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Ageratina adenophora invasions are associated with microbially mediated differences in biogeochemical cycles

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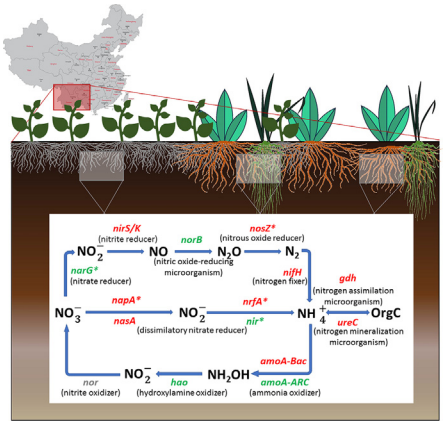
HIGHLIGHTS

- Microbial and nutrient dynamics differed in invaded and native plant soil.
- Many microbial-mediated nitrogen process rates were higher in invaded soil.
- Relative abundances of labile carbon decomposition genes were higher in invaded soil.

GRAPHICAL ABSTRACT

Soils impacted by *A. adenophora* presence and invasion have increased microbial functional gene potential for several key processes important in soil N cycling. Statistical significance of differences was conducted by *t*-distribution tests. *, $p < 0.05$. Genes involved in nitrogen fixation (*nifH*), ammonification (*gdh* and *ureC*), nitrification (*amoA/B*, *hao*,and *nor*), nitrate reduction (*napA**, *nrfA**, *nasA*, and *nir**), and denitrification (*narG**, *nirS/K*, *norB*, and *nosZ**) had significantly different relative abundances. Those that were significantly higher in *A. adenophora* monoculture soils compared to non-invaded plant soils are shown in red and those significantly lower are in green. The names of the communities were shown in the brackets under genes.



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ABSTRACT

Invasive plant species may alter soil nutrient availability to facilitate their growth and competitiveness. However, the roles and functional mechanisms of plant-associated microbes that mediate these soil biogeochemical cycles

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remain elusive. Here, we studied how soil microorganisms and their functional processes differed between soils invaded by *Ageratina adenophora* and adjacent non-invaded soils in a region of China with heavy invasion. Our results indicated that soil nitrogen contents were over 4.32 mg/kg higher ($p < 0.05$) in both rhizosphere soils and bulk soils dominated by *A. adenophora* as compared with those in soils dominated by non-invaded plants. Concurrently, soil microbial-mediated functional processes, i.e. nitrogen fixation rate, nitrification rate and ammonification rate, were also significantly ($p < 0.05$) higher in either rhizosphere soils or bulk soils of invasive *A. adenophora*. Using a functional gene microarray, we found higher relative abundances of soil microbial genes involved in N cycling processes in *A. adenophora* soils, e.g. *nifH*, required for nitrogen fixation, which significantly correlated with ammonia contents ($r = 0.35$ in bulk soils, $r = 0.37$ in rhizosphere soils, $p < 0.05$) and the nitrogen fixation rate ($r = 0.44$, $p < 0.05$). We also found that the relative abundances of labile carbon decomposition genes were higher in invasive *A. adenophora* soils, implying a potential higher availability of carbon. These results suggest that the soil surrounding the invasive plant *A. adenophora* is a self-reinforcing environment. The plant litter and rhizosphere environment of the invasive may influence soil microbial communities, promoting self-supporting soil processes. Alternatively, the regions invaded by *A. adenophora* may have already had properties that facilitated these beneficial microbial community traits, allowing easier invasion by the exotics. Both scenarios offer important insights for the mitigation of plant invasion and provide an ecosystem-level understanding of the invasive mechanisms utilized by alien plants.

1. Introduction

Invasion by exotic plants has severe biological impacts on agricultural and natural ecosystems worldwide and is largely responsible for huge economic costs attributed to ecology restoration (Paini et al., 2016; Thapa et al., 2018). How exotic plants species invade and establish is elusive, despite decades of research (Kolar and Lodge, 2001; Pyšek et al., 2012).

To date, most studies addressing species invasiveness have focused on definable plant characteristics, rather than taking an ecosystem-level approach to understanding invasive mechanisms, which is fundamental for the control of exotic plant species. It is commonly thought that the success of an invasive species depends on its performance-related functional traits (Van Kleunen et al., 2010) and strong reproductive ability (Colautti et al., 2006). In addition, fast evolutionary adaptation (Lee, 2002), greater resistance to enemies, higher nutrient-use efficiency (Heckman et al., 2017), and species-driven biological traits (Kourtev et al., 2002) also aid the establishment and spread of exotic species. These mechanisms of invasion may interact or even co-evolve in different habitats (Erfmeier and Bruelheide, 2010; Hornoy et al., 2011; Lankau, 2012). Thus far, however, less is known about the elucidation of microbial mediated nutrient cycles underlying plant invasion.

Soil microbes have important influences on plant species distributions in terrestrial ecosystems (Keymer et al., 2017; van der Heijden et al., 2008a). They can shape plant community structure and dynamics via microbial mediation of niche differentiation in resource use (Reynolds et al., 2003). The niche differentiation theory claims that differential plant-associated microbes can access different nutrient pools. Therefore, alien plant colonization is accompanied by changes to nutrient-cycling microbial communities, which reallocate soil nutrients to different plant niches, resulting in growth advantages for the invasive plants. For example, the invasion of *Elaeagnus umbellate* significantly altered the composition of soil ammonia oxidizing microorganisms (Malinich et al., 2017) and *Sapium sebiferum* invasion accelerated soil nitrification and denitrification potentials (Zou et al., 2006). In general, plant invasions increase nitrogen pools and accelerate nitrogen fluxes (Castro-Diez et al., 2014; Vila et al., 2011).

