodes; Fu, fungi-feeding nematodes; Pl, plant-feeding nematodes; Om&Ca, omnivores and carnivores.

The seedlings were transplanted in February 2010 and harvested in December 2010, following a growth period of 10 months. Finally (at the end of the growing season), all samples were harvested and the plant biomass (above and belowground biomass measured separately), soil total organic carbon (TOC), total *N* (Table S1) and the nematode communities were examined. Plants were collected and dried at 80 °C to constant weight.

2.2. Isolation and identification of nematodes

Nematodes were extracted from 100 g soil samples from each pot via a modified cotton–wool filter method [23], and were counted and identified under a dissecting microscope. The nematode trophic groups were characterized by feeding habits: (1) bacterial-feeding nematodes (Ba), (2) fungal-feeding nematodes (Fu), (3) plant-feeding nematodes (Pl), and (4) omnivores and carnivores (Om&Ca) [24]. Additionally, several ecological indices were calculated to assess the nematode community. These included the Shannon–Weaver diversity index $H' = -\sum p_i (\ln p_i)$, where p_i is the proportion of individuals in the *i*th taxon; maturity index $MI = \sum(v_i)\cdot(f_i)$, where (v_i) is the c–p value of taxon *i* according to their *r* and *K* characteristics [25], and (f_i) is the frequency of taxon *i* in a sample; enrichment index $EI = 100 \times (\sum k_e n_e/(\sum k_e n_e + \sum k_b n_b))$; structural index $SI = 100 \times (\sum k_s n_s/(\sum k_s n_s + \sum k_b n_b))$, where k_b is the weight assigned to guilds Ba_2 and Fu_2, and n_b represents the abundance of nematodes in guilds Ba_2 and Fu_2, k_e is the weight assigned to guilds Ba_1 and Fu_2, n_e is the abundance of nematodes in these guilds (indicating an enriched condition of the food web), k_s is the weight assigned to guilds Ba_3–Ba_5, Fu_3–Fu_5, Om_4–Om_5, and Ca_2–Ca_5; and n_s is the abundance of nematodes in these guilds, representing the structural condition of the food web [26].

2.3. Statistical analysis

There were three factors in this experiment, CO_2 concentration (ambient and elevated), invasive feature (invasive and native) and plant species. The invasive feature had two treatments, with two plant species in each (invasive *A. adenophora* and *C. odorata*, and native *E. heterophyllu* and *E. japonicum*). As invasiveness was nested within species, a three-way nested ANOVA analysis method was used to analyze effects of elevated CO_2, invasive feature and plants on nematode abundance and ecological indices. Canonical correspondence analysis (CCA) was use to compare nematode communities among treatments performed with Canoco 4.5. MANOVA was used to determine whether elevated CO_2 and invasive feature had significant effect on nematode communities. Differences in plant-root biomass, nematode abundance, proportion of trophic groups and ecological indices among the various plant species under the environmental conditions were tested by one-way ANOVA. Duncan's test was employed to determine if differences were significant among treatments. The significance level was set at $P < 0.05$. Statistical analyses were performed with SPSS 13.0 software.

3. Results

3.1. Plant biomass and nematode communities

Under ambient or elevated CO_2, root biomasses of the invasive species were significantly lower than two native species. Elevated CO_2 significantly increased the root biomass of all the plant species (Fig. 1). No significant difference of aboveground biomass was observed among four plant species under ambient conditions. Under elevated CO_2, aboveground biomass of *C. odorata* was significantly higher than *E. heterophyllum* or *E. japonicum* (Fig. S2).

Twenty-four nematode genera were found in this study, and *Cephalobus*, *Helicotylenchus*, and *Hirschmanniella* were dominant throughout (Table 2).

Three-way nested ANOVA results (Table 3) showed that elevated CO_2 significantly affected the ecological indices of MI, EI, and SI. Invasive feature affected the total nematode abundance and the abundance of fungal-feeding nematodes (Fu), plant-feeding nematodes (Pl), and the ecological index H′. Plant species significantly affected the abundance of bacteria-feeding nematode (Ba) and Pl. Significant interaction effects between CO_2 and invasive feature were observed with the abundance of Ba, H′ and MI. Significant interaction effects between CO_2 and plant species were also observed with Ba, MI, SI and EI.

Nematode absolute abundances were shown in Fig. S3. Nematode communities were sharply different among treatments (Fig. 2). Both CO_2 and invasive feature had significant effects on nematode communities ($P < 0.001$).

3.2. Proportion (%) of nematode trophic groups in invasive and native plants under elevated CO_2

Comparing proportions (%) of nematode trophic groups among the four plant species (Fig. 3) showed that under ambient CO_2, the proportion of Ba with *C. odorata* treatment was significantly higher than that with the three other plant species, and the proportion of Ba with *E. heterophyllum* and *E. japonicum* was significantly higher than *A. adenophora*. The proportion of Fu with *C. odorata* was significantly lower than with the three other plant species. The proportion of Pl with *A. adenophora* was significantly higher than that with *C. odorata*. The proportion of O&C with *A. adenophora* was significantly higher than that with *E. heterophyllum*. Under elevated CO_2, the proportion of Ba was highest with *C. odorata*, and was lowest with *E. heterophyllum* among the four plant species. However, the proportion of Pl was lowest with *C. odorata* and highest with *E. heterophyllum*. The proportion of Om&Ca with *C. odorata* was significantly higher than that with the other plant species.

