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Impacts of the invasive annual herb *Ambrosia artemisiifolia* L. on soil microbial carbon source utilization and enzymatic activities

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ABSTRACT

There is currently much interest in the interactions between exotic plants and soil organisms. Exotic invasive species can have profound effects on the microbial community of the soil and positive feedback of soil biota to invasive plants may facilitate their successful invasion. To better understand the impacts of *Ambrosia artemisiifolia* L. invasion on microbial carbon source utilization and related microbiological parameters, soils were sampled from two invaded sites, i.e., historically-invaded (HINVA), recently-invaded (LINVA) sites and two non-invaded sites, i.e., grassland (NINVA) and native-plant (NATIV) sites in late April. Soil biochemical properties, enzyme activities, and microbial biomass were determined. Meanwhile, carbon source utilization intensity was examined based on the Biolog community-level physiological profile (CLPP) method. The two invaded sites had significantly higher total phosphorus, available nitrogen and phosphorus than non-invaded sites. Microbial biomass nitrogen and phosphorus, and invertase and catalase activities were also significantly higher in soils from invaded sites. The soil microbial community from the HINVA site most profoundly improved soil fertility. Microbial utilization of carbohydrate groups significantly increased in the invaded sites relative to non-invaded sites, especially the utilization of carbohydrates and amines/amides. Soil from the HINVA site had higher efficiency in carbon source utilization, especially for carbohydrates and amino acids. Principal components analysis (PCA) of carbon substrate utilization data revealed distinct differentiation in soil microbial community functions among the four studied sites. Redundancy analysis (RDA) indicated that better soil biochemical conditions, especially the microbial quotient (Cmic/Corg) and available nitrogen values were associated with higher soil carbon utilization in *A. artemisiifolia* invaded sites. The improvement of soil fertility as well as microbial community function in invaded soils may be beneficial to *A. artemisiifolia* and contribute to its establishment in new habitats.

1. Introduction

Exotic plant species have direct and indirect effects on the composition and function of soil communities which may create a feedback that influences aboveground communities [1]. There are increasing reports that invasive plants can alter the composition and abundance of microbial communities [2–4], stimulate or inhibit microbial activity [5,6], increase or decrease the rate of nutrient cycling in the soil [7–9] and therefore impact ecosystem functions. Invasive plants may alter soil biota in a new habitat in ways which lead to either positive or negative plant–soil biota feedback effects [10,11]. Positive soil feedback may facilitate exotic plant invasion by providing greater benefits from these mutual interactions to invasive species in their adventive ranges than in their native ranges [12,13] which might be an important factor contributing to competitive success and dominance. For instance, the invasive species *Ageratina adenophora* can alter soil chemistry and soil biota in invaded sites, creating conditions that favor itself but inhibit natives through positive plant–soil biota feedback effects [10].

Ambrosia artemisiifolia L. (common ragweed – Asteraceae), a North American native annual herb, has now been widely

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introduced and has expanded into numerous regions of the world, including European countries such as Italy [14], Croatia [15], Hungary [16], Ukraine and Russia [17,18]. The species has also rapidly established and expanded its range in Asia (Japan, China) [19,20] and Australia [21]. *A. artemisiifolia* has deleteriously impacted the structure, biodiversity and function of numerous ecosystems in these regions including cropping systems. This noxious weed is also responsible for causing allergies because of its abundant allergenic pollen release [22,23]. Numerous studies have determined possible factors contributing to the increased prevalence and distribution of this species into new habitats and regions. These work has focused on factors such as evolutionary adaptive traits [24–26], genetic variability [19,27], allelopathic inhibitory effects [28,29], facilitation through association with arbuscular mycorrhizal fungi (AMF) [30,31] as well as the absence of natural enemies [32]. Relatively few studies have investigated the impact of *A. artemisiifolia* on soil microbial communities and especially their functioning. Recently, the soil enzyme activity and impact on soil fertility of the invasive *A. artemisiifolia* and a native annual monocot weed (*Digitaria chinensis*) were compared in a common garden experiment in China and showed that *A. artemisiifolia* was more effective at improving soil fertility and activities of soil enzymes such as urease and phosphatase than the native weed [33]. However, knowledge of the direct and indirect impacts of *A. artemisiifolia* invasion on soil nutrient availability and cycling as well as microbial composition and function is limited. Little is known about the soil microbiological mechanism or driving process which may be contributing to the rapid range expansion of *A. artemisiifolia* in numerous regions of the world including southern China.

Based on previous findings related to plant–soil microbe interactions, we hypothesized that *A. artemisiifolia* invasion may exert influences on soil biochemical properties and microbial function characteristics, which would be beneficial and contribute to its spread in introduced regions. The aims of our study were to: (1) explore changes in soil fertility, enzyme activities, and microbial function of soils having different *A. artemisiifolia* invasion histories; (2) investigate the possible soil microbiological mechanism facilitating invasion of *A. artemisiifolia* in new habitats.

2. Materials and methods

2.1. Experimental sites

The study was conducted in 2010 in a sequence of old fields in the town of Li in Zhengjiang district, located in Shaoguan city, Guangdong Province of southern China (24°48′N, 113°36′E). The region has a humid and subtropical monsoon climate with an average annual air temperature of 18 °C–22 °C. Monthly mean minimum air temperatures fluctuate between 8 °C and 11 °C, and maximum air temperatures range between 27 °C and 29 °C. Annual precipitation ranges from 1400 mm to 2400 mm. There are two main seasons: a wet season from March to August and a dry season from September to February. Mean number of sunlight hours in the region ranges from 1473 h to 1925 h, with an average 310 frost-free days.