The weed *Ageratina adenophora* (Sprengel) R. King & H. Robinson (synonym: *Eupatorium adenophorum* Sprengel), or crofton weed, is a notorious, worldwide, invasive weed originating from Mexico and Costa Rica (Qiang, 1998). First introduced into Yunnan Province in China in the 1940s, this species has extensively colonized southwestern China and is spreading rapidly (20 km/year) eastward and northward (Wan et al., 2010). It threatens native forests, rangelands, and farmlands by causing degeneration of native plants and establishment of monocultures (Wang and Wang, 2006). Owing to its strong, aggressive effects on native species and its global significance, *A. adenophora* is an ideal system for studying the mechanisms of plant invasion.

Much effort has been exerted to detect alterations to soil after *A. adenophora* invasion and to reveal these feedbacks on plant diversity. Invasion of *A. adenophora* increased soil fertility (C_{org}, NO_3-N, NH_4-N, available P and available K contents) and soil enzyme activities (urease, phosphatase and invertase). In fact, *A. adenophora* altered soil environments to an extent that growth of native plants was inhibited (Li et al., 2011). Arbuscular mycorrhizal fungi, the fungal/bacterial ratio and nitrogen cycle-related microorganisms (azotobacteria, ammonia-oxidation bacteria and denitrification bacteria) are also affected, as was evidenced using fatty acids analysis, phylogenetic sequencing, and traditional culture-based methods (Li et al., 2009; Niu et al., 2007a; Niu et al., 2007b; Xu et al., 2012). In particular, highly invaded soils by *A. adenophora* had significantly greater NO_3-N and NH_4-N contents and higher relative abundance and diversity of nitrogen fixing bacteria compared with non-invaded soils (Niu et al., 2007a; Xu et al., 2012). Consequently, variations in soil biotic and abiotic properties, which promote the new species and inhibit native plants, may be critical components of the *A. adenophora* invasion process.

In this study, we aimed to identify factors that regulate microbial functional gene diversities in soil and to provide insights into the importance of the factors for invasive *A. adenophora* dynamics in terrestrial ecosystems. We hypothesized that soil microbial and nutrient dynamics and microbial-mediated functional processes would differ in the invaded, a mixture of invaded and non-invaded plants, and non-invaded plant soils. To test these hypotheses, we examined how soil properties and microbial phylogenetic and functional communities responded to exotic *A. adenophora* invasions.

2. Materials and methods

2.1. Site description and soil sampling

The study area was located in southwestern Yunnan Province, China in an evergreen, broad-leaved-deciduous mixed forest region dominated by *Machilus pingii* Cheng ex Yang, *Cyclobalanopsis glaucoides* Schottky, *Lithocarpus dealbatus* Rehder, *Alnus cremastogyne* Burkill and *Broussonetia papyrifera* (L.) L'Hér ex Vent. The location of the experiment was 24°41′86″–91.8″ N and 102°52′37.6″–48.5″, with an average elevation of 2000 m and a regional mean annual precipitation and temperature of 952 mm and 16.5 °C, respectively. The study area was characterized by a subtropical hot and arid valley climate with pronounced wet and dry seasons. The study area was chosen for two reasons: 1) it was once dominated by native plant communities and has experienced substantial turnover to *A. adenophora* domination in the past 30 years

and 2) the *A. adenophora* expansion appears to have followed an obviously center-to-border pattern with different displacement intensities (Fig. S1).

Soil samples from *A. adenophora* expansion blocks were collected in December 2010 (Fig. S1). We do not have pre-invasive soil samples, so to avoid pseudoreplication (Hurlbert, 1984), we used a randomized block design with five blocks, each containing a replicate of the following samples: rhizosphere soils (AR samples) or bulk soils (AB samples) dominated only by *A. adenophora* (>60% coverage), rhizosphere soils (AXR samples) or bulk soils (AXB samples) harboring a mixture of *A. adenophora* and non-invaded plants (coverage of *A. adenophora* is 10–30%, coverage of non-invaded plant species is 30–50%), and rhizosphere soils (NR samples) or bulk soils (NB samples) dominated only by non-invaded plant species (>40% coverage), resulting in 30 samples in total. The five blocks were 17 m – 72 m away from each other. The native vegetation consisted of several local weeds: *Imperata cylindrica* (L.) Beauv., *Oplismenus compositus* (L.) P. Beauv., *Arthraxon hispidus* (Thunb.) Makino, *Capillipedium assimile* Steudel, *Artemisia carvifolia* Buch.-Ham. ex Roxb., *Elsholtzia ciliata* (Thunb.) Hyland., *Cyperus duclouxii* E. G. Camus, *Dicranopteris dichotoma* (Thunb.) Bernh. and *Lygodium yunnanense* Ching. For each block, soil samples were collected as follows: 1 cm of litter was removed from the soil surface and underlying soil within a 30-cm radius of each plant was loosened with a shovel. Then plants were pulled up and shaken vigorously in situ until approximately 80% of the soil was removed. All soil remaining on the roots was then collected as a rhizosphere sample. Bulk soil samples were collected from the soil that fell off the roots. All cores for each subsample were deposited in a polyethylene bag and stored on ice for transport to the lab. Samples were homogenized and sieved (2 mm) to remove stones, roots and large soil animals. The fresh soils were used for biogeochemical analysis, with the remaining samples stored at −80 °C for DNA extraction.

