

Changes in soil microbial community and enzyme activity along an exotic plant *Eupatorium adenophorum* invasion in a Chinese secondary forest

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The exotic plant, *Eupatorium adenophorum*, has invaded rapidly across southwest China, damaging native ecosystems and causing great economic losses. Soil microbes, as an important component of belowground community, can drive nutrient cycling and regulate plant competition in terrestrial ecosystems. Therefore, knowledge of the soil microbial community and function will enhance our understanding of the mechanism of exotic plant invasive process in natural ecosystems. In this study, we examined the soil microbial community, soil enzyme activity, soil property and plant community along an invasive gradient of *E. adenophorum* in a southwest Chinese secondary forest. The soil analysis demonstrated that heavy invasion significantly increased the total P and NO₃-N contents, whereas it significantly decreased the total N and soil organic matter contents. The available P content was significantly decreased by moderate invasion. The *E. adenophorum* invasion significantly decreased the biomass of total soil microbes, as well as Gram-negative bacteria, actinomycetes, arbuscular mycorrhizal (AM) fungi and non-AM fungi. However, *E. adenophorum* invasion significantly increased the activities of soil urease, acid phosphatase, polyphenol oxidase and peroxidase. Non-metric multidimensional scaling showed that soil microbial composition and soil enzyme composition were significantly different in the three *E. adenophorum* invaded sites. Partial Mantel tests indicated that plant composition was the most important factor for structuring soil microbial and enzyme compositions. The results suggest that changes in soil microbial community structure and enzyme activity may play an important role in the process of *E. adenophorum* invasion in a Chinese secondary forest ecosystem.

Crofton weed, invasion density, microbial biomass, community composition, enzyme activity

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Rapid expansion of invasive plants is threatening biodiversity, productivity, and ecosystem health throughout the world [1,2]. Understanding the strategies employed by invasive plants for rapid spread is one of the most important fields in ecology. Although multiple hypotheses have been put forward to explain the remarkable success of many invasive plant species, most of these studies have mainly focused on aboveground features [3]. As an important component of belowground community, soil microbes can drive nutrient cycling and regulate plant competition in terrestrial

ecosystems [4,5], they therefore can play an important role in driving plant invasion [5,6].

An increasing number of studies have shown that exotic invasive plants can influence the abundance, composition and functioning of soil microbial communities [6–13]. Furthermore, these changes in soil communities usually promote the growth of invasive plants, yet inhibit native plant performance, mainly driven by the lack of soil pathogens to invasive plants in the new habitats [14,15], the preferential enrichment of microbial pathogens to native plants [16,17], or the inhibition of microbial mutualism with native plants [18–20].

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Eupatorium adenophorum Spreng. (croftonweed), native to Mexico and Costa Rica of Central America, is a world-wide invasive weed. This plant has invaded southwest China since the 1940s and become one of the most noxious invaders, damaging native ecosystems and causing great economic losses in China [21,22]. Several mechanisms have been proposed to explain the success of *E. adenophorum* invasion, such as rapid genetic differentiation [23], extraordinary biological characteristics including high seed production and germination [24,25], wide biogeographic adaptation [26,27], and production of allelopathic compounds that reduce native plant growth [28–30]. However, little information has been available on the responses of soil microbial community structure and function to *E. adenophorum* invasion, except for a few studies of its effect on soil bacterial composition [31,32].

In order to understand the response of soil microbial community structure and function to *E. adenophorum* invasion, in this study we selected three invasive intensities of *E. adenophorum* in a secondary forest of Yunnan Province, southwestern China. The soil microbial communities were examined based on the analysis of phospholipid fatty acids (PLFA). Soil enzyme activities concerning the cycling of carbon (C), nitrogen (N) and phosphorus (P) were tested, and soil characteristics and plant community were investigated. The objectives were to (1) explore how the soil microbial community and enzyme activity change along a *Eupatorium adenophorum* invasion gradient, and (2) find out the important factors that affect soil microbial community and enzyme compositions during the *E. adenophorum* invasion in a Chinese secondary forest.