In terms of plant species and invasiveness (Fig. 3), elevated CO_2 strongly increased the proportion of Ba in both invasive plant species but decreased the proportion of Ba with *E. heterophyllum* significantly. Elevated CO_2 also significantly decreased the proportion of Fu with *A. adenophora*. In addition, the proportions of Pl and Om&Ca were affected significantly by elevated CO_2. Plant-feeding nematodes were decreased significantly with *C. odorata* and increased significantly with *E. heterophyllum*. Om&Ca were decreased significantly with *A. adenophora*.

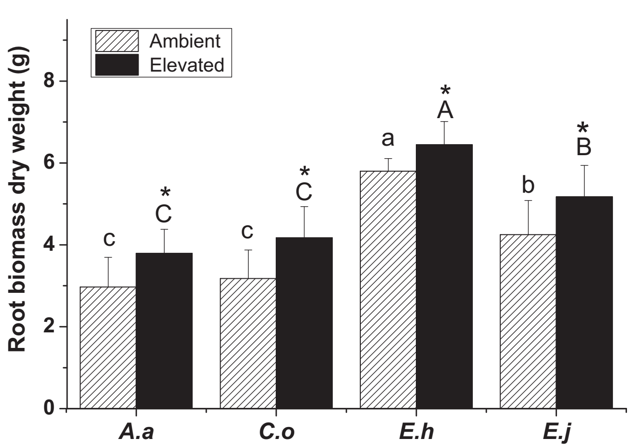

Fig. 1. Root biomass of four plant species under ambient and elevated CO_2 conditions. Lowercase letters indicates significant differences ($P < 0.05$) among plant species under ambient CO_2. Capital letters indicates the significant difference among different plant species under elevated CO_2 condition. Asterisk indicates the significant difference between different CO_2 treatments in the same plant species. *A.a*, *Ageratina adenophora*; *C.o*, *Chromolaena odorata*; *E.h*, *Eupatorium heterophyllum*; and *E.j*, *Eupatorium japonicum*. Error bars represent standard errors ($n = 5$).

3.3. Nematode ecological indices in invasive and native plant under elevated CO_2

Differences in nematode ecological indices among the four plant species were compared in Fig. 4. Under ambient CO_2, no significant difference was observed in H′. The values of MI and SI with *A. adenophora* were significantly higher than that with *E. heterophyllum*. The value of EI with *A. adenophora* and *E. heterophyllum* was significantly higher than that with *C. odorata*. Under elevated CO_2, the value of H′ with *A. adenophora* was significantly higher than that with *E. japonicum*, and the value of H′ with *C. odorata* was significantly higher than that with *E. heterophyllum* and *E. japonicum*. No significant differences were observed among plant species for MI, or SI. The value of EI with E. *japonicum* was significantly higher than that with *A. adenophora* and *E. heterophyllum*.

In terms of plant species and invasiveness (Fig. 4), elevated CO_2 increased the value of H′ with *C. odorata* significantly, but substantially decreased the value of H′ with two native plant species. It also significantly decreased the value of MI in all the treatments except in *E. heterophyllum*. In addition, elevated CO_2 significantly increased the value of EI with *C. odorata* and *E. japonicum*, but decreased the value of SI with *A. adenophora*.

4. Discussion

4.1. Nematode communities with invasive and native plant species under ambient and elevated CO_2

At the beginning of the experiment, bacterial-feeding nematodes were the dominant trophic group and they represented 52.2% of the total nematode numbers (Table 1). However, at the end of the experiment, plant-feeding nematodes replaced bacterial-feeding nematodes as the dominant trophic group (Fig. S2). These results indicated that plant roots significantly stimulated the growth of plant-feeding nematodes. Plant-root biomass and TOC results indicated that elevated CO_2 significantly increased the allocation of C to the soil (Fig. 1) (Table S1) [27,28]. However, increased resource allocation did not significantly increase the total number of nematodes under elevated CO_2, indicating that the total number of nematodes may not simply follow a pattern of increase with belowground C allocation [29].

Table 2
Nematode functional guilds and genera present in this study (individuals per 100 total nematode).