2.2. Plant, soil and soil biogeochemical property analyses

For every subsample at the different blocks, species richness was measured by counting plant species types within a 100 × 100 cm subplot. The coverage of the invasive species *A. adenophora* was also evaluated in the same sub-plot. Plant height was calculated as the mean value of the tallest and shortest branches of the shrubs. To determine aboveground and belowground biomass, the 100 × 100 cm plot was clipped just above the soil surface, sorted into live material (aboveground biomass) and senesced litter (litter biomass), dried and weighed. Roots were sampled at a depth of 0–20 cm using 5-cm diameter cores in the area used for the aboveground biomass clipping. Roots were washed, dried and weighed.

The soil samples were oven-dried to determine the mass water content of field-wet soils by percent weight loss at 105 °C after 24 h. Soil pH was measured from a 1:2 soil-to-H_2O ratio, and organic carbon (C_{org}) was analyzed using the potassium dichromate-sulfuric acid oxidation method. The total nitrogen (TN), total phosphorus (TP), total potassium (TK), available nitrogen (AN), available phosphorus (AP) and available potassium (AK) were determined using standard methods (Lu, 1999). Soil mineral N was extracted with 2 $mol \cdot L^{-1}$ KCl, and concentrations of NH_4^+-N and NO_3^--N in the KCl were measured by a Lachat Quick Chem II flow-injection analyzer (Zellwegger Analytical, Milwaukee, WI, USA).

The potential N-fixation rates were determined by measuring the ^{15}N enrichment of soils receiving $^{15}N_2$ compared to controls receiving $^{14}N_2$ (Buckley et al., 2007). Briefly, each soil sample was placed into a 100 mL bottle and the headspace was replaced by synthetic air of 20% O_2 and 80% $^{15}N_2$. Controls were prepared with unlabeled N_2. After incubation at room temperature in the dark for around 30 days, each bottle was opened and approximately 2 g of soil were extracted and oven dried (<65 °C) until a constant weight was attained. Then, 200 mg subsamples were weighed and packaged into tin capsules and sent for analysis of N stable isotope contents.

To determine ammonification rates, 20 g of each fresh soil was mixed with 0.04 g peptone nitrogen source in test tubes and adjusted to about 60% of the field water capacity. Tubes were stored at 30 °C for 7 d and tested for the production of NH_4^+-N with the auto analyzer method described above. Samples without peptone addition were used as controls.

Nitrification analyses were carried out according to a modified method of Smolders et al. (2001) as follows: 20 g of each fresh soil was added with 4 mL $(NH_4)_2SO_4$ of 1 $mg \bullet mL^{-1}$g in test tubes and adjusted to about 65% of the field water capacity. Tubes were stored at 30 °C for 7 d and tested for the production of NH_4^+-N and NO_3^--N using the auto analyzer method, described above. Samples without $(NH_4)_2SO_4$ additions were used as controls.

Denitrification potentials were measured according to a modified method (Ellis et al., 1998) as follows: 20 g of each fresh soil was added to 9 mg KNO_3 in water solution and moistened until there was a thin water layer on the surface. Tubes were vacuumed with drying apparatus and then stored at 21–23 °C for 1 d and tested for the loss of NO_3^--N with the auto analyzer method described above. Samples without KNO_3 addition were used as controls.

2.3. DNA extraction, purification and quantification

DNA was extracted from 5 g soil using freeze-grinding and sodium dodecyl sulfate (SDS) for cell lysis (Zhou et al., 1996). The extracted DNA was purified by low melting agarose gel electrophoresis, and followed by phenol-chloroform-butanol extraction. DNA quality was assessed by the ratios of absorbance at 260/280 nm and 260/230 nm with ND-1000 spectrophotometer (Nanodrop Inc.), and the final concentrations were quantified using a PicoGreen fluorometric method (Invitrogen, Grand Island, NY, USA).

2.4. Illumina MiSeq sequencing of 16S rRNA gene

The 16S rRNA gene amplification and sequencing was conducted as previously described (Caporaso et al., 2012; Caporaso et al., 2011). The PCR primers (F515/R806) were used to amplify the V4 hypervariable regions of bacterial 16S rRNA genes. The PCR amplification system and cycling conditions were modified from a previous protocol (Caporaso et al., 2011). Briefly, the PCR reaction mix contained 5 units of AccuTaq™ LA DNA Polymerase (Sigma, St. Louis, MO), 2.5 μl reaction buffer (contains 100 μM dNTPs), and a 0.1 μM concentration of each primer in a volume of 25 μl. Genomic DNA (10 ng) was added to each amplification mix. Cycling condition was an initial denaturation at 94 °C for 1 min, 35 cycles of 94 °C 20 s, 50 °C for 25 s, and 72 °C for 45 s, a final 10-min extension at 72 °C. The PCR products were pooled together and purified through QIAquick Gel Extraction Kit (Qiagen, Chatsworth, CA). The purified PCR products were recovered and then quantitated with PicoGreen using a FLUOstar Optima (BMG Labtech, Jena, Germany). The DNA amplicon mixture was denatured and then run on MiSeq for paired-end, 150 bp reads (Illumina, Inc., San Diego, CA, USA).