1 Materials and methods

1.1 Study site and sampling

The study was conducted in an evergreen mixed forest in Xishan of Kunming, Yunnan Province, southwest China (24°58'33.1"N, 102°37'04.9"E), with an average elevation of 2200 m above sea level, a mean annual precipitation of 932.7 mm, and a mean annual temperature of 15.6°C. This location used to be dominated by the native plant community, but has been invaded by *E. adenophorum* for the last 30 years. The location is southwest-facing with an average slope of 25°. The *E. adenophorum* invasion has obviously started from roadside to hillside with different displacement intensities, i.e. the cover of *E. adenophorum* decreased from the roadside to hillside. Three sites with different covers of *E. adenophorum* were selected. The first site was not invaded by *E. adenophorum* (hereafter referred to as NI). There were about 18 plant species, including three woody trees, 12 shrubs and three grasses in this site dominated by *Myrsine africana* with an average cover of 59%. The second site, with 40%–50% cover of *E. adenophorum*, was termed as the moderately invaded site (MI). There were about 19

plant species, including four woody trees, 12 shrubs and three grasses in this site. The third site, with more than 70% cover of *E. adenophorum*, was termed as the heavily invaded site (HI). There were about 16 plant species, including four woody trees, nine shrubs and three grasses. The distances between the sites were more than 100 m.

Three plots (20 m × 20 m each) were randomly set in each site and the distances between plots were more than 100 m. Five composite soil samples, each consisting of three soil cores (3.5 cm in diameter, 15 cm deep), were randomly collected from each plot in April, 2008. The soil samples were immediately transported to the laboratory and sieved (2-mm mesh) to remove plant roots and debris. A part of fresh soil (ca. 20 g each sample) was stored at –80°C for PLFA analysis. The remaining soil samples (ca. 300 g) were stored at 4°C or air-dried according to the requirement of analyses of soil characteristics and soil enzyme activities.

1.2 Soil characteristics

The soil organic matter (SOM) was determined using K₂Cr₂O₇-H₂SO₄ oxidation method [33]. Total nitrogen (N) was measured using the Kjeldahl method [34]. Soil mineral N was extracted with 2 mol L⁻¹ KCl, then the concentrations of NO₃⁻-N and NH₄⁺-N in the KCl extracts were determined by hydrazine sulfate colorimetry and indophenol blue colorimetry, respectively [35]. The total phosphorus (P) and available P were determined using the colorimetric Molybdenum-blue-method [36]. The pH value (1:2.5 solution, soil to water) was measured using a Glass Electrode-calomel Electrode pH meter (Mettler-Toledo International Inc., China).

1.3 PLFA analysis

PLFAs were analyzed following a procedure described by Frostegård et al. [37]. Briefly, lipids were extracted from 4.0 g of freeze-dried soil with a chloroform:methanol:citrate buffer mixture (1:2:0.8, v/v/v), and the lipids were separated into neutral lipids, glycolipids and phospholipids on a silicic acid column. Phospholipids were then converted to fatty acid methyl esters by mild alkaline methanolysis. Fatty acid methyl esters were analyzed using a gas chromatograph (GC 7890, Agilent Technologies, Inc., USA) coupled with a mass spectrometer (5973N MSD, Agilent Technologies, Inc., USA). The individual fatty acid peaks were quantified based on peak area converted to nmol lipid g⁻¹ soil DW using methyl nonadecanoate fatty acid (19:0) as an internal standard. Fatty acids were designated in terms of total number of carbon atoms: number of double bonds, followed by the position of the double bond from the methyl end (ω) of the molecule. The prefixes 'i' and 'a' indicate iso- and anteiso branching, 'br' indicates an unknown methyl branching position, 'cy' indicates cyclopropane fatty acid, and 'Me' refers to the position of methyl group from the carboxyl end of the chain.

The sum of PLFAs with no more than 20 carbon atoms in length was used to calculate total microbial biomass [38]. Furthermore, we used a sum of indicative PLFAs to represent the biomass of different microbial groups, including Gram-negative bacteria (G^-) [39], Gram-positive bacteria (G^+) [39], actinomycetes [10,37], arbuscular mycorrhizal fungi (AMF) [40], and fungi (excluding AMF) [38,41].

1.4 Soil enzyme activity

The activities of eight enzymes which are related to the cycling of C, N, and P were measured in this study, including β -glucosidase (E.C. 3.2.1.21), invertase (E.C. 3.2.1.26), cellulase (E.C. 3.2.1.4), urease (E.C. 3.5.1.5), acid phosphatase (E.C. 3.1.3.2), alkaline phosphatase (E.C. 3.1.3.1), polyphenol oxidase (E.C. 1.10.3.1), and peroxidase (E.C. 1.11.1.7).