Bacterial feeders	*A.a*		*C.o*		*E.h*		*E.j*	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Ba_1								
Mesorhabditis	0.23 ± 0.45	0.90 ± 0.81	0.91 ± 1.17	0.87 ± 0.72	0 ± 0	0.46 ± 0.93	0.44 ± 0.88	0.95 ± 1.09
Panagrolaimus	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.18 ± 0.36	0 ± 0	0.21 ± 0.43	0 ± 0
Protorhabditis	0 ± 0	0.44 ± 0.88	0.63 ± 0.73	1.74 ± 0.72	0.57 ± 0.70	0 ± 0	0.21 ± 0.43	0.47 ± 0.55
Ba_2								
Cephalobus[a]	5.74 ± 0.48	10.28 ± 3.27	14.70 ± 2.16	19.19 ± 1.77	11.22 ± 2.48	3.63 ± 2.66	8.31 ± 3.21	12.80 ± 1.74
Acrobeles	0 ± 0	0.43 ± 0.51	0.31 ± 0.60	1.52 ± 1.09	0.24 ± 0.49	0.68 ± 0.86	0.22 ± 0.44	0.48 ± 0.56
Chiloplacus	0 ± 0	0.60 ± 0.79	0.63 ± 0.73	0.66 ± 0.85	0.21 ± 0.43	0 ± 0	0.22 ± 0.44	0 ± 0
Eucephalobus	1.19 ± 0.42	1.59 ± 1.58	4.09 ± 1.31	4.79 ± 3.62	1.98 ± 0.75	2.92 ± 0.88	0.87 ± 0.70	1.88 ± 1.31
Prismatolaimus	1.15 ± 0.86	0 ± 0	0.92 ± 0.62	1.29 ± 1.65	3.51 ± 0.58	1.53 ± 1.47	4.81 ± 1.51	0.24 ± 0.47
Tylocephalus	0 ± 0	0 ± 0	0 ± 0	0.22 ± 0.44	0.24 ± 0.49	0 ± 0	0.22 ± 0.44	0 ± 0
Ba_4								
Alaimus	1.23 ± 0.95	0.82 ± 0.68	4.05 ± 1.54	4.35 ± 1.85	1.25 ± 0.93	1.59 ± 1.16	2.18 ± 1.09	2.36 ± 1.82
Fungal feeders								
Fu_2								
Aphelenchoides	1.94 ± 0.84	3.19 ± 0.73	1.51 ± 1.18	1.52 ± 0.43	3.09 ± 0.77	1.59 ± 1.37	1.55 ± 1.51	5.53 ± 2.66
Aphelenchus	1.47 ± 0.99	0.60 ± 0.78	0.33 ± 0.65	0.66 ± 0.84	2.12 ± 0.88	1.61 ± 2.19	1.74 ± 1.23	0.97 ± 1.33
Fu_4								
Leptonchus	3.99 ± 2.63	1.19 ± 0.97	2.79 ± 1.15	0.65 ± 0.82	7.57 ± 2.25	4.12 ± 4.68	3.46 ± 3.02	0.25 ± 0.50
Plant feeders								
Pl_2								
Filenchus	5.77 ± 4.07	5.13 ± 2.85	10.29 ± 2.17	4.14 ± 0.86	8.94 ± 1.65	7.55 ± 3.38	7.02 ± 1.32	3.15 ± 1.20
Paratylenchus	0.92 ± 0.74	3.10 ± 1.43	1.60 ± 1.67	8.49 ± 2.53	0.28 ± 0.57	4.27 ± 0.99	1.31 ± 1.13	3.61 ± 0.37
Tylenchus	0.76 ± 0.95	1.32 ± 1.24	0.91 ± 1.17	1.09 ± 0.42	0.24 ± 0.49	0.90 ± 1.04	0.88 ± 0.72	0.45 ± 0.90
Pl_3								
Helicotylenchus[a]	33.04 ± 3.34	25.46 ± 5.66	8.16 ± 1.78	4.14 ± 1.09	38.92 ± 8.08	52.22 ± 7.27	35.65 ± 2.06	42.75 ± 10.01
Hirschmanniella[a]	31.91 ± 5.81	31.33 ± 11.17	42.78 ± 2.01	33.15 ± 4.02	19.44 ± 7.85	16.36 ± 3.70	22.38 ± 7.01	19.61 ± 4.97
Pratylenchus	2.66 ± 0.60	9.01 ± 5.66	0.61 ± 1.22	3.91 ± 2.25	0.78 ± 1.07	0.45 ± 0.51	2.20 ± 2.10	0.24 ± 0.47
Rotylenchus	0.70 ± 0.87	0.58 ± 0.72	0 ± 0	0 ± 0	0.57 ± 0.70	0 ± 0	0 ± 0	0 ± 0
Tylenchorhynchus	0.78 ± 1.01	1.07 ± 0.85	0.6 ± 0.70	1.54 ± 0.86	0.24 ± 0.49	0.68 ± 0.45	0.88 ± 1.25	0.93 ± 1.28
Omnivores and Carnivores								
$O\&C_4$								
Dorylaimus	4.57 ± 0.95	1.12 ± 0.88	2.53 ± 1.82	3.06 ± 1.84	1.20 ± 0.87	1.10 ± 0.84	3.06 ± 1.14	2.17 ± 0.90
Mononchus	0.72 ± 0.48	0.77 ± 1.05	0.33 ± 0.66	0.43 ± 0.49	0.24 ± 0.49	0.66 ± 0.83	0 ± 0	0.47 ± 0.55
$O\&C_5$								
Aporcelaimus	1.23 ± 0.60	2.96 ± 0.96	3.45 ± 0.68	2.60 ± 1.55	3.26 ± 0.86	0.88 ± 0.70	2.18 ± 1.52	0.70 ± 0.87

[a] Indicates the dominant genera. Ba_x, Fu_x, Pl_x, $O\&C_x$ (where $x = 1–5$) represent the nematode functional guilds: Bacterial-feeding nematodes, Fungi-feeding nematodes, Plant-feeding nematodes, and Omnivores and Carnivores, respectively; and *x* represents the colonizer–persister (*c*-*p*) value according to their *r* and *K* characteristics following Bongers [25]. *A.a*, *Ageratina adenophora*; *C.o*, *Chromolaena odorata*; *E.h*, *Eupatorium heterophyllum*; *E.j*, *Eupatorium japonicum*.