Four old fields, about 1 km apart and having different *A. artemisiifolia* L. invasion histories and vegetation composition were selected as experimental sites: (1) a site (herein referred to as HINVA) colonized by *A. artemisiifolia* six years prior to the start of this study. This site was dominated by *A. artemisiifolia* with an 85%–95% coverage of the sample area; (2) a site (LINVA) colonized by *A. artemisiifolia* about three years prior to the start of this study with the highest single species coverage of 20%–35%. Also present but less dominant were native species including *Urena lobata*, *Smilax bonanox*, *Paederia scandens*, and *Garcinia mangostana*; (3) a site not colonized by *A. artemisiifolia* (NINVA) and dominated (80%–90% cover) by a mixture of native herbaceous species including *Cynodon dactylon*, *Festuca arundinacea*, *Paspalum natatu*, and *Commelina communis*; (4) A site not colonized by *A. artemisiifolia* (NATIV) and dominated (>80% cover) by a mixture of native species including *Toxicodendron vernicifluum*, *Sapium discolor*, *Lygodium japonicum*, *Gardenia jasminoides*, *Pinus massoniana*, *G. mangostana*, *U. lobata*, *Mallotus apelta*, *Vitex negundo*, *Melia azedarach*, *S. bonanox*, *Broussonetia papyrifera*, *Paederia scandens* and *Rhus chinensis*. Laterictic red soil with similar texture, depth, and landscape position was present at all four sites. Within each of the sites, four sample plots (5 m × 5 m) were randomly established.

2.2. Soil sampling and measurement

Soil samples were taken from the experimental sites in late April 2010. For each sample, surface litter (if present) was removed and the top 20 cm of soil was collected using a 5-cm diameter circular soil corer. In HINVA and LINVA sites, soil adhering to the roots of *A. artemisiifolia* was collected. Since *A. artemisiifolia* was not present within the NINVA and NATIV sites, soil samples were taken from roots of randomly selected plant species. Five soil samples were taken from each of the four plots at each site and thoroughly mixed. Using the cone and quartering method, an aliquot of the four soil samples per site was stored at field moisture content in a refrigerator at 4 °C between the time of sampling and analysis of microbial biomass and enzyme activities. Another aliquot of soil was air-dried at room temperature, ground to pass through a 2-mm sieve to remove large organic matter fragments and stored in a lined paper bag for chemical analyses. The remainder of each soil was stored at −20 °C for analysis of community level physiological profiles within a week.

Soil chemical analyses were conducted at the Soil Ecology Laboratory of South China Agricultural University. Soil organic matter (SOM) was determined by the potassium dichromate volumetric method [34]. Total nitrogen (TN) was determined using the Kjeldahl method [35]. The total amount of phosphorus (TP) in soil was measured at 700 nm by spectrophotometry of ammonium molybdate after the soil sample was digested with melting NaOH [36]. Total potassium (TK) content was measured by the NaOH flame photometry method. Soil alkali-hydrolyzable nitrogen (AN), available phosphorus (AP) and available potassium (AK) were analyzed [37]. Four replicates were performed for each analysis.

Soil microbial biomass C, N, and P were measured by the fumigation extraction method [38,39]. Soil microbial C was extracted with 100 ml 0.5 M K_2SO_4 determined using a heated $K_2Cr_2O_7$–H_2SO_4 digestion and estimated as the difference in extractable organic C between fumigated and unfumigated soils using a correction factor (K_c) of 0.45. Microbial biomass N was extracted with 50 ml of 2 M KCl and measured photometrically at 570 nm after the ninhydrin reaction. The correction factor (K_{en}) was 3.1, which indicates 1 mg biomass nitrogen per g dry soil per 3.1 mg ninhydrin-reactive nitrogen per g dry soil [40]. Microbial biomass P was extracted with 100 ml 0.5 M $NaHCO_3$ and was determined photometrically at 882 nm as a blue phosphate molybdic acid complex using a correction factor (K_c) of 0.40 [41]. All microbiological and biochemical determinations were replicated three times and reported on a soil oven (105 °C) dry basis. The soil microbial quotient, i.e., Cmic/Corg (the ratio of the microbial biomass C to organic C), Nmic/Nt (the ratio of the microbial biomass N to total N), and Pmic/Pt (the ratio of the microbial biomass P to total P) were also calculated.

Soil catalase activity was measured using the 0.1 N $KMnO_4$ titration method [42]. The urease activity was determined by a

modified method [43]. Data for urease activity were recorded with a spectrophotometer at 578 nm and expressed as mg NH_4–N released g^{-1} dry soil in 24 h. Invertase activity was assayed using the 3,5-dinitrosalicylic acid technique [44]. Data were read with a spectrophotometer at 508 nm and expressed as μg of glucose produced g^{-1} dry soil in 24 h. For the determination of proteinase activity, a colorimetric ninhydrin procedure was applied, data were read at 500 nm and expressed as μg NH_3–N produced g^{-1} dry soil in 24 h [45].

2.3. Biolog substrate utilization data

The potential metabolic diversity of soil microbial communities was evaluated using Biolog ECO plates (Biolog Inc., Hayward, CA) through the inoculation of 95 individual carbon sources plus a water control on a 96 well plate [46]. The field-moist soil (equivalent to 10 g of dry soil) was added to 100 ml of sterile 0.85% NaCl solution in a glass bottle and shaken for 20 min with a rotary shaker (160 rpm) at 25 °C. The soil samples was then diluted to a final 10^{-3} suspension in sterile 0.1 mol L^{-1} phosphate buffer (adjust to pH 7.0). Each well of the microplate was inoculated with 150 μl of diluted soil suspension for 9 d at 25 °C. Color development was quantified by determining absorbance at 590 nm with a microplate reader at regular time intervals for 24 h. Each Biolog plate had a control well (blank) that was subtracted from all other wells to correct for background color. Wells that had negative values were set to zero for the analyses. Optical density values obtained at 72 h incubation represented the most significant difference among the soils from experimental sites and were used to assess bacterial functional diversity and for statistical analyses. Three replicates per field site were performed.