Results were processed using an in-house pipeline http://ieg.ou.edu/. To minimize effects of random sequencing errors, we eliminated (i) sequences with quality scores (Q score) <20 with a window size (number of continuous low quality bases) of 5; (ii) with <140 bp after the proximal PCR primer if they terminated before reaching the distal primer, and (iii) sequence with possible chimeras using U-Chime (Edgar et al., 2011) to check against a 16S core set selected from NCBI database. The singleton OTUs (with only one read) were removed, and the sequences were resampled to 2500 sequences per sample. The qualified sequences were clustered into OTUs using Uclust (Edgar, 2010) algorithm at 97% identity. Based on the OTU data set, if an OTU only appeared in two or fewer samples among the total of 5 samples for

each sample, it was removed for data reliability. Finally, sequences were normalized to relative abundance and used for statistical analyses.

2.5. GeoChip assay

Three replicates were randomly selected to analyze functional gene structures, resulting in 18 functional gene arrays (FGAs; GeoChip 4.2). Each array contains approximately 28,000 probes covering approximately 57,000 gene variants from 292 functional gene families involved in carbon, nitrogen, phosphorus, and sulfur cycles, soil-borne pathogen, antibiotic resistance, metal resistance, and organic contaminant degradation (Tu et al., 2014). Each probe represents a sequence generated from a microorganism.

For each soil sample, 1.5 μg purified DNA was used for microarray hybridization. DNA was denatured and then fluorescently labeled with cyanine-5 using random primers and purified using a QIAquick purification kit (Qiagen), as described previously (Wu et al., 2008). Purified, labeled DNA was then hybridized to microarrays on HS4800 Hybridization Station (TECAN US, Durham, NC) at 42 °C for 10 h. After hybridization, the arrays were imaged by a ScanArray 5000® Microarray Analysis System (Perkin Elmer, Wellesley, MA) at 95% laser power and 68% PMT (photomultiplier tube gain).

Signal intensities of each slide were measured with NimbleScan software (2.5 version, Biodiscovery Inc., EI Segundo, CA). Raw data from NimbleScan were analyzed using a GeoChip data analysis pipeline http://ieg2.ou.edu/NimbleGen/. Spots with signal to noise ratio [SNR = (signal mean-background mean)/background standard deviation] of ≥2 were considered as positive signal. Probes that were detected in only 1 out of 3 samples were removed to improve data reliability. Signal intensities were then normalized by dividing the total intensity of one sample and multiplying by a constant. Finally, the normalized signal intensities were logarithmically transformed and were considered to be the relative abundances of functional genes.

2.6. Statistical analyses

Pre-processed data (e.g. GeoChip and Illumina sequencing) were analyzed with different statistical methods. Microbial diversity was calculated by Simpson's reciprocal index (1/D) and Shannon-Weaner index (H′). To examine differences between microbial communities, detrended correspondence analyses (DCA) of microbial community compositions and functional structures were carried out and a dissimilarity test was employed to determine significances of the differences. To ensure our results are robust when conducting dissimilarity tests, we used three distance metrics, Bray-Curtis, Euclidean, and Morisita-Horn with different functions as following to analyze the variance.

Bray-Curtis	*d[jk] = (sum abs(x[ij]-x[ik]))/(sum (x[ij] + x[ik]))* *binary: (A + B-2*J)/(A + B)*
Euclidean	*d[jk] = sqrt(sum(x[ij]-x[ik])^2)* binary: *sqrt(A + B-2*J)*
Morisita-Horn	*d[jk] = 1–2*sum(x[ij]*x[ik])/((lambda[j] + lambda[k]) * sum(x[ij]) *sum(x[ik]))*, where *lambda[j] = sum(x[ij]^2)/(sum(x[ij])^2)* binary: *(A + B-2*J)/(A + B)*

where *x[ij], x[ik]* refer to the quantity on species (column) *i* and sites (rows) *j* and *k*. In binary versions *A* and *B* are the numbers of species on compared sites, and J is the number of species that occur on both compared sites. The Similarity Percentages (SIMPER) analysis was used to evaluate the contribution of individual species to the microbial community dissimilarity between pairwise samples, in which Bray-Curtis distance was used (Warton et al., 2012). Mantel tests and canonical correspondence analyses (CCA) linked functional microbial community structures to plant or soil variables and partial Mantel tests and partial CCA's were analyzed for co-variation analysis of soil and plant variables. In the Mantel test, we used Bray-Curtis distance matrices for the OTU table or the functional gene table, and Euclidean distance matrices for the environment variable table. All the above analyses were performed by R software (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria). Statistical significance of differences in environmental variables or functional processes were determined using one-way ANOVA's (analysis of variance) with 95% confidence intervals in SAS (SAS Inc., Cary, NC, USA) followed by LSD tests. Statistical significance of differences in nitrogen or carbon cycling genes between sites was conducted by *t*-distribution tests in Microsoft Excel.