Elevated CO_2 and plant invasive status significantly changed nematode communities (Fig. 2). This result was consistent with the previous studies [29–31]. Three-way nested ANOVA results showed that elevated CO_2 mainly affected nematode ecological indices (MI, SI and EI). Invasive feature affected both nematode trophic group and ecological indices (Fu, Pl and H′), while plant species only affected nematode trophic group (Ba and Pl). Single factors (elevated CO_2 or invasive feature) had no significant effect on Ba (Table 3). However, significant interaction effects between

Table 3
Summary of three-way nested analysis of variance (ANOVA) to test the effects of elevated CO_2, invasive feature and plants on nematode abundance and ecological indices.

	TNEM	Ba	Fu	Pl	Om&Ca	*H′*	MI	EI	SI
C	ns	ns	ns	ns	ns	ns	**	**	**
F	**	ns	*	**	ns	**	ns	ns	ns
F(P)	ns	**	ns	**	ns	ns	ns	ns	ns
C*F	ns	**	ns	ns	ns	**	*	ns	ns
C* F(P)	ns	*	ns	ns	ns	ns	*	**	*

Note: *C* indicates the elevated CO_2 effect. F indicates invasive feature. *P* indicates different plant species. TNEM is the total number of nematodes; Ba refers to bacterial-feeding nematodes; Fu indicates the fungi-feeding nematodes; Pl refers to plant-feeding nematodes; Om&Ca indicates the omnivores and carnivores; *H′* is the Shannon–Weaver diversity; MI is the maturity index; EI is the enrichment index; SI is the structure index. ** and * indicate significance at $P < 0.01$ and $P < 0.05$, respectively; ns indicates no significant difference.

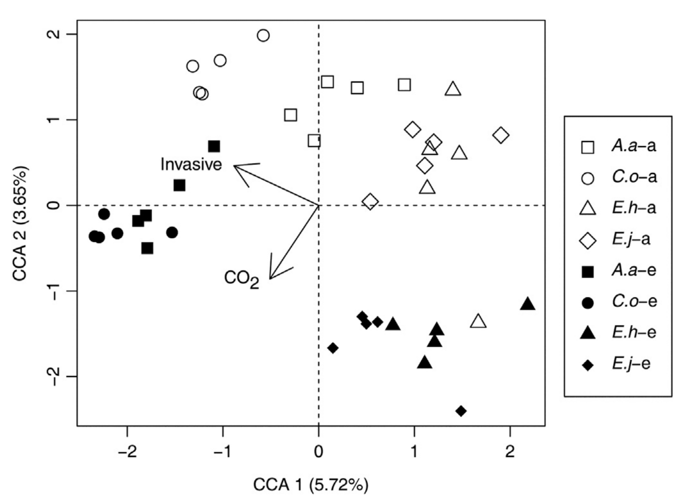

Fig. 2. Canonical correspondence analysis (CCA) of the nematode communities under ambient and elevated CO_2. *A.a*-a, *Ageratina adenophora* under ambient CO_2; *C.o*-a, *Chromolaena odorata* under ambient CO_2; *E.h*-a, *Eupatorium heterophyllum* under ambient CO_2; *E.j*-a, *Eupatorium japonicum* under ambient CO_2. *A.a*-e, *Ageratina adenophora* under elevated CO_2; *C.o*-e, *Chromolaena odorata* under elevated CO_2; *E.h*-e, *Eupatorium heterophyllum* under elevated CO_2; *E.j*-e, *Eupatorium japonicum* under elevated CO_2.

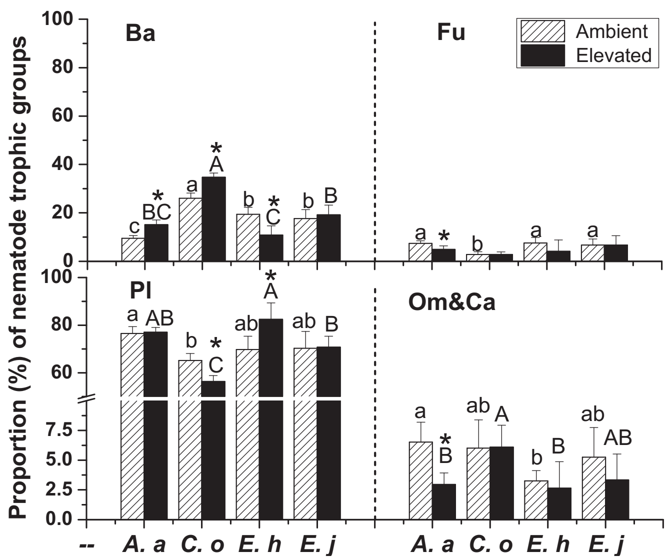

Fig. 3. Proportion (%) of nematode trophic groups in soils with invasive and native plants under ambient and elevated CO_2. Lowercase letters indicates significant differences ($P < 0.05$) among plant species under ambient CO_2. Capital letters indicates significant differences among different plant species under elevated CO_2. Asterisks indicates significant differences between CO_2 treatments in the same plant species. Ba, bacterial-feeding nematodes; Fu, fungi-feeding nematodes; Pl, plant-feeding nematodes; Om&Ca, omnivores and carnivores; *A.a*, *Ageratina adenophora*; *C.o*, *Chromolaena odorata*; *E.h*, *Eupatorium heterophyllum*; and *E.j*, *Eupatorium japonicum*. Error bars represent standard errors ($n = 5$).

elevated CO_2 and invasive feature were observed with the abundance of Ba, which indicated that invasive plants can significantly affect Ba under elevated CO_2.