Average well color development (AWCD) of substrate utilization at A590 was calculated as the average optical density across all wells per plate with the equation: AWCD = n_i/31, where n_i is the relative optical density of the *i*th well, which was corrected by the color development in the control well [47].

2.4. Statistical analyses

Since the four experimental sites were not field replicated, the four sampling plots within each site were used as pseudo-replicates for statistical analysis, and the analysis was treated as if it was a randomized experiment. Differences in soil biochemical characteristics, enzyme activities, utilization of carbon substrates groups among the four experimental sites were analyzed with one-way ANOVA followed by Duncan's multiple range test (at the 0.05 level) for post hoc comparisons. Data that were not normally distributed were log-transformed prior to analysis. All figures show non-transformed data and standard errors (SE). Community level physiological profiles (CLPP) data were analyzed by principal components analysis (PCA). Statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA).

The relationship between soil biochemical characteristics and carbon substrate utilization was analyzed using redundancy analysis (RDA), a canonical community ordination method. This method was selected according to an initial detrended correspondence analysis with gradient length <3.0, which suggested a linear ordination model should be used. The significance of soil biochemical variables to explain the variance in carbon substrate utilization was assessed using Monte Carlo simulations. In the RDA diagram, the length and direction of the arrows denoting different soil biochemical variables indicate their approximate correlation with the ordination axis. A more detailed description of this method has been provided [48,49]. RDA was performed using CANOCO 4.5 [48] and results visualized with the extension CanoDraw for Windows.

3. Results

3.1. Soil fertility properties

Amounts of SOM, total and available N, P and K of soils in the four experimental sites are shown in Table 1. All of the variables (except total potassium) tested from the HINVA soils were significantly higher than soils in the two sites not colonized by *A. artemisiifolia*. Total P, available N and P of soils with the presence of *A. artemisiifolia* were significantly higher than soils without *A. artemisiifolia*. The contents of available N, total and available P in HINVA soils were 6.4%, 23.3%, 9.6% higher than those in the LINVA soils, while SOM, total N, total and available *K* contents were significantly higher than those in the LINVA soils by 18.9%, 23.4%, 22.6%, 42.4% respectively. No significant differences in the various parameters tested were detected between the NINVA and NATIV soils except for available P and total *K*.

3.2. Soil microbial biomass

Microbial biomass C, N and P in *A. artemisiifolia* colonized soils were significantly higher than in NATIV soils as well as in NINVA soils (except microbial biomass C) (Table 1). The highest concentration of microbial biomass C, N and P were detected in the HINVA

Table 1
Soil bio-chemical properties in the four experimental sites located in Shaoguan City, Guangdong Province, China in late April 2010 (mean ± SE, $n = 4$).

Soil bio-chemical properties	HINVA	LINVA	NINVA	NATIV
Soil organic carbon (SOM, g kg^{-1})	26.92 ± 0.67a	22.65 ± 0.74b	22.08 ± 0.66b	21.83 ± 0.54b
Total nitrogen (TN, g kg^{-1})	1.37 ± 0.041a	1.11 ± 0.065b	1.19 ± 0.015b	1.14 ± 0.061b
Available nitrogen (AN, mg kg^{-1})	115.67 ± 3.36a	108.75 ± 3.95a	93.42 ± 5.88b	79.11 ± 5.79b
Total phosphorus (TP, g kg^{-1})	0.53 ± 0.016a	0.43 ± 0.021a	0.28 ± 0.0037b	0.29 ± 0.0026b
Available phosphorus (AP, mg kg^{-1})	8.53 ± 0.35a	7.78 ± 0.75a	0.69 ± 0.03c	1.05 ± 0.08b
Total potassium (TK, g kg^{-1})	19.59 ± 0.38a	15.98 ± 0.59b	18.94 ± 0.60a	16.67 ± 0.85b
Available potassium (AK, mg kg^{-1})	78.18 ± 2.03a	54.90 ± 1.27b	46.53 ± 4.16b	53.23 ± 2.20b
Microbial biomass C	614.62 ± 6.64a	587.01 ± 45.95a	624.01 ± 53.40a	309.48 ± 19.95b
Microbial biomass N	37.39 ± 1.16a	36.42 ± 0.45a	30.04 ± 1.08b	29.39 ± 1.61b
Microbial biomass P	10.98 ± 1.04a	8.96 ± 0.63a	5.02 ± 0.71b	4.38 ± 0.58b
Cmic/Corg	39.45 ± 1.28b	44.71 ± 3.22 ab	48.63 ± 3.63a	24.53 ± 1.87c
Nmic/Nt	27.34 ± 1.58b	33.25 ± 1.95a	25.31 ± 1.10b	25.99 ± 2.31b
Pmic/Pt	20.83 ± 1.80a	20.79 ± 0.79a	17.69 ± 2.35a	15.17 ± 2.04a

HINVA, site invaded at least 6 years by *A. artemisiifolia*; LINVA, site invaded no more than 4 years by *A. artemisiifolia*; NINVA, non-invaded site, dominated by herbaceous species; NATIV, non-invaded, dominated by a mixture of native species. Cmic/Corg: the microbial biomass C to total organic C ratios; Nmic/Nt: the ratio of the microbial biomass N to total N; Pmic/Pt: the ratio of the microbial biomass P to total P. Different letters indicate significant differences between means within a row according to Duncan's multiple range tests at the 0.05 level after ANOVA.

Table 2
Soil enzyme activities in the four experimental sites (means ± SE, $n = 4$).