β -glucosidase and phosphatase activities were measured using the p-nitrophenol (pNP) method [42]. The substrates were pNP- β -D-glucopyranoside for β -glucosidase and pNP-phosphate for acid phosphatase and alkaline phosphatase. The urease activity was determined using urea as substrate, and the soil mixture was incubated at 37°C for 5 h. The residual urea was determined by a colorimetric method [43]. Cellulase and invertase activities were analyzed using CM-cellulose and sucrose as substrates, respectively, following the method described by Schinner and von Mersi [44]. Polyphenol oxidase activity was determined using pyrogallol as substrate and oxidation products were determined colorimetrically at 430 nm [45]. Peroxidase activity was determined using pyrogallol and hydrogen peroxide as substrates following the same procedure as measure of polyphenol oxidase activity.

1.5 Data analysis

The sum of indicative PLFAs that represent different taxonomic microbial groups was used instead of individual PLFA data to visualize the real status of soil microbial composition [46]. One-way analysis of variance (ANOVA) followed by a Least Square Difference (LSD) *post hoc* test was carried out to explore the differences in soil characteristic variables (SOM, Total N, NO_3^- -N, NH_4^+ -N, total P, available P, and pH), biomass of total soil microbes and every microbial group (G^+ , G^- , actinomycetes, AMF, and fungi) and soil enzyme activities (β -glucosidase, invertase, cellulase, urease, acid phosphatase, alkaline phosphatase, polyphenol oxidase, and peroxidase) among the three *E. adenophorum* invaded sites. To assure normality and homogeneity of PLFA and enzyme activity data, PLFA data were $\log(x_i+1)$ transformed, and soil enzyme activity data were x_i/x_{mean} standardized in ANOVA analysis.

Distance matrices of soil microbial community (PLFA content dataset, wisconsin-sqrt transformed) and soil enzyme composition (enzyme activity datasets, wisconsin-sqrt

transformed) were generated by calculating Bray-Curtis dissimilarity. Non-metric multidimensional scaling (NMDS) was carried out to visualize the distance matrices of soil microbial composition and soil enzyme composition of all plots. *Eupatorium adenophorum* invasion intensity were fitted as centroids, and soil characteristic variables and plant species were fitted as vectors onto the ordination plots using the 'envfit' function. Distance matrices of plant composition and soil characteristics were also generated by calculating Bray-Curtis dissimilarity. The relationships of soil microbial composition or soil enzyme composition with soil characteristics and plant composition were explored with Mantel tests. The independent effects of soil characteristics and plant composition on soil microbial composition and soil enzyme composition were explored with partial Mantel tests by controlling plant composition and soil characteristics, respectively.

All statistical analyses were carried out in R 2.15.1 [47]. NMDS and 'envfit' were carried out in the package *Vegan* [48]. Mantel and partial Mantel tests were carried out in the package *Ecodist* [49].

2 Results

2.1 Soil characteristics and plant community

One-way ANOVA indicated that soil NO_3^- -N ($F = 65.89$, $P < 0.001$), total P ($F = 41.67$, $P < 0.001$), SOM ($F = 16.11$, $P = 0.001$), total N ($F = 9.47$, $P = 0.001$) and available P ($F = 5.55$, $P = 0.01$) contents were significantly different among the NI, MI and HI, but the NH_4^+ -N content and pH were not significantly different. For example, total P and NO_3^- -N contents were significantly higher in HI than in MI and NI, whereas SOM content was significantly lower in HI than in MI and NI (Table 1). The total N content was significantly higher in NI than in MI and HI. The available P content was significantly higher in NI than in MI, but there was no significant difference between NI and HI and between MI and HI (Table 1).

NMDS analysis indicated that *E. adenophorum* invasion significantly influenced plant composition ($r^2 = 0.53$, $P = 0.001$). For example, the abundance of *Hypericum acmosepalum*, *M. Africana*, *Rhododendron speciferum*, *Rubus foliolosus*, and *Tetrastigma obovatum* was significantly decreased by *E. adenophorum* invasion (Table 2).