The changes on the proportion of different nematode trophic groups is much more informative than that the changes on their absolute abundances [22]. Under ambient CO_2, the proportion of Ba was highest, but Pl was lowest with the invasive plant *C. odorata*. A high proportion of Ba may be favorable to *C. odorata* because it could promote the mineralization of soil nutrients, thereby increasing nutrient access [32,33]. Meanwhile, as Pl feed on plant roots, a lower proportion of Pl could reduce damage to *C. odorata* roots. Under elevated CO_2, this trend became more pronounced, indicating that higher levels of CO_2 may strengthen interactions between *C. odorata* and soil nematodes. Soil C/N (Table S1) was also consistent with nematode results. Soil C/N with *C. odorata* was lower than that with other plant species. This may be the reason why *C. odorata* have higher proportion of Ba because lower C/N favors the bacteria-feeders [32–34]. Although *A. adenophora* is also an invasive species, the abundance and proportion of Pl in the soil planted with it did not change with elevated CO_2 in this study. This result suggested that the response of nematodes to plants under elevated CO_2 was species-specific.

Elevated CO_2 significantly decrease Pl proportion with *C. odorata*, suggesting that elevated CO_2 had a negative effect on Pl with invasive *C. odorata*. Previous studies have demonstrated that many invasive plants excrete allelochemicals into the soil as root exudates that could impede the growth of soil fauna, including nematodes [15,16]. For example, *C. odorata* excretes flavonoids and alkaloids that have strong allelopathic effects on soil biota [35]. Plant-feeding nematodes are most closely associated with plant roots and thus potentially most sensitive to allelochemicals. For this reason they may suffer stronger suppression by C. *odorata*. Furthermore, some defensive chemical compounds in invasive plants may have anti-herbivore properties in addition to allelopathic [36]. As a result, invasive plants can experience less root herbivory [37] and gain advantage in competition with native plant species. Engelkes et al. [38] also suggested that successful range-expanding plants such as invasives often experience less belowground herbivory compared with native species due to their defensive characteristics. On the contrary, a significant increase in the proportion of Pl with *E. heterophyllum* indicated that elevated CO_2 had more positive effects on Pl with the native plant *E. heterophyllum*. This is consistent with our hypothesis that positive or negative impact of elevated CO_2 through plants on nematodes depends on plant species. Overall, our results suggested that invasive plant *C. odorata* benefited from elevated CO_2, which was consistent with the findings of other studies [39,40].

Elevated CO_2 increases the C allocation from plants to soil, usually increasing the abundance of fungi [27,41]. However, in our study, fungal-feeding nematodes did not significantly increase under elevated CO_2. In contrast, elevated CO_2 significantly decreased the proportion of fungi-feeding nematodes with *A. adenophora*. Elevated CO_2 significantly decreased the abundance and proportion of Om&Ca with *A. adenophora* as well. This agrees with the findings of Neher et al. [30], who found that the predatory nematode abundance and omnivore biomass declined substantially with elevated CO_2 in Sweet Gum soils.

In this study, *Helicotylenchus* and *Hirschmanniella* were the dominant Pl taxa in the soil (Table 2). However, we found few *Helicotylenchus* in soil planted with *C. odorata*. This result was similar to that reported by Van der Putten et al. [37], who found that in newly colonized areas, the invasive plant *Ammophila arenaria* had fewer feeding-specialist nematodes *Heterodera* sp. than did native plants there. We speculate that *C. odorata* may secrete chemical compounds that inhibit the growth of *Helicotylenchus*. Additionally, *Helicotylenchus* was generally more abundant than other Pl taxa; thus it may feed on more plant roots and subsequently cause greater damage to plants. *C. odorata* inhibits the growth of *Helicotylenchus* and so avoids their herbivory, which may be related to its success as an invasive species.