Soil microbiological properties	HINVA	LINVA	NINVA	NATIV
Urease (g amino nitrogen g^{-1} 24 h^{-1})	298.93 ± 21.51a	180.38 ± 9.58c	228.79 ± 16.25 b	131.83 ± 9.44d
Proteinase (mg kg^{-1})	1.45 ± 0.05a	1.39 ± 0.01a	1.22 ± 0.04b	1.30 ± 0.06 ab
Invertase (mg glucose equivalents g^{-1} 24 h^{-1})	16.32 ± 0.65a	9.80 ± 0.56 b	7.63 ± 0.62c	6.21 ± 0.60c
Catalase (0.1 N $KMnO_4$ g^{-1} 20 min^{-1})	1.67 ± 0.05a	1.69 ± 0.03a	1.45 ± 0.08b	1.36 ± 0.05b

Different letters indicate significant differences between means within a row according to Duncan's multiple range tests at the 0.05 level after ANOVA. Acronyms for experimental sites are as for Table 1.

site which was 4.7%, 2.7% and 22.5% higher than in the LINVA site, respectively, though differences between these two sites were not significant. Soil in the NINVA site had significantly higher levels of microbial biomass C than that in the NATIV site. Concentrations of microbial biomass N and P in soils at the NINVA site were 2.2%, 14.6% higher than in the NATIV site, respectively.

The ratio of microbial biomass C to soil organic C (Cmic/Corg) in the NINVA site was 8.8% higher than the LINVA site and significantly higher than in the HINVA and NATIV sites. The ratio of Cmic/Corg in the HINVA site was significantly higher than in NATIV site. The ratios of microbial biomass N to total soil N (Nmic/Nt) in the LINVA site were significantly higher than in the other three sites, but no significant differences were detected among the HINVA, NINVA and NATIV sites. Soils in the HINVA site had a relatively higher Pmic/Pt value, but no significant difference was found among the four experimental sites. The ratio of Nmic/Nt and Pmic/Pt in the HINVA site was 5.2% and 37.3% higher than in the NATIV site.

3.3. Soil enzymes activities

Soils in the two sites colonized by *A. artemisiifolia* exhibited significantly higher urease, invertase and catalase activities than soils in the NATIV site (Table 2). The activities of protease for the HINVA and LINVA sites were 11.2% and 6.4% higher than the NATIV site respectively. Protease activities were significantly higher for the HINVA and LINVA sites compared with the NINVA site. Among the four experimental sites, the HINVA site had relatively higher soil enzyme activities except for catalase, which was 1.0% lower than that in the LINVA site. Significant differences in urease and invertase activities were observed between the HINVA and LINVA sites. Soil enzyme (except urease) activities for the NINVA site remained at the same level as for the NATIV site.

3.4. Community level physiological profiles of soil microbial communities

The development of average utilization (AWCD) of carbon sources for soil samples collected from the experimental sites followed a similar pattern with time during the 192 h incubation period (Fig. 1). Microbial activity, as measured by AWCD, increased rapidly from 24 to 72 h incubation period followed by relatively large variations among the four treatments until 96 h, and then increased steadily with incubation time. The AWCD values of soils from the HINVA and LINVA sites reached 1.23, 1.57 respectively after 72 h of incubation, significantly higher values than for the NINVA and NATIV sites. Remarkably, higher AWCD values were maintained in soils colonized by *A. artemisiifolia* relative to non-colonized sites until the end of the incubation period. The microbial communities from the NATIV soil consistently exhibited the lowest AWCD values while communities from the LINVA soils generally showed higher AWCD values, indicating that substrate utilization rates might be improved by colonization of *A. artemisiifolia*, especially in sites with lower intensity of invasion and shorter invasion histories. However, there were no significant differences in AWCD between microbial communities for soils collected from the two sites colonized by *A. artemisiifolia*, nor between soils from the two sites not colonized by this invasive species.

Soil microbial substrate utilization for the six substrate categories [50] in Biolog plates are shown in Fig. 2. The highest utilization of all carbon sources (except phenols) occurred for soils from the LINVA site. Mean carbohydrate and amino acid utilization of soil from the LINVA site were significantly greater than those in the other three sites. Carboxylic acid and polymers utilization from this site were also detected to be the highest. For the two sites invaded by *A. artemisiifolia*, carbohydrates were the most strongly utilized, and phenols least utilized substrates. In contrast, soil from the NATIV site had the lowest utilization of carbohydrates, carboxylic acid and polymers, while soil from the NINVA site had lowest amino acid and amine/amide utilization. There was no significant difference in the utilization of amines/amides among the four experimental sites.

Principal components analysis (PCA) based on AWCD data after 72 h of incubation differentiated the samples from sites with and without *A. artemisiifolia* on canonical variate axis 2 (Axis 2). Samples from the LINVA and NATIV sites clustered to the upper end of axis 1, and samples from the HINVA and NINVA sites clustered below axis 1 (Axis 1). The four sites were distinctly divided by the two axes, and showed 58.8% of the variance between sites accounted for on the first axis, and an additional 28.9% of the variation accounted for on the second axis (Fig. 3). Further analysis of the carbon source loadings on the PCA axes indicated that amines/amides (Phenylethylamine), carbohydrates (Glucose-1-

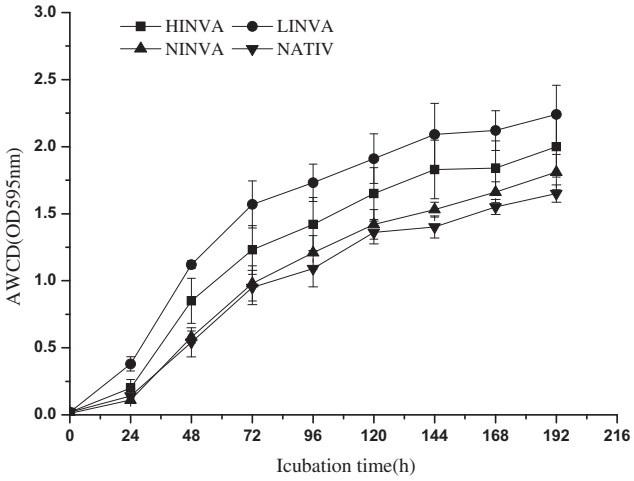

Fig. 1. Average well color development (AWCD) of substrate utilization profiles in BIOLOG EcoPlates™ for soil microorganisms in the four study sites ($n = 4$; SE bars are shown). HINVA, site invaded at least 6 years by *A. artemisiifolia*; LINVA, site invaded no more than 4 years by *A. artemisiifolia*; NINVA, non-invaded site, dominated by herbaceous species; NATIV, non-invaded, dominated by a mixture of native species.