3. Results

3.1. Effect of A. adenophora invasion on vegetation and soil properties

Ageratina adenophora established a monoculture community in the study area as its invasion progressed, with its vegetation coverage eventually reaching 93% (Table 1). The average height of *A. adenophora* was nearly 1 m, which was 7 times higher than that of most native plants. In addition, the average aboveground and belowground biomass was respectively 6 and 14 times greater than those of native plants. The litter productivity of *A. adenophora* was also 44 times higher than that of native species. Because *A. adenophora* was frequently the only species in the invasive plots, the plant diversity indices, including Shannon's Index, Simpson's Index, Pielou's Evenness and Simpson's Evenness were significantly smaller than those in the non-invaded plant plots.

Monocultures of *A. adenophora* had the highest soil inorganic nitrogen contents, which were significant for available nitrogen (AN), NO_3-N and NH_4-N (Table 1). Most other soil geochemical properties, including soil pH, soil moisture, TK, AP, and AK were similar between *A. adenophora* soils and the mixture of *A. adenophora* and non-invaded plant soils. The only exception was TP, which was significantly ($p < 0.05$) smaller in the *A. adenophora* soils. In addition, although the differences were not statistically significant, there were obvious trends wherein both exotic monoculture of *A. adenophora* soils and the mixture of *A. adenophora* and non-invaded plant soils possessed higher C_{org} and TN than non-invaded plant soils.

According to a ^{15}N-isotope labeling experiment, potential N-fixation rates were significantly higher in the exotic monoculture soils (Fig. 1a). Potential nitrification rates in bulk soils of invasive *A. adenophora* were higher ($p < 0.05$) than those in non-invasive soils (Fig. 1b). Ammonification potentials were significantly higher, by up to 50%, in *A. adenophora* rhizosphere soils (Fig. 1c), whereas denitrification potentials were noticeably lower in exotic monoculture soils compared with those in non-invaded plants (Fig. 1d).

3.2. Effect of A. adenophora invasion on soil microbial communities

Microbial taxonomic communities as determined by 16S *r*RNA gene sequencing, which revealed 6696 OTUs in total, were significantly ($p < 0.05$) different in both rhizosphere and bulk *A. adenophora* soils from those in non-invaded plant soils based on the results of dissimilarity tests (Table S1) and DCA (Fig. S2a). The SIMPER analysis was used to evaluate the contribution of individual OTU's to the dissimilarity between invaded and non-invaded samples. We found that *Gp6* of the *Acidobacteria* phylum contributed the most (8.60%) to the dissimilarity of soil microbial communities between invaded and non-invaded soils, followed by *Sphingomonas* (6.09%) and *Spartobacteria* (5.10%). The three genera also contributed the most to the dissimilarity of soil microbial communities between rhizosphere soils and bulk soils, accounting to a total of 20.96%.

We used GeoChip 4.2 to evaluate soil functional gene structures and got 51,672 functional genes across all samples. The invasion of *A. adenophora* also had significantly ($p < 0.05$) different soil functional gene structures from those of native plant (Table S1, Fig. S2b). Although microbial taxonomic diversities remained stable after exotic invasion,

functional gene diversities were significantly ($p < 0.05$) higher in the rhizosphere soils, but not in the bulk soils (Table 2). We then focused on the specific functional genes identified by GeoChip in the following results.

3.2.1. Nitrogen fixation genes

The relative abundances of nitrogen fixation (*nifH*) gene were higher in both rhizosphere soils and bulk soils of *A. adenophora* comparing to those in the non-invasive soils (Fig. 2). The *nifH* genes with the greatest response to exotic invasion were derived from bacteria, e.g. *Cyanobacteria*, *Azotobacter*, and *Clostridium*, which enhanced the nitrogen availability to the non-leguminous invasive plant. Furthermore, *nifH* genes showed significant correlations with potential N-fixation rates ($r = 0.44$, $p < 0.05$ in the rhizosphere soils) and ammonia contents ($r = 0.35$–0.37, $p < 0.05$, Table 3) based on Mantel test.

3.2.2. Ammonification genes

The *ureC* (encoding urease) genes were significantly ($p < 0.05$) lower in relative abundance in the bulk soil of *A. adenophora* comparing to those in the bulk non-invasive soils (Fig. 2), suggesting that microbial conversion of organic nitrogen into ammonium (NH_4^+) is more pronounced in the substance-rich plant root zone. In the bulk soils, gdh and ureC genes were significantly correlated with AN ($r = 0.35$–0.39, $p = 0.02$) and NH_4^+-N ($r = 0.47$–0.52, $p = 0.001$–0.006, Table 3).

3.2.3. Nitrification genes

The relative abundance of *amoA* genes has no significant difference between invasive and non-invasive soils (Fig. 2). Mantel test revealed that *amoA*-Bac significantly correlated with nitrification potential ($r = 0.36$, $p < 0.05$) and *amoA* genes significantly correlated with soil NH_4^+-N ($r = 0.28$–0.56, $p = 0.001$–0.07, Table 3).

3.2.4. Denitrification genes

Nitrate reductase genes (*narG*) were lower ($p < 0.05$) in relative abundance in the presence of *A. adenophora* (Fig. 2), suggesting that the conversion of nitrate to nitrite was attenuated in the invasive plant soils. The relative abundances of *narG*, *nirS*, *nirK*, *norB* and *nosZ*, key enzyme-encoding genes in the denitrification pathway, were significantly correlated with the denitrification potential ($r = 0.26$–0.45, $p = 0.006$–0.094) in the rhizosphere soils. In addition, the relative abundances of *narG*, *nirS*, *nirK* and *nosZ* were significantly correlated with soil NH_4^+-N ($r = 0.33$–0.54, $p = 0.002$–0.066, Table 3).