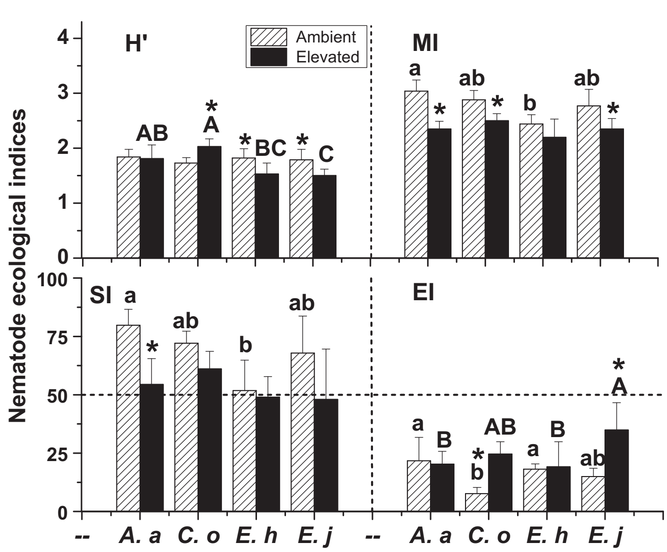

Fig. 4. Nematode ecological indices of response to invasive and native plants under ambient and elevated CO_2. Lowercase letters indicate significant differences ($P < 0.05$) among plant species under ambient CO_2. Capital letters indicates the significant difference among different plant species under elevated CO_2. Asterisks indicates significant differences between CO_2 treatments in the same plant species. H′, Shannon–Weaver diversity; MI, maturity index; EI, enrichment index; SI, structure index. *A.a*, *Ageratina adenophora*; *C.o*, *Chromolaena odorata*; *E.h*, *Eupatorium heterophyllum*; *E.j*, *Eupatorium japonicum*. Error bars represent standard errors ($n = 5$).

4.2. Nematode ecological indices under ambient and elevated CO_2

Elevated CO_2 significantly affected the ecological indices of MI, EI, and SI. Invasive feature affected the ecological index H′. Significant interaction effects between elevated CO_2 and invasive feature were also observed with H′. Here, H′ is an ecological index for nematode diversity. Under elevated CO_2, the value of H′ with *A. adenophora* was significantly higher than that with *E. japonicum*, and the value of H′ with *C. odorata* was significantly higher than two native species, indicating that invasive plants can increase nematode diversity. Previous studies have also reported that invasive plants had higher belowground biological diversity compared with native plants [31,42,43]. Generally, invasive plants reduce aboveground plant diversity. Why do these invasive plants cause higher belowground biodiversity than native plants under elevated CO_2? Our explanation is that higher belowground biodiversity can enhance the stability of soil functions [44,45]. Additionally, Elton's classic hypothesis considered that diversity increases the resistance ability of ecosystem to alien species invasion [46]. Thus, it is reasonable to infer that invasive species increase belowground biological diversity, thereby promoting their own colonization.

Changes in MI, SI, and EI reflect changes in the nematode food-web structure and the extent of soil disturbance [26]. Under elevated CO_2, MI levels with the four plant species were decreased, which indicated that soil food-web structures in all treatments were altered. Bongers and Ferris [21] found MI to be reduced in soils contaminated by heavy metals. EI and SI less than 50 indicated the highest level of disturbance. EI greater than 50 and SI less than 50 indicated intermediate disturbance. Both EI and SI greater than 50 indicated a slightly disturbed level, while EI less than 50 and SI greater than 50 indicated an undisturbed level [26,47]. Our results (see Fig. 4) showed that under elevated CO_2, EI was less than 50 and SI was greater than 50 in the treatments with invasive plants. Both SI and EI were less than 50 in the treatments with native plants, indicating that the soil food web structure in native-plant treatments was more disturbed than that in the invasive-plant treatments. This supports our hypothesis that invasive plants created more stable belowground ecosystems in our experiments.

5. Conclusions

Although limited in scope, this study showed that nematode communities were significantly affected by elevated CO_2 and plants invasive characteristics. Elevated CO_2 significantly decreased the proportion of Pl with invasive plant *C. odorata*, but strongly increased the proportion of Pl with native plant *E. heterophyllum*. Thus, elevated CO_2 exacerbated the effects of invasive plant *C. odorata* on the belowground ecosystem, which might lead to an advantage by causing the invasive to experience less belowground herbivory in competition with native-plant species. Nematode ecological indices demonstrated that soil nematode diversity was higher with invasives than with native plants under elevated CO_2. The results of EI and SI indicated that, compared with native plants, invasive plants developed more stable belowground ecosystems that improved their colonization success under CO_2 enrichment. If invasive plants more strongly benefit from elevated CO_2, their effects on belowground ecosystems may further increase their success.

Conflict of interest

All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report. We certify that the submission is not under review at any other publication.

Author contributions

Y.L. Feng contributed to experimental design. Hai F. Xiao performed experiments and wrote the manuscript. D.A. Schaefer and X.D. Yang provided technical support. Y.L. Zhen Yan B. Lei and Yang P. Li discussed the results and commented on the manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejsobi.2013.07.002.

References

[1] C.D. Thomas, A. Cameron, R.E. Green, M. Bakkenes, L.J. Beaumont, Y.C. Collingham, B.F.N. Erasmus, M.F. de Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley, A.S. van Jaarsveld, G.F. Midgley, L. Miles, M.A. Ortega-Huerta, A.T. Peterson, O.L. Phillips, S.E. Williams, Extinction risk from climate change, Nature 427 (2004) 145–148.

[2] J.J. Hellmann, J.E. Byers, B.G. Bierwagen, J.S. Dukes, Five potential consequences of climate change for invasive species, Conserv. Biol. 22 (2008) 534–543.