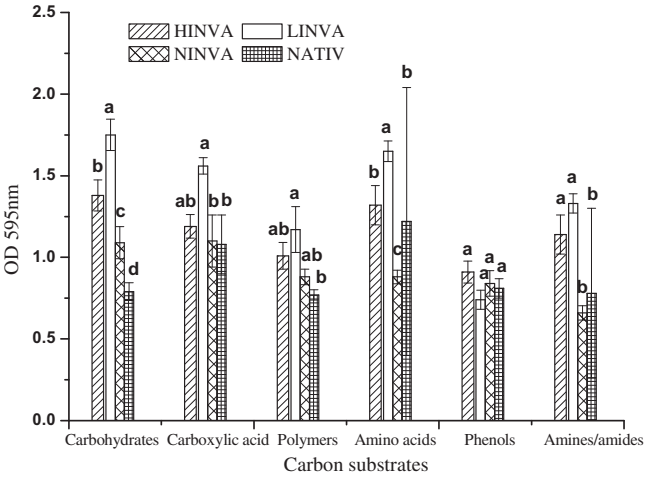

Fig. 2. Average utilization of carbon substrates from different substrate groups by soil microorganisms from the four study sites ($n = 4$; SE bars are shown). All values were based on 72 h incubation. Data with the same letter indicate no significant difference according to Duncan's multiple range tests at the 0.05 level. Acronyms for experimental sites are as for Fig. 1.

Phosphate, D-Mannitol, α-D-Lactose), carboxylic (γ-Hydroxybutyric Acid, D-Malic Acid, Pyruvic Acid Methyl Ester, Itaconic Acid) and amino acids (Glycyl-L-Glutamic Acid, L-Asparagine) had greater influences than the other substrates. These carbon resource components were most responsible for the separation between sites along axis 1 (Table 3). Similarly, carbohydrates (D-Galactonic Acid γ-Lactone, D-Xylose, *N*-Acetyl-D-Glucosamine), polymers (Tween 40), carboxylic (D-Glucosaminic Acid, α-Ketobutyric Acid) and amino acids (L-Threonine) were most responsible for treatment differences across axis 2, and thus were important variables for the discrimination between the presence and absence of *A. artemisiifolia*.

3.5. Correlation analysis of soil carbon utilization with soil biochemical variables

Redundancy analysis (RDA) results quantified the association between soil carbon substrates utilization and specific soil

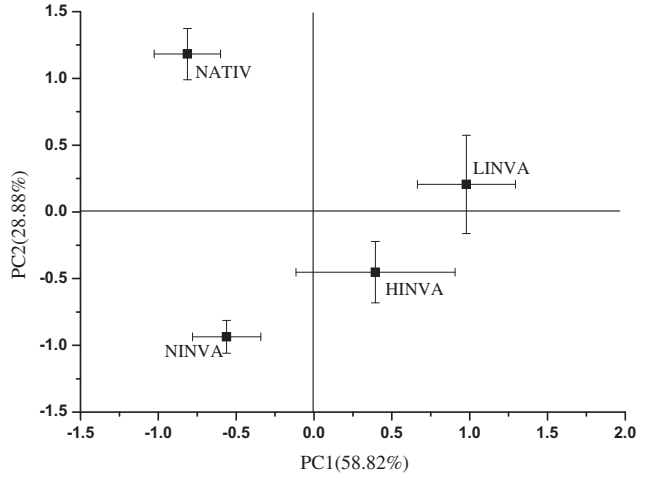

Fig. 3. PCA-ordination biplot of the first two principal component axes (PC1 and PC2) based on average utilization data of carbon substrate groups at 72 h in BIOLOG Ecoplates™ for four study sites ($n = 4$; SE bars are shown). Acronyms for experiment sites are the same as for Fig. 1.

Table 3
Correlations between carbon substrate utilization in Biolog EcoPlates and the first two principal components (PC 1 and PC 2).

Carbon substrate	R_{PC1}	Substrate class	Carbon substrate	R_{PC2}	Substrate class
Phenylethylamine	0.9918	AM	D-Glucosaminic acid	0.9996	CA
Glycyl-L-glutamic acid	0.9871	AA	D-Galactonic acid γ-lactone	0.9407	CH
L-Asparagine	0.9812	AA	α-Ketobutyric acid	0.9314	CA
Glucose-1-phosphate	0.9795	CH	Tween 40	0.7958	PL
γ-Hydroxybutyric acid	0.9626	CA	D-Xylose	0.7815	CH
D-Malic acid	0.9551	CA	*N*-Acetyl-D-glucosamine	0.7265	CH
D-Mannitol	0.9422	CH	L-Threonine	0.7014	AA
α-Cyclodextrin	0.9419	PL	β-Methyl-D-glucoside	0.6213	CH
Pyruvic acid methyl ester	0.9373	CA	D-Galacturonic acid	0.6199	CA
α-D-Lactose	0.9364	CH	L-Phenylalanine	0.6065	AA
Itaconic acid	0.9057	CA	D-Cellobiose	0.6063	CH
Putrescine	0.8968	AM	Glycogen	0.6023	PL
L-Serine	0.8795	AA			
i-Erythritol	0.8703	CH			
4-Hydroxy benzoic acid	0.8444	PH			