2.2 Soil microbial biomass and composition

In total, 30 PLFAs characterizing soil microbes were obtained, including 12:0, i12:0, 13:0, a13:0, 14:0, i14:0, i15:0, a15:0, 16:0, i16:0, 16:1 ω 5, 16:1 ω 7, 17:0, a17:0, i17:0, cy17:0, 10Me17:0, 18:0, i18:0, 18:2 ω 6, 9, 18:1 ω 5, 18:1 ω 6, 18:1 ω 7, 18:1 ω 8, 18:1 ω 9, 18:1 ω 10, 10Me18:0, cy19:0, br19:0, 20:0. Within the 30 PLFAs, 22 were used

Table 1 Soil characteristics in the different *Eupatorium adenophorum* invaded sites

Variable	NI ^{a)}	MI ^{b)}	HI ^{c)}
Soil organic matter (SOM, g kg ⁻¹)	47.02±6.27a	43.85±1.32a	31.66±2.41b
Total N (g kg ⁻¹)	1.77±0.25a	1.43±0.04b	1.40±0.03b
NO ₃ ⁻ -N (mg kg ⁻¹)	3.01±0.42a	2.46±0.41a	8.08±1.32b
NH ₄ ⁺ -N (mg kg ⁻¹)	9.31±0.62a	9.88±0.65a	8.29±1.34a
Total P (g kg ⁻¹)	0.59±0.02a	0.48±0.03a	0.76±0.08b
Available P (mg kg ⁻¹)	5.93±0.12a	4.27±0.75b	5.10±0.50ab
pH value	6.57±0.15a	6.31±0.13a	6.56±0.16a

a) no *E. adenophorum* invaded site; b) *E. adenophorum* moderately invaded site; c) *E. adenophorum* heavily invaded site. Values are means ± SE of 3 replicates. Values with different letters in the same row indicate significant difference at $P < 0.05$.

Table 2 Plant abundance in the different *Eupatorium adenophorum* invaded sites

Plant species	NI ^{a)}	MI ^{b)}	HI ^{c)}
<i>Alnus nepalensis</i> D. Don	2.4±1.0	0.7±0.5	0.8±0.5
<i>Buddleja officinalis</i> Maxim.	1.3±0.9	0.0±0.0	0.0±0.0
<i>Coniogramme intermedia</i> Hieron.	0.0±0.0a	0.0±0.0a	3.9±0.9b
<i>Cornus capitata</i> Wall.	1.9±1.2	0.0±0.0	0.0±0.0
<i>Cotoneaster microphyllus</i> Wall. ex Lindl.	0.0±0.0a	3.9±1.6b	1.3±0.9ab
<i>Cupressus duclouxiana</i> Hickel	0.0±0.0a	5.8±1.2b	2.7±1.1a
<i>Echinochloa colonum</i> (Linn.) Link	0.7±0.5	1.9±1.0	0.3±0.2
<i>Elsholtzia ciliata</i> (Thunb.) Hyland.	1.2±0.8	0.0±0.0	0.0±0.0
<i>Eupatorium adenophorum</i> Spreng.	0.0±0.0a	52.0±2.6b	98.4±1.8c
<i>Hypericum acmosepalum</i> N. Robson	2.7±0.7a	0.5±0.3b	0.0±0.0b
<i>Ilex chamaebuxus</i> C.Y. Wu ex Y.R. Li	6.1±2.6	3.9±2.0	0.0±0.0
<i>Lindera communis</i> Hemsl.	3.8±2.6	0.0±0.0	0.0±0.0
<i>Michelia yunnanensis</i> Franch. ex Finet & Gagnep.	0.0±0.0	0.0±0.0	1.3±0.9
<i>Microstegium ciliatum</i> A. Camus	1.6±0.6	1.8±1.2	1.2±0.5
<i>Myrsine africana</i> L.	70.2±3.6a	13.2±1.9b	7.5±1.5b
<i>Osyris wightiana</i> Wall. ex Wight	0.0±0.0	0.0±0.0	2.2±1.5
<i>Pinus armandii</i> Franch.	0.0±0.0	1.0±0.7	1.3±0.9
<i>Pinus yunnanensis</i> Franch.	1.2±0.8	2.0±0.8	1.3±0.9
<i>Prinsepia utilis</i> Royle	0.0±0.0	2.8±1.2	3.4±1.7
<i>Pyracantha fortuneana</i> (Maxim.) H.L. Li	1.8±1.2	0.0±0.0	0.0±0.0
<i>Quercus variabilis</i> Blume	0.0±0.0	2.4±1.6	1.3±0.9
<i>Rhododendron speciferum</i> Franch.	9.4±1.6a	1.1±0.6b	3.3±1.8b
<i>Rosa longicuspis</i> Bertol.	5.1±3.4	1.4±0.9	0.0±0.0
<i>Rubus foliolosus</i> D. Don	3.7±1.6a	0.6±0.4b	0.0±0.0b
<i>Rumex hastatus</i> D. Don	3.1±1.5ab	4.0±1.2a	0.0±0.0b
<i>Setaria plicata</i> T. Cooke	2.3±0.8a	2.6±1.0a	0.0±0.0b
<i>Tetrastigma obovatum</i> (Laws.) Gagnep.	1.9±0.8a	0.0±0.0b	0.0±0.0b
<i>Viburnum foetidum</i> Wall.	0.0±0.0	5.1±2.1	2.7±1.8
Total abundance	120.0±6.0a	106.7±9.9b	133.3±5.0c
Species richness	7.2±1.2ab	8.2±1.5a	6.2±1.3b