[3] G.R. Walther, A. Roques, P.E. Hulme, M.T. Sykes, P. Pyšek, I. Kűhn, M. Zobel, S. Bacher, Z. Botta-Dukát, H. Bugmann, B. Czúcz, J. Dauber, T. Hickler, V. JarošÍk, M. Kenis, S. Klotz, D. Minchin, M. Moora, W. Nentwig, J. Ott, V.E. Panov, B. Reineking, C. Robinet, V. Semenchenko, W. Solarz, W. Thuiller, M. Vilà, K. Vohland, J. Settele, Alien species in a warmer world: risks and opportunities, Trends Ecol. Evol. 24 (2009) 686–693.

[4] B.A. Bradley, D.M. Blumenthal, D.S. Wilcove, L.H. Ziska, Predicting plant invasions in an era of global change, Trends Ecol. Evol. 25 (2010) 310–318.

[5] M. Bonkowski, Protozoa and plant growth: the microbial loop in soil revisited, New Phytol. 162 (2004) 617–631.

[6] D.A. Wardle, R.D. Bardgett, J.N. Klironomos, H. Setälä, W.H. Van der Putten, D.H. Wall, Ecological linkages between aboveground and belowground biota, Science 304 (2004) 1629–1633.

[7] L.A. Biederman, T.W. Boutton, Biodiversity and trophic structure of soil nematode communities are altered following woody plant invasion of grassland, Soil Biol. Biochem. 41 (2009) 1943–1950.

[8] K. Ekschmitt, G. Bakonyi, M. Bongers, T. Bongers, S. Boström, H. Dogan, A. Harrison, P. Nagy, A.G. O'Donnell, E.M. Papatheodorou, B. Sohlenius, G.P. Stamou, V. Wolters, Nematode community structure as indicator of soil functioning in European grassland soils, Eur. J. Soil Biol. 37 (2001) 263–268.

[9] J.G. Ehrenfeld, Effects of exotic plant invasions on soil nutrient cycling processes, Ecosystems 6 (2003) 503–523.

[10] B.J. Wilsey, H.W. Polley, Aboveground productivity and root-shoot allocation differ between native and introduced grass species, Oecologia 150 (2006) 300–309.

[11] Y.L. Feng, Y.B. Lei, R.F. Wang, R.M. Callaway, A. Valiente-Banuet, Inderjit, Y.P. Li, Y.L. Zheng, Evolutionary tradeoffs for nitrogen allocation to photosynthesis versus cell walls in an invasive plant, Proc. Natl. Acad. Sci. USA 106 (2009) 1853–1856.

[12] L.Y. Song, C.H. Li, S.L. Peng, Elevated CO_2 increases energy-use efficiency of invasive *Wedelia trilobata* over its indigenous congener, Biol. Invasions 12 (2010) 1221–1230.

[13] C.S. Denton, R.D. Bardgett, D. Richard, R. Cook, P.J. Hobbs, Low amounts of root herbivory positively influences the rhizosphere microbial community of a temperate grassland soil, Soil Biol. Biochem. 31 (1999) 155–165.

[14] J. Kao-Kniffin, T.C. Balser, Elevated CO_2 differentially alters belowground plant and soil microbial community structure in reed canary grass-invaded experimental wetlands, Soil Biol. Biochem. 39 (2007) 517–525.

[15] J.L. Hierro, R.M. Callaway, Allelopathy and exotic plant invasion, Plant Soil 256 (2003) 29–39.

[16] D.L. Porazinska, P.D. Pratt, R.M. Giblin-Davis, Consequences of *Melaleuca quinquenervia* invasion on soil nematodes in the Florida Everglades, J. Nematol 39 (2007) 305–312.

[17] J.D. Weidenhamer, R.M. Callaway, Direct and indirect effects of invasive plants on soil chemistry and ecosystem function, J. Chem. Ecol. 36 (2010) 59–69.

[18] M.F. Cotrufo, A. Gorissen, Elevated CO_2 enhances below-ground allocation in three perennial grass species at different levels of N availability, New Phytol. 137 (1997) 421–431.