Data represent principal components analysis of combined data for 72-h incubations of Biolog EcoPlate™ plates for soil samples collected in four study sites. Only substrates with factor loadings >0.80, >0.60 in PC1 and PC2 respectively are presented.
PC 1 and PC 2 refer to principal components 1 and 2 from principal components analysis. R_{PC1}, R_{PC2} values refer to the eigenvector loading factor for each substrate. Substrates are grouped into six different guilds based on type of substrate. Abbreviations: CH: carbohydrates, CA: carboxylic acids, AM: amines/amides, AA: amino acids, PL: polymers, PH: phenols.

biochemical variables (SOM, TK, AN, AK, Cmic/Corg, Nmic/Nt and Pmic/Pt). The Tri-plot of the RDA from the full model is shown in Fig. 4. The first two canonical axes explained 65.8% and 3.8% of the variation in the carbon utilization data, and a total of 97.7% of soil carbon utilization − biochemical variables relations were loaded along axis 1 and axis 2 (axis 1 and axis 2 were responsible for 92.4% and 5.3% of the variation respectively) (Table 4). Approximately 71.2% of the variation in soil carbon utilization data was explained by all four significant canonical axes. Monte Carlo tests for the first and all canonical axes were significant (RDA, after 499 permutations, *F*-ratio = 15.407, *P*-value = 0.044), indicating that these biochemical properties may be important in explaining soil carbon utilization.

Results indicated that Cmic/Corg was the variable responsible for a higher contribution to the sample variability in soil carbon utilization ($r^2 = 0.6880$), followed by AN ($r^2 = 0.4656$). Nmic/Nt was also a statistically significant explanatory variable ($r^2 = 0.4323$, *P*-value = 0.010). The other four soil biochemical variables had limited influence on carbon utilization (Table 5). Nmic/Nt showed a strong positive relationship with utilization ability of major carbon sources except phenols. Significant positive correlations were observed between Nmic/Nt and amines/amides ($r = 0.690$, *P*-value = 0.003). AN and Cmic/Corg were significantly correlated with carbohydrate utilization levels (AN: $r = 0.692$, *P*-value = 0.003; Cmic/Corg: $r = 0.616$, *P*-value = 0.011). Soil samples from the four experimental sites (each with four replicates) could barely be grouped according to their similarity in substrate utilization patterns, except that samples from the NATIV site clustering on the lower left side of the triplot were separated completely from the other samples. Samples from the two sites colonized by

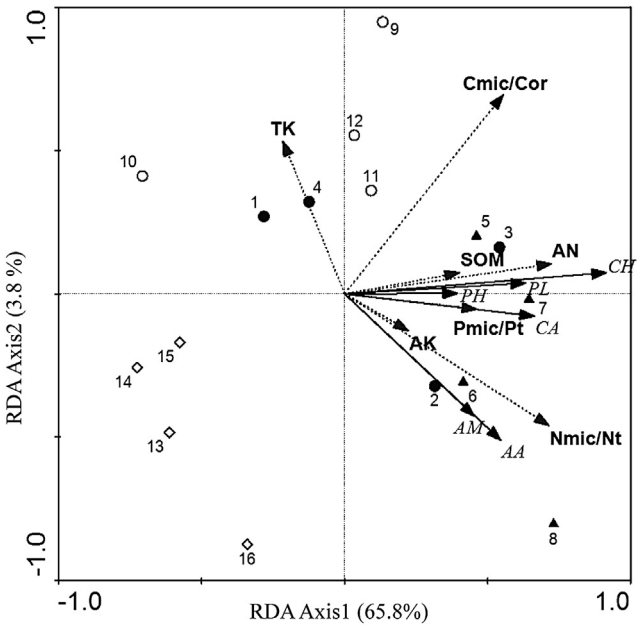

Fig. 4. RDA-ordination triplot of the first two axes (RDA axis 1 and RDA axis 2) of soil carbon utilization and biochemical parameters for four study sites. The soil biochemical parameters (expressed as explanatory variables) are presented as dotted line vectors, and the soil carbon utilization (response variables) are presented as solid line vectors. Four study sites, each with four replicates were denoted by: filled circles—HINVA; filled triangles—LINVA; empty circles—NINVA; empty diamond—NATIV. Acronyms for experimental sites are as for Fig. 1. Abbreviations: CH: carbohydrates, CA: carboxylic acids, AM: amines/amides, AA: amino acids, PL: polymers, PH: phenols.

A. artemisiifolia were more scattered in response to more variable soil nutrient conditions (Fig. 4).

4. Discussion

4.1. Changes in soil biochemical characteristics with *A. artemisiifolia* invasion

Significant differences in soil nutrient concentrations and soil enzyme activities were found between sites that were invaded by *A. artemisiifolia* compared with non-invaded sites. These results are consistent with findings from a comparative study between *A. artemisiifolia* and a native non-invasive annual grass weed, *Digitaria chinensis* in Shenyang, China [33]. In their study, *A. artemisiifolia* had a greater capacity to improve soil fertility (i.e. increased concentrations of NO_3—N, NH_4—N, available P and K) and activities of soil enzymes (urease, invertase and phosphatase) than the native weed (*D. chinensis*), thus increasing its competitive performance relative to the native species. Our work also revealed

Table 4
Eigen values of ordination axes for redundancy analysis (RDA). Contribution percentage of the RDA axes as well as correlations of soil carbon utilization and soil biochemical parameters.

Axis	Eigenvalue	Spec.-env. Correlation	Cumulative % variation of species	Cumulative % variation of spec.-env.	Sum of all canonical Eigen values
Axis 1	0.658	0.879	65.8	92.4	0.713
Axis 2	0.038	0.758	69.6	97.7	
Axis 3	0.010	0.413	70.6	99.1	
Axis 4	0.006	0.628	71.2	99.8	

Monte Carlo significance tests for soil carbon utilization data sets: sum of all eigen values, 1.000; Significance of first canonical axis, F value = 15.407, P = 0.044; Significance of all canonical axes, F value = 2.834, P = 0.048. Soil enzymes/carbon utilization was used as "species". Soil biochemical properties were used as "env."