a) no *E. adenophorum* invaded site; b) *E. adenophorum* moderately invaded site; c) *E. adenophorum* heavily invaded site. Values are means ± SE of 3 replicates. Values with different letters in the same row indicate significant difference at $P < 0.05$.

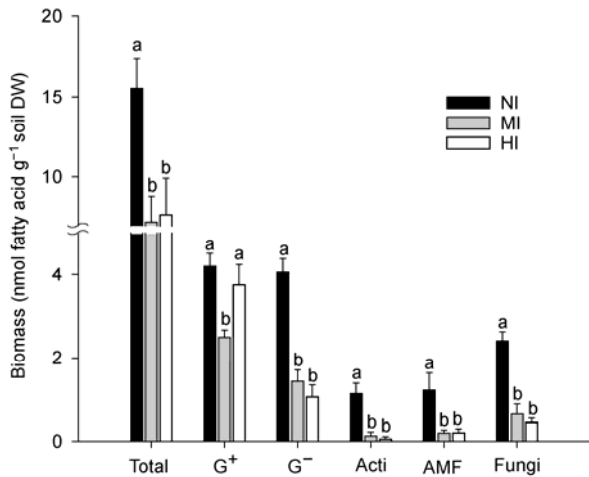
to indicate taxonomic microbial groups, in which G⁺ were represented by nine, G⁻ by eight, actinomycetes by two, AMF by one, and fungi by two (excluding AMF) (Table 3). The biomass of total microbes ($F = 15.09$, $P < 0.001$), G⁻ ($F = 29.97$, $P < 0.001$), actinomycetes ($F = 14.19$, $P < 0.001$), AMF ($F = 5.65$, $P = 0.015$) and fungi ($F = 30.99$, $P < 0.001$) were significantly higher in NI than in MI and HI, but there was no significant difference between MI and HI (Figure 1). In addition, the biomass of G⁺ ($F = 6.26$, $P = 0.011$) was

significantly lower in MI than in NI and HI, but there was no significant difference between NI and HI (Figure 1).

NMDS analysis demonstrated that soil microbial composition was significantly different among the NI, MI and HI ($r^2 = 0.53$, $P = 0.001$ in *envfit*, Figure 2(a)). Furthermore, the soil microbial composition was significantly related to available P ($r^2 = 0.45$, $P = 0.002$), total N ($r^2 = 0.38$, $P = 0.004$), total P ($r^2 = 0.27$, $P = 0.015$), and seven plant species: *E. adenophorum* ($r^2 = 0.63$, $P = 0.001$), *M. africana* (r^2

Table 3 Phospholipid fatty acid (PLFA) markers used for taxonomic microbial groups in this study

Microbial group	Specific PLFA marker	References
Gram-negative bacteria (G ⁻)	Sum of 16:1 ω 7, cy17:0, 18:1 ω 5, 18:1 ω 6, 18:1 ω 7, 18:1 ω 8, 18:1 ω 10, and cy19:0	[39]
Gram-positive bacteria (G ⁺)	Sum of i12:0, a13:0, i14:0, i15:0, a15:0, i16:0, a17:0, i17:0, and i18:0	[39]
Actinomycetes	Sum of 10Me17:0 and 10Me18:0	[10,37]
Arbuscular mycorrhizal fungi (AMF)	16:1 ω 5	[40]
Fungi (excluding AMF)	Sum of 18:2 ω 6,9 and 18:1 ω 9	[38,41]

**Figure 1** Biomass of soil microbes in the different *Eupatorium adenophorum* invaded sites. Bars with different letters indicate significant difference at $P < 0.05$. NI, no *E. adenophorum* invaded site; MI, *E. adenophorum* moderately invaded site; HI, *E. adenophorum* heavily invaded site; Total, total soil microbes; G⁺, Gram-positive bacteria; G⁻, Gram-negative bacteria; Acti, actinomycetes; AMF, arbuscular mycorrhiza fungi; Fungi, fungi (excluding AMF).