[19] K.J. van Groenigen, A. Gorissen, J. Six, D. Harris, P.J. Kuikman, J.W. Van Groenigen, C. van Kessel, Decomposition of ^{14}C-labeled roots in a pasture soil exposed to 10 years of elevated CO_2, Soil Biol. Biochem. 37 (2005) 497–506.
[20] Y.P. Li, Y.L. Feng, G. Barclay, No evidence for evolutionarily decreased tolerance and increased fitness in invasive *Chromolaena odorata*: implications for invasiveness and biological control, Plant Ecol. 213 (2012) 1157–1166.
[21] T. Bongers, H. Ferris, Nematode community structure as a bioindicator in environmental monitoring, Trends Ecol. Evol. 14 (1999) 224–228.
[22] H.L. Chen, B. Li, C.M. Fang, J.K. Chen, J.H. Wu, Exotic plant influences soil nematode communities through litter input, Soil Biol. Biochem. 39 (2007) 1782–1793.
[23] W.J. Liang, Y.L. Lou, Q. Li, S. Zhong, X.K. Zhang, J.K. Wang, Nematode faunal response to long-term application of nitrogen fertilizer and organic manure in Northeast China, Soil Biol. Biochem. 41 (2009) 883–890.
[24] G.W. Yeates, T. Bongers, R.G.M. De Goede, D.W. Freckman, S.S. Georgieva, Feeding habitats in soil nematode families and genera-an outline for soil ecologists, J. Nematol 25 (1993) 315–331.
[25] T. Bongers, The maturity index, an ecological measure of environmental disturbance based on nematode species composition, Oecologia 83 (1990) 14–19.
[26] H. Ferris, T. Bongers, R.G.M. de Goede, A framework for soil food web diagnostics: extension of the nematode faunal analysis concept, Appl. Soil Ecol. 18 (2001) 13–29.
[27] T.H. Jones, L.J. Thompson, J.H. Lawton, T.M. Bezemer, R.D. Bardgett, T.M. Blackburn, K.D. Bruce, P.F. Cannon, G.S. Hall, S.E. Hartley, G. Howson, C.G. Jones, C. Kampichler, E. Kandeler, D.A. Ritchie, Impacts of rising atmospheric carbon dioxide on model terrestrial ecosystems, Science 280 (1998) 441–443.
[28] E. Paterson, R. Neilson, A.J. Midwood, S.M. Osborne, A. Sim, B. Thornton, P. Millard, Altered food web structure and C-flux pathways associated with mineralisation of organic amendments to agricultural soil, Appl. Soil Ecol. 48 (2011) 107–116.
[29] J.D. Hoeksema, J. Lussenhop, J.A. Teeri, Soil nematodes indicate food web responses to elevated atmospheric CO_2, Pedobiologia 44 (2000) 725–735.
[30] D.A. Neher, T.R. Weicht, D.L. Moorhead, R.L. Sinsabaugh, Elevated CO_2 alters functional attributes of nematode communities in forest soils, Funct. Ecol. 18 (2004) 584–591.
[31] W.J. Liang, F.P. Li, Q. Li, W.D. Zhang, Temporal dynamics of soil nematode community structure under invasive *Ambrosia trifida* and native *Chenopodium serotinum*, Helminthologia 44 (2006) 29–33.
[32] X.F. Mao, F. Hu, B.S. Griffiths, H.X. Li, Bacterial-feeding nematodes enhance root growth of tomato seedlings, Soil Biol. Biochem. 38 (2006) 1615–1622.
[33] 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.
[34] Q. Li, C.G. Xu, W.J. Liang, S. Zhong, X.H. Zheng, J.G. Zhu, Residue incorporation and N fertilization affect the response of soil nematodes to the elevated CO_2 in a Chinese wheat field, Soil Biol. Biochem. 41 (2009) 1497–1503.
[35] S.R. Ambika, Allelopathic plants.5. *Chromolaena odorata* (L.) King and Robinson, Allelopathy J. 9 (2002) 35–41.
[36] N. Cappuccino, D. Carpenter, Invasive exotic plants suffer less herbivory than non-invasive exotic plants, Biol. Lett. 1 (2005) 435–438.
[37] W.H. van der Putten, G.W. Yeates, H. Duyts, C. Schreck Reis, G. Karssen, Invasive plants and their escape from root herbivory: a worldwide comparison of the root-feeding nematode communities of the dune grass *Ammophila arenaria* in natural and introduced ranges, Biol. Invasions 7 (2005) 733–746.
[38] T. Engelkes, E. Morriën, K. Verhoeven, T.M. Bezemer, A. Biere, J.A. Harvey, L. McIntyre, W. Tamis, W.H. van der Putten, Successful range expanding plants experience less above-ground and below-ground enemy impact, Nature 456 (2008) 946–948.
[39] C.C. Daehler, Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration, Annu. Rev. Ecol. Evol. Syst. 34 (2003) 183–211.
[40] D. Blumenthal, C. Mitchell, P. Pyšek, V. Jarošík, Synergy between pathogen release and resource availability in plant invasion, Proc. Natl. Acad. Sci. USA 106 (2009) 7899–7904.
[41] P. Fortuna, L. Avio, S. Morini, M. Giovannetti, Fungal biomass production in response to elevated atmospheric CO_2 in a *Glomus mosseae*–*Prunus cerasifera* model system, Mycol. Prog. 11 (2012) 17–26.
[42] J.J. Duda, D. Carl Freeman, J.M. Emlen, J. Belnap, S.G. Kitchen, J.C. Zak, E. Sobek, M. Tracy, J. Montante, Differences in native soil ecology associated with invasion of the exotic annual chenopod, *Halogeton glomeratus*, Biol. Fertil. Soils 38 (2003) 72–77.
[43] N.L. Angeloni, K.J. Jankowski, N.C. Tuchman, J.J. Kelly, Effects of an invasive cattail species (*Typha*×*glauca*) on sediment nitrogen and microbial community composition in a freshwater wetland, FEMS Microbiol. Lett. 263 (2006) 86–92.
[44] S. Yachi, M. Loreau, Biodiversity and ecosystem productivity in a fluctuating environment: the insurance hypothesis, Proc. Natl. Acad. Sci. USA 96 (1999) 1463–1468.
[45] M. Loreau, Biodiversity and ecosystem functioning: recent theoretical advances, Oikos 91 (2000) 3–17.
[46] C. Elton, The Ecology of Invasion by Plant and Animal, Methuen, London, 1958.
[47] Y.J. Li, J.H. Wu, H.L. Chen, J.K. Chen, Nematodes as bioindicator of soil health: methods and application, Chin. J. Appl. Ecol. 16 (2005) 1541–1546.