Table 5
Correlations of soil biochemical parameters and the first two ordination axes of RDA (RDA1 and RDA2) for soil carbon utilization, the influence of soil biochemical parameters on soil carbon utilization.

Parameters included in the model	RDA1	RDA2	r^2	Pr (>r)	Symbol
SOM	0.9467	0.3220	0.1469	0.366	ns
TK	−0.2765	0.9610	0.1713	0.292	ns
AN	0.9538	0.3006	0.4656	0.012	*
AK	0.9721	−0.2345	0.0407	0.752	ns
Cmic/Corg	0.7426	0.6697	0.6880	0.002	**
Nmic/Nt	0.9571	−0.2897	0.4323	0.010	**
Pmic/Pt	0.9912	0.1324	0.1691	0.258	ns

r^2 values and Pr values (based on 499 permutations) represented the explanatory power of soil biochemical variables to the variation of soil carbon utilization and the corresponding significant level. Significance levels of the Pr values (based on 499 permutations): $P \leq 0.001$ = ***; ≤0.01 = **; ≤0.05 = *; ≤1.0 = ns.
SOM: soil organic matter; TK: total potassium; AN: alkali-hydrolyzable nitrogen; AK: available potassium; Cmic/Corg, Nmic/Nt and Pmic/Pt represent microbial biomass carbon, nitrogen and phosphorus.

non-significant differences in SOM content and total N between the recently colonized (LINVA) site and native sites. Moreover, SOM content, total N and P concentrations as well as soil enzyme activities in the historically invaded (HINVA) site were greatly enhanced relative to non-invaded sites suggesting that effects of *A. artemisiifolia* colonization on soil fertility are related to the amount of time a site has been colonized. Similar results have been reported for other exotic invasive plants [51]. However, soil available nitrate concentration in bare plots of a one-year-old field was reported to be 4.6 times higher than levels in plots dominated by *A. artemisiifolia* [52]. This result is not consistent with our finding that soil available nitrate concentrations were significantly higher in *A. artemisiifolia*-invaded sites than non-colonized sites. Lower soil available nitrate content reported during the early stages of invasion might be attributed to the high uptake of nitrogen by exotic plants (for instance, *Lantana camara*) and intense competition with soil microbes [53]. *A. artemisiifolia* was also shown to reduce nitrogen losses very early in succession by taking up and storing nitrogen, most of which was probably readily available to plants in subsequent years since the nitrogen was mostly present in leaf tissue [52]. However, additional work is needed to determine whether the increase in soil nitrogen concentration occurs as a result of the release of nitrogen taken up by *A. artemisiifolia* or is due to increased litter levels and soil organic matter.

4.2. Changes of soil microbial biomass with *A. artemisiifolia* invasion

Soil microbes play a significant role in plant-nutrient transformation and act as a small but labile reservoir of nutrients, providing important sources of soil carbon and available nutrients to plants [54]. In our study, *A. artemisiifolia* invasion resulted in a pronounced increase in soil microbial biomass N and P, while microbial biomass C levels did not differ from the herbaceous mixed species-dominated site (NINVA). Similarly, in a study conducted in Guangdong Province, China, soil biomass N and P beneath the exotic invader *L. camara* were significantly higher than those sampled underneath the edge of the canopy and 2–5 m away from *L. camara* individuals, while concentrations of microbial biomass C were similar in the three experimental soil sample locations [55]. The specific soil microbial community present beneath exotic plants such as *L. camara* and *A. artemisiifolia* may be responsible for the observed effects and would be beneficial because of their ability to immobilize nutrients thus directly reducing nutrient losses from surface runoff or leaching. In addition, phenolic acids (i.e., caffeic,

chlorogenic acids) and carbohydrates excreted from *A. artemisiifolia* roots may be beneficial to the growth and reproduction of soil micro-organisms.

Ratios of Cmic/Corg, Nmic/Nt, and Pmic/Pt were used to further investigate the effects of *A. artemisiifolia* invasion on soil microorganism performance since these indexes may reflect the efficiency of microbial incorporation, and the stabilization of organic C, N, and P by the soil mineral fractions [56]. The two invaded sites did not exhibit the highest Cmic/Corg ratios, but these were significantly higher than the ratio in the NATIV site. These results are consistent with previous findings showing improved average availability of organic C and accelerated cycling of the soil carbon pool in the presence of *A. artemisiifolia* [55]. The slightly elevated Pmic/Pt ratio in soil invaded by *A. artemisiifolia* suggested that the availability of soil P in these sites was nearly the same as a result of soil microbial activities. Arbuscular mycorrhizal fungi (AMF) which are believed to facilitate phosphorus uptake in plants may have played an important role in the soil P cycle beneath *A. artemisiifolia* plants. The ratio of Nmic/Nt in *A. artemisiifolia* invaded sites was higher than in non-colonized sites, especially the LINVA site, which had a significantly higher Nmic/Nt ratio than the other three sites (Table 1). This result suggests that the average availability of nitrogen source is highest after *A. artemisiifolia* monoculture stands were established, which might be associated not only with activities of soil microorganisms, but also the higher release rate of N in soil at invaded sites. The Cmic/Corg and Nmic/Nt ratios obtained indicate that *A. artemisiifolia* may cause more rapid C and N cycles thereby improving the efficiency of organic C and N stabilization.