$= 0.60$, $P = 0.001$), *H. acmosepalum* ($r^2 = 0.41$, $P = 0.002$), *R. speciferum* ($r^2 = 0.38$, $P = 0.003$), *Michelia yunnanensis* ($r^2 = 0.31$, $P = 0.009$), *Cotoneaster microphyllus* ($r^2 = 0.27$, $P = 0.013$) and *Pinus armandii* ($r^2 = 0.26$, $P = 0.014$).

Mantel tests indicated that soil microbial composition

was significantly correlated with plant composition ($r = 0.47$, $P = 0.001$, Table 4). Furthermore, plant composition was still significant after the effect of soil characteristics variables was controlled for by partial Mantel tests ($r = 0.48$, $P = 0.001$). Soil characteristics exhibited no significant influence on microbial composition (Table 4).

2.3 Soil enzyme activity and composition

One-way ANOVA indicated that polyphenol oxidase ($F = 69.66$, $P < 0.001$), peroxidase ($F = 40.27$, $P < 0.001$), urease ($F = 26.24$, $P < 0.001$) and acid phosphatase ($F = 3.52$, $P = 0.044$) activities were significantly different among the NI, MI and HI. For example, urease and acid phosphatase activities were significantly higher in HI than in NI and MI, but there was no significant difference between NI and MI (Figure 3). Polyphenol oxidase and peroxidase activities significantly increased with invasion intensity of *E. adenophorum*. However, the activities of invertase, β -glucosidase, cellulase and alkaline phosphatase were not significantly different among the NI, MI and HI (Figure 3).

NMDS analysis showed that soil enzyme composition was significantly different among the NI, MI and HI ($r^2 = 0.68$, $P = 0.001$ in *envfit*, Figure 2(b)). Furthermore, the soil enzyme composition was significantly related to NO_3^- -N ($r^2 = 0.55$, $P = 0.001$), total P ($r^2 = 0.45$, $P = 0.001$), total N ($r^2 = 0.26$, $P = 0.026$), and seven plant species *E. adenophorum*

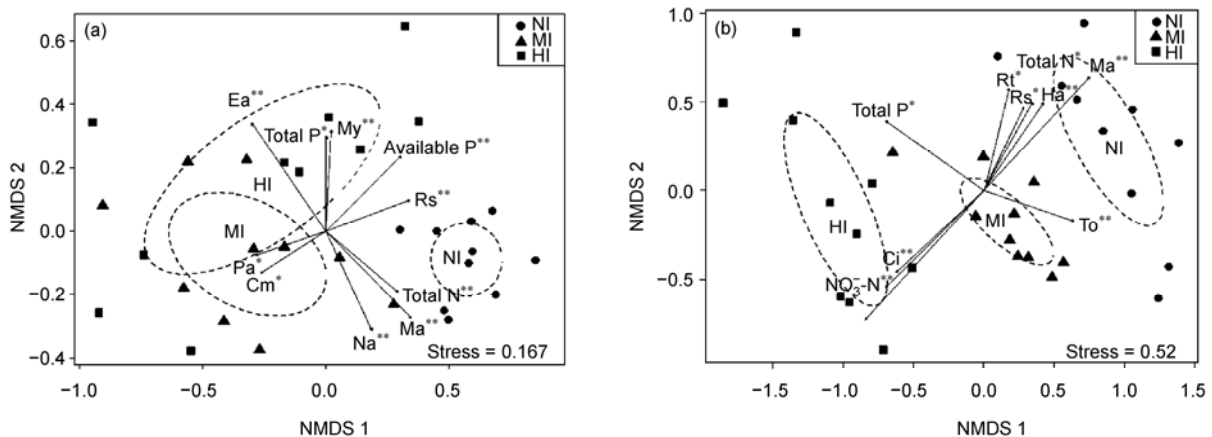
**Figure 2** Non-metric multidimensional scaling (NMDS) ordinations of (a) soil microbial composition ($r^2 = 0.53$, $P = 0.001$) and (b) soil enzyme composition ($r^2 = 0.68$, $P = 0.001$) of all plots. Vectors shown on the ordination plots were significant at $*P < 0.05$ and $**P < 0.01$. NI, no *E. adenophorum* invaded site; MI, *E. adenophorum* moderately invaded site; HI, *E. adenophorum* heavily invaded site; Ci, *Coniogramme intermedia*; Cm, *Cotoneaster microphyllus*; Ea, *Eupatorium adenophorum*; Ha, *Hypericum acmosepalum*; Ma, *Myrsine africana*; My, *Michelia yunnanensis*; Pa, *Pinus armandii*; Rf, *Rubus foliolosus*; Rs, *Rhododendron speciferum*; To, *Tetrastigma obovatum*.