3.2.5. Carbon cycling genes

The invasion of *A. adenophora* had differential impacts on various carbon decomposition genes (Fig. 3). Most labial carbon degradation genes, including those involved in degrading hemicelluloses and cellulose, were significantly ($p < 0.05$) higher in both rhizosphere soils and bulk soils in *A. adenophora* invaded plots. However, the relative abundances of most genes involved in degrading recalcitrant carbon (e.g. lignin) were significantly ($p < 0.05$) lower in the rhizosphere soils, and remained the same in the bulk soils when *A. adenophora* was present. In addition, carbon fixation genes were significantly ($p < 0.05$) lower in relative abundance in both rhizosphere soils and bulk soils of the exotic plant compared to those genes in the non-invasive soils (Fig. S3).

3.3. Relationships between plant or soil properties and soil microbial communities

Community dissimilarity matrices based on both taxonomic and functional gene markers showed significant correlations with soil and plant properties (Table 4). Notably, plant richness, coverage of *A. adenophora* and plant height were significantly correlated with the microbial functional gene dissimilarity matrices ($r = 0.31$–0.68, $p = 0.001$–0.039) in both rhizosphere soils and bulk soils, but had no significant correlation with microbial taxonomic compositions described by the dissimilarity matrix of the OTU table ($p > 0.05$). In contrast, soil pH was significantly correlated with microbial taxonomic compositions ($r = 0.61$–0.71, $p = 0.001$) in both rhizosphere soils and bulk soils, but had no significant correlation with functional gene structures ($p > 0.05$). In addition, soil NH_4-N significantly correlated with microbial functional gene structures ($r = 0.36$–0.45, $p = 0.008$–0.047) in both rhizosphere soils and bulk soils, but only had a significant correlation with microbial taxonomic compositions in the rhizosphere soil ($r = 0.26$, $p = 0.021$). Two significant CCA models ($p = 0.005$–0.017) were constructed using soil properties, i.e. soil pH, moisture, C_{org}, NO_3-N, NH_4-

Table 1
Morphological traits of *A. adenophora* and soil geochemical properties (mean ± standard error, $n = 5$). Different letters indicate significant differences among samples ($p < 0.05$), tested by one-way ANOVA.

		Rhizosphere			Bulk		
		A	*AX*	*N*	*A*	*AX*	*N*
Plant properties	Richness				1.00 ± 0c	6.20 ± 0.58a	4.40 ± 0.40b
	Coverage of *A.adenophora* (%)				92.6 ± 0.81a	49.2 ± 2.31b	1.2 ± 0.58c
	Height (m)				0.99 ± 0.04a	0.75 ± 0.05a	0.14 ± 0.01c
	Aboveground Biomass (g/m^2)				1518.92 ± 121.17a	544.44 ± 36.0b	248.04 ± 43.28c
	Belowground Biomass (g/m^2)				155.48 ± 20.05a	36.92 ± 2.24b	10.87 ± 1.37b
	Litter (g/m^2)				495.56 ± 64.89a	47.84 ± 4.55b	11.13 ± 1.25b
	Shannon's Index (H′)				0 ± 0b	1.31 ± 0.13a	1.25 ± 0.09a
	Simpson's Index (1/*D*)				1.00 ± 0b	2.98 ± 0.42a	3.21 ± 0.29a
	Pielou's Evenness				0 ± 0b	0.31 ± 0.03a	0.36 ± 0.01a
	Simpson's Evenness				0.01 ± 0c	0.04 ± 0.01b	0.11 ± 0.02a
Soil properties	pH	7.53 ± 0.04a	7.52 ± 0.05a	7.62 ± 0.02a	7.1 ± 0.18b	7.28 ± 0.12ab	6.99 ± 0.23b
	M (%)	27.59 ± 0.65ab	26.69 ± 0.84abc	28.05 ± 0.61a	25.04 ± 0.75c	26.16 ± 0.91abc	25.81 ± 0.78bc
	C_{org} (g/kg)	60.36 ± 6.83ab	74.76 ± 7.35a	59.77 ± 6.29ab	37.8 ± 8.08bc	44.91 ± 16.27bc	29.11 ± 6.6c
	TP (g/kg)	1.86 ± 0.06ab	1.94 ± 0.08a	1.98 ± 0.07a	1.7 ± 0.07b	1.92 ± 0.07a	1.91 ± 0.07a
	TK (g/kg)	8.89 ± 0.08a	9.03 ± 0.12a	9.13 ± 0.1a	9.07 ± 0.07a	9.12 ± 0.16a	9.13 ± 0.12a
	TN (g/kg)	4.23 ± 0.26a	4.36 ± 0.41a	3.59 ± 0.28ab	2.46 ± 0.46bc	2.34 ± 0.62c	1.95 ± 0.41c
	AP (mg/kg)	9.33 ± 1.03ab	13.24 ± 2.37a	14.12 ± 3.2a	4.98 ± 1.22b	9.18 ± 1.82ab	8.27 ± 1.64ab
	AK (mg/kg)	203.41 ± 22.16a	148.03 ± 12.95ab	180.56 ± 18.79a	104.68 ± 38.64b	89.99 ± 11.65b	89.04 ± 17.02b
	AN (mg/kg)	34.81 ± 1.07a	24.13 ± 3.37c	26.51 ± 1.37bc	32.73 ± 2.64ab	12.71 ± 2.4d	13.03 ± 1.98d
	NO_3-N (mg/kg)	11.37 ± 1.69a	7.69 ± 0.91bc	7.05 ± 0.69c	10.56 ± 0.83ab	3.82 ± 1.01d	3.04 ± 0.91d
	NH_4-N (mg/kg)	15.73 ± 2.01a	3.67 ± 1.16c	7.17 ± 1.94bc	19.18 ± 1.69a	7.07 ± 1.2bc	9.85 ± 1.16b