4.3. Changes of soil enzymes activities

Soil enzymes play an essential role in catalyzing reactions necessary for organic matter decomposition and nutrient cycling. The activities of soil enzymes, together with microbial biomass, are often used as potential indicators of soil quality mainly because of their rapid responses to temporary soil changes caused by management and environment factors [57,58]. Four soil enzymes sampled beneath *A. artemisiifolia* in this study showed generally enhanced activities in comparison with soils beneath native plant species. This is consistent with the findings that soil enzyme activities (e.g. invertase, protease, urease, acid phosphatase) were highest in plots beneath the invasive *Mikania micrantha* and lowest in plots comprised of native plant species [59]. The greater N cycling-related enzyme activities in our study are also consistent with previous studies [55,60]. Increases in soil enzyme activities involved in C and N cycling in invaded soils suggest that *A. artemisiifolia* may play a role in accelerating decomposition of soil organic matter and enhancing mineralization rates of organic N. Despite the substantial differences in soil enzyme activities between soils colonized by *A. artemisiifolia* and soils colonized by native species observed in this study, knowledge of the mechanisms driving these changes or patterns is less clear. Differences in plant tissue nutrient concentrations, size of *A. artemisiifolia* plants and litter decomposition rates may explain, in part, the observed changes in soil enzyme activities [61]. Although we did not examine the activities of soil enzymes involved in phosphorus cycling (i.e., acid or alkaline phosphatase), our results do demonstrate that invasive *A. artemisiifolia* can alter soil enzymes activities (Table 2). These changes were accompanied and correlated with soil microbial biomass as well as soil nutrients (such as SOM, total and available N and K). Our findings indicate that invasive *A. artemisiifolia* has a greater capacity to improve soil fertility and activities of soil enzymes than native plants in the study area, which may create conditions that favor invasion in new areas and its competitive dominance over native plants.

4.4. Changes in soil carbon utilization with A. artemisiifolia invasion

Comparison of carbon utilization and activity profiles of the soil microbial community from *A. artemisiifolia*-colonized and non-colonized sites provided a measurable and useful analysis of microbial functional differences. The AWCD reflects the oxidative capacity of soil microorganisms developing in Biolog and has often been used as an indicator of microbial activity [62,63]. In this study, AWCD values in sites colonized by *A. artemisiifolia* were significantly greater than in non-colonized sites (Fig. 1), indicating that *A. artemisiifolia* invasion greatly altered the carbon substrate utilization by soil microorganisms, especially in the LINVA site, where utilization of carbohydrates, carboxylic and amino acids, amines/amides and polymers increased significantly. Similar results have been reported for soils invaded by other exotic species including *Mikania micrantha* [59] and *Solidago canadensis* [53]. However, variation patterns for the main carbon source were species specific. For instance, *S. canadensis* invasion resulted in significant decreases in utilization of soil carboxylic acids and amines/amides but in a significant increase in utilization of carbohydrates which differs from our findings with *A. artemisiifolia*.

The presence of *A. artemisiifolia* led to changes in the CLPP of the soil microbial community which were due mainly to differences in carbohydrate, amine/amide, carboxylic and amino acid usage, suggesting that *A. artemisiifolia* colonization may enhance the decomposition of these compounds. These readily metabolized compounds may result in a greater concentration of microbial biomass. On the other hand, the differential use of carbon substrates allowed a distinct separation of the exotic *A. artemisiifolia* invaded sites from non-colonized sites (Fig. 3). There was also a difference in the metabolic profile of the microbial community between the HINVA and LINVA sites, which are associated with increased invasion history and pressure by *A. artemisiifolia*. The carbon utilization patterns we observed were similar to those reported in previous studies in which the catabolic abilities of microbial communities could be significantly modified by the resident plant communities [64–66]. However, it is still unclear whether the changes in the CLPP and the differences among soils in the experimental sites can be attributed to a change in soil microbial functional abilities only, or also to a change in community composition [67]. Measures of soil microbial community function and structure are necessary to gain more detailed information on the impacts of *A. artemisiifolia* invasion on soil microorganisms and subsequently nutrient cycling.

4.5. Relationship between soil carbon substrate utilization and biochemical variables

RDA in this study quantified the association between carbon substrate utilization and specific soil biochemical variables. Improved soil biochemical conditions (especially Cmic/Corg, AN values) in *A. artemisiifolia* invaded sites were associated with higher soil carbon utilization. Ratios of Cmic/Corg, Nmic/Nt, and Pmic/Pt were effective in explaining carbon utilization of soil microbial functional groups, thereby seven biochemical parameters were combined in the RDA model, accounting for significant variation in soil carbon utilization. Despite the successful assessment of these different biochemical parameters, linking the quantification of microbial community function with its composition is necessary since interpreting the changes in microbial community composition using statistical analyses will be a valid way to prioritize the complex factors influencing microbial community function [68]. Comparison of the carbon utilization and activity profiles of the soil microbial community in our study, combined

with microbial community structure data, can lead to a more comprehensive assessment of changes occurring in *A. artemisiifolia* invaded soils from multiple aspects of the soil microbial community.

In conclusion, our results revealed general differences in soil biochemical properties and microbial community function in sites colonized by the exotic invader *A. artemisiifolia* relative to non-colonized sites. The presence of *A. artemisiifolia* was correlated with higher soil nutrient availability and enzyme activities as well as average utilization of some carbohydrate, amino acid, and amines/amides substrates. This enhancement of soil fertility, in turn, may have facilitated its establishment and dominance in colonized habitats. The results are consistent with the hypothesis that invasibility of a habitat is positively correlated with resource availability [69]. Distinctive changes in soil biochemical properties and microbial community function among the four study sites may be due to the presence of *A. artemisiifolia* since soil texture was similar in both invaded and non-invaded sites. As for the two invaded sites, higher soil nutrient concentrations, microbial biomass and soil enzyme activities were observed at the HINVA site while a higher AWCD value and more efficiency in all groups of carbon compounds, except for phenols, were detected at the LINVA site, indicating that the length of time interactions between soil microbes and *A. artemisiifolia* have occurred may affect the degree to which soil biochemical properties are altered. However, because of the pseudo-replication of this experiment, we need to be cautious about any generalizations we make from our results. Nonetheless, based on extensive soil sampling in random locations within the different plots of the two non-invaded sites in our study (data not presented), we confirmed that the soil biochemical and microbial parameters of the two non-invaded sites were fairly representative.

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