Table 4 Mantel and partial Mantel tests of soil microbial composition and enzyme composition with plant composition and soil parameters

	Plant composition				Soil characteristics			
	Mantel test		Partial Mantel test ^{a)}		Mantel test		Partial Mantel test	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Microbial composition	0.47	0.001	0.48	0.001	0.09	0.107	-0.12	0.971
Soil enzymes	0.56	0.001	0.49	0.001	0.17	0.029	-0.07	0.785

a) Plant composition was partialled out when calculating partial Mantel *r* of soil characteristics, and soil characteristics was partialled out when calculating partial Mantel *r* of plant composition.

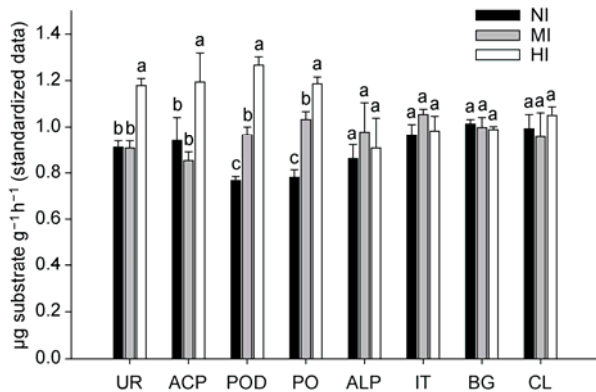


Figure 3 Soil enzyme activities in the different *Eupatorium adenophorum* invaded sites. Bars with different letters indicate significant difference at $P < 0.05$. NI, no *E. adenophorum* invaded site; MI, *E. adenophorum* moderately invaded site; HI, *E. adenophorum* heavily invaded site; UR, urease; ACP, acid phosphatase; PPO, polyphenol oxidase; PO, peroxidase; ALP, alkaline phosphatase; IT, invertase; BG, β -glucosidase; CL, cellulase.

($r^2 = 0.87$, $P = 0.001$), *M. africana* ($r^2 = 0.68$, $P = 0.001$), *Coniogramme intermedia* ($r^2 = 0.42$, $P = 0.001$), *H. acmosepalum* ($r^2 = 0.33$, $P = 0.006$), *T. obovatum* ($r^2 = 0.31$, $P = 0.003$), *R. foliolosus* ($r^2 = 0.25$, $P = 0.015$) and *R. speciferum* ($r^2 = 0.21$, $P = 0.038$).

Mantel tests indicated that soil enzyme composition was significantly correlated with soil characteristics ($r = 0.17$, $P = 0.029$) and plant composition ($r = 0.56$, $P = 0.001$, Table 4). Furthermore, plant composition was still significant after the effect of soil characteristics variables was controlled for by partial Mantel tests ($r = 0.49$, $P = 0.001$), but soil characteristics exhibited no significant influence after the effect of plant composition was partialled out (Table 4).

3 Discussion and conclusions

The biomass of total soil microbes and major microbial groups were significantly lower in *E. adenophorum* invaded sites than in the no *E. adenophorum* invaded site in this study. Similarly, fungal biomass was reduced by invasion of *Berberis thunbergii* [10] and *Centaurea maculosa* [50], and the biomass of AMF was decreased by *Alliaria petiolata* invasion [19] in sites in North America. Furthermore, our results showed that the soil microbial composition was significantly different in the three *E. adenophorum* invaded

sites, agreeing with previous studies that bacterial composition was significantly different between lightly and heavily *E. adenophorum* invaded sites in China [31,32]. Similarly, soil microbial compositions were dramatically altered by the invasion of the exotic plants *B. thunbergii* in New Jersey [10], *Mikania micrantha* in southeast China [12], *Falcataria moluccana* in Hawaii [51], *Centaurea solstitialis* and *Aegilops triuncialis* in Northern California [52], and *Lonicera maackii* in Kentucky [8].

Soil microbial community can be directly shaped by allelopathy of exotic plants [5,53]. *Eupatorium adenophorum*, as a strong allelopathic plant [28–30], can release some chemical compounds that have significant antibacterial and antifungal effects [54], which can decrease soil microbial biomass and alter microbial composition. In addition, our results indicated that *E. adenophorum* invasion significantly influenced plant communities, which are likely to produce litter, root exudates and organic matter that differ in their chemical composition and role in maintaining nutrient pools. Therefore, the soil microbial community could be indirectly affected by *E. adenophorum* invasion via changing the local plant community [53,55].