M: Moisture; C_{org}: Organic carbon; TN: Total N; TP: Total P; TK: Total K; AN: Available N; AP: Available P; AK: Available K.
Rhizosphere, rhizosphere soil; Bulk, bulk soil. A, *A. adenophora*; AX, mixture of *A. adenophora* and non-invaded plants; N, non-invaded plants.
Native plants included several local weeds: *Imperata cylindrica*, *Oplismenus compositus*, *Arthraxon hispidus*, *Capillipedium assimile*, *Artemisia carvifolia*, *Elsholtzia ciliata*, *Cyperus duclouxii*, *Dicranopteris dichotoma* and *Lygodium yunnanense*.

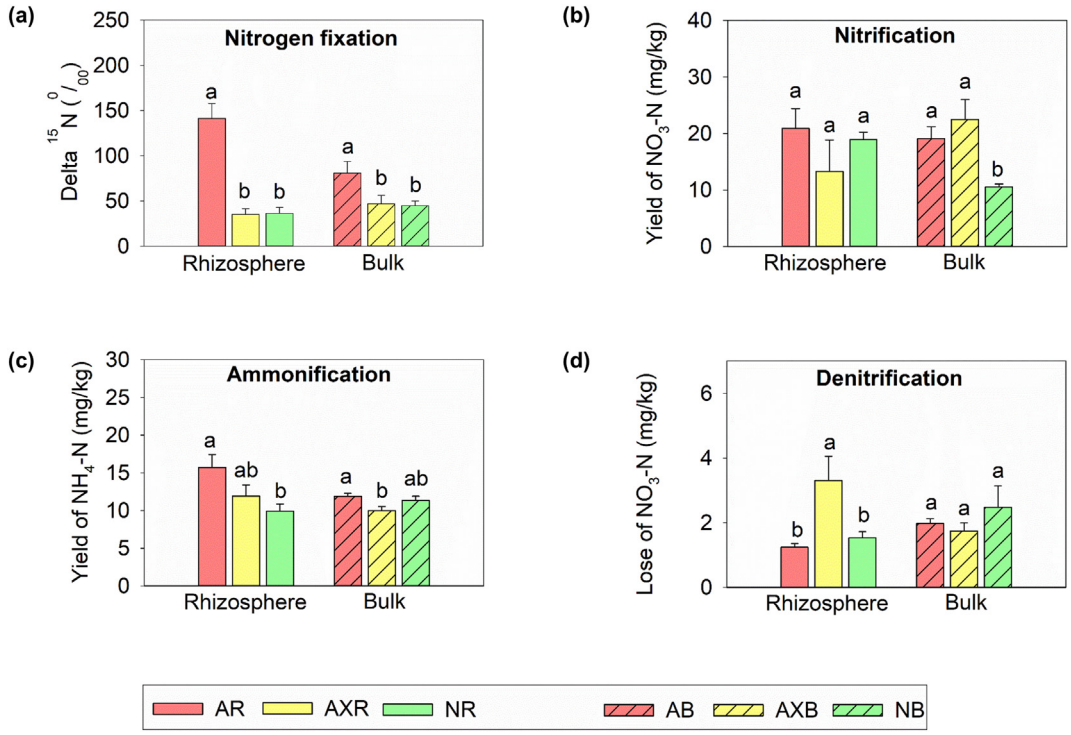

Fig. 1. Soil nitrogen cycle-related biogeochemical processes. (a) Potential nitrogen-fixation rates. (b) Nitrification potential. (c) Ammonification potential. (d) Denitrification potential. All data are presented as mean ± standard error, $n = 5$. Different letters indicate significant differences among samples ($p < 0.05$) as tested by one-way analysis of variance. A: *A. adenophora*; AX: mixture of *A. adenophora* and non-invaded plants; N: non-invaded plants; R, rhizosphere soil; B, bulk soil.

N etc., and plant properties (plant species richness, plant height and litter input) (Fig. S4), which verified the results of Mantel test.

4. Discussion

4.1. Key findings

Evidence of invasive plant-induced microbial shifts has been broadly reported and has revealed that the soil microbial community can play critical roles in invasion processes (Klironomos, 2002; Mitchell et al., 2006; Reinhart and Callaway, 2006; van der Putten et al., 2007). Nevertheless, this study uniquely reports an in-depth understanding of soil microbial mediated nutrient cycling related under the influence of an invasive plant. In general, we found that soil microbial functional gene diversity in *A. adenophora* soils was dramatically different from that in non-invaded plant soils. Our research revealed microbial taxonomic groups and functional genes involved in soil nutrient cycling to differ in *A. adenophora* soils compared with non-invaded soils. Additionally, we revealed that soil nitrogen cycling processes, i.e. nitrogen fixation, nitrification, ammonification and denitrification were different in invaded *A. adenophora* soils from those in non-invaded plant soils.