Soil enzyme composition was significantly different in the three *E. adenophorum* invaded sites in this study. Similarly, some previous studies have reported that soil enzyme compositions were significantly changed by the exotic plant *M. micrantha* invasion in southeast China [12] and *B. thunbergii* in New Jersey [10]. Furthermore, soil enzyme composition was significantly correlated with plant composition in our study. Plant released-compounds such as polysaccharides, aromatic compounds and esters, together with detritus and root exudates serve as sources of substrates for enzymatic degradation and provide the energy and elements' necessary for enzyme synthesis [56]. Thus, variation in plant inputs to soil, which arise from changes in plant composition by *E. adenophorum* invasion, has the potential to cause variation in enzyme composition [56].

Urease and acid phosphatase activities were significantly higher in the site heavily invaded with *E. adenophorum* compared with the moderately and no invaded sites. Similarly, urease and acid phosphatase activities were increased by *M. micrantha* invasion in southeast China [12] and *F. moluccana* invasion in Hawaii [51]. Elevated activities of related P and N degrading enzymes in the invaded sites indicated that *E. adenophorum* may accelerate nutrient

mineralization rates of organic P and N [12,51]. In addition, the polyphenol oxidase and peroxidase activities were significantly increased with invasion intensity of *E. adenophorum*. Similarly, phenol oxidase activity was increased by the invasion of *M. micrantha* in southeast China [12] and *B. thunbergii* in New Jersey [10]. These oxidative enzyme activities, degrading more complex materials, often correlate with high decomposition rate and mass loss rate in SOM [57], which is consistent with our result that SOM content significantly decreased with *E. adenophorum* invasion. In addition, *E. adenophorum* invasion did not significantly influence the activities of alkaline phosphatase, β -glucosidase, cellulase, and invertase in the present study. Previous studies also have reported some inconsistent results of these enzyme activities affected by different exotic plants. For example, alkaline phosphatase and β -glucosidase activities were significantly decreased by *B. thunbergii* invasion in New Jersey [10], whereas they were significantly increased by *M. micrantha* invasion in southeast China [12]. It suggests that soil enzymes show various responses to different exotic plant invasions in ecosystems.

In addition, some soil chemical properties were significantly different in the three *E. adenophorum* invaded sites. For example, the total P and NO_3^- -N contents were significantly higher whereas the total N content was significantly lower in *E. adenophorum* invaded sites than in the no *E. adenophorum* invaded site. The results are consistent with other studies that found similar changes in soil properties with exotic plant invasion [58–60]. For example, *Amaranthus viridis* invasion significantly increased concentration of soil total P in Senegal [58], and *Agropyron cristatum* invasion decreased total N at the northern edge of the Great Plains, USA [59]. Soil NO_3^- -N content was 30% higher in invaded ecosystems than in native ecosystems based on the meta-analysis of 94 experimental studies [60]. The reason for the differences in soil chemical property might be the effect of *E. adenophorum* invasion or also might be a spatial heterogeneity effect. Furthermore, there was a reverse changing trend between microbial biomass and some soil enzyme activities with increasing *E. adenophorum* invasion. This is because soil enzymes not only come from living and dead microbes, but also from plant roots and residues and soil animals. Enzymes stabilized in the soil matrix accumulate or form complexes with humus, clay, and humus-clay complexes. It is thought that 40% to 60% of enzyme activity can come from stabilized enzymes, thus soil enzyme activity does not necessarily correlate highly with microbial biomass [61].

In conclusion, *E. adenophorum* invasion conspicuously influenced local plant species composition and some soil properties such as NO_3^- -N, total N, available P, total P and SOM contents. The soil microbial composition and soil enzyme composition were significantly different in the three *E. adenophorum* invaded sites. Furthermore, there were inversely changing trends between microbial biomass and

some soil enzyme activities with increasing *E. adenophorum* invasion. Plant composition was the most important factor for structuring soil microbial and enzyme compositions. Our results suggest that changes in soil microbial community structure and enzyme activity may play an important role in the invasive process of *E. adenophorum* in a southwest Chinese secondary forest. However, we also recognize that further studies, concerning more exotic plant species in wide geographic scales, will promote our understanding of the mechanism of exotic plant invasive processes in natural ecosystems.

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