Table 2
Microbial taxonomic diversities detected by 16S *r*RNA gene sequencing (mean ± standard error, $n = 5$) and functional gene diversities detected by GeoChip (mean ± standard error, $n = 3$).

		16S rRNA gene		GeoChip	
		$H^{‡}$	$1/D^{§}$	$H^{‡}$	$1/D^{§}$
Rhizosphere	AR	5.40 ± 0.05	106.1 ± 9.2.	10.68 ± 0.01a	42,680 ± 475 a
	AXR	5.41 ± 0.05	107.1 ± 7.3.	10.65 ± 0.00 ab	41,807 ± 167 ab
	NR	5.49 ± 0.03**	106.1 ± 3.9*	10.63 ± 0.00 b	41,274 ± 278 b
Bulk	AB	5.33 ± 0.04	78.5 ± 12.3	10.70 ± 0.02	44,194 ± 721
	AXB	5.28 ± 0.10	81.5 ± 13.0	10.70 ± 0.04	43,968 ± 1615
	NB	5.20 ± 0.05	72.4 ± 10.5	10.64 ± 0.00	41,565 ± 358

A, *A. adenophora*; AX, mixture of *A. adenophora* and non-invaded plants; N, non-invaded plants; R, rhizosphere; B, Bulk.
Different letters indicate significant differences among A/AX/N communities at rhizosphere or bulk soils ($p < 0.05$) as tested by one-way analysis of variance. Significance differences between rhizosphere and bulk soil samples as assessed by the one-tailed *t*-test are indicated as follows: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; '.', $p < 0.1$.
‡ Shannon-Weiner index, higher number represents higher diversity.
§ Reciprocal of Simpson's index, higher number represents higher diversity.

4.2. Functional microbial groups

Previous research determined that exotic plants had greater biomass than native plants, which was attributed to higher specific leaf area and net photosynthetic rates of the invasive species (Bottollier-Curtet et al., 2013; Ehrenfeld et al., 2001; Liao et al., 2008). Consistently, exotic *A. adenophora* in our study had higher biomass and average height than adjacent native plants (Table 1). The vigorous growth of *A. adenophora* has been attributed to potential evolutionary changes in N allocation, i.e., increased nitrogen allocation to photosynthesis with corresponding faster plant growth, and decreased allocation to costly cell walls (Feng et al., 2009). The stimulated carbon input not only directly increases litter biomass but also further enhances carbon accumulation in soil. Accordingly, plant litter was significantly greater in *A. adenophora* invaded soils, which is consistent with previous studies demonstrating that invasive plants generally have much higher leaf litter density (Belnap and Phillips, 2001; Evans et al., 2001).

Litter decomposition rates and nutrient availability has been shown to increase in regions threatened by invasive plant spread (Martin et al., 2010; Standish et al., 2004; Trammell et al., 2012). For example, the decomposition of both exotic and native plant litters were accelerated in the presence of many invasive plants including *Acer platanoides* L., *Ampelopsis brevipedunculata*, *Lonicera morrowii*, *Rosa multiflora*, and *Alliaria petiolata* (Ashton et al., 2005; Rodgers et al., 2008). In addition, a survey of 94 experimental studies showed that higher concentrations in plant nitrogen, soil NH_4-N and soil NO_3-N were present in invaded

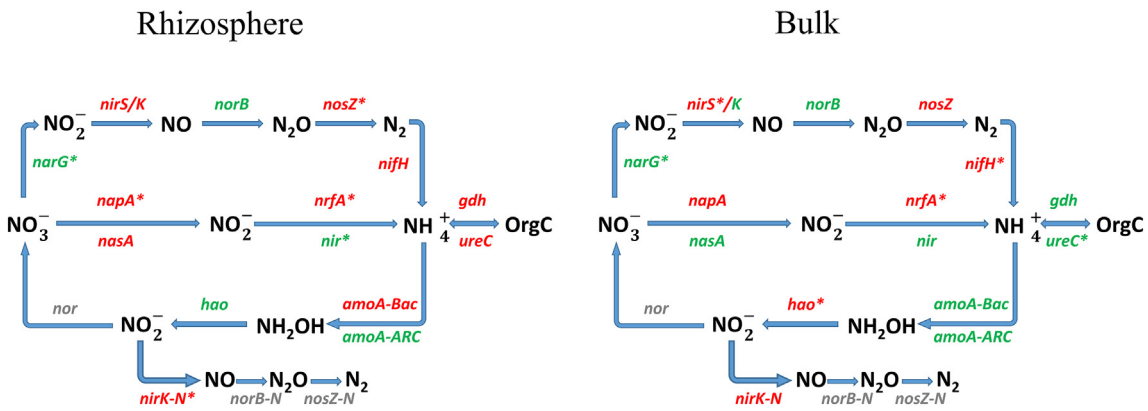

Fig. 2. Relative changes of nitrogen cycling gene abundances between monoculture *A. adenophora* soils and non-invaded plant soils. The relative abundance for each gene detected was normalized by dividing the total intensity of one sample and multiplying by a constant. Those that were significantly higher in *A. adenophora* monoculture soils compared to non-invaded plant soils are shown in red and those significantly lower are in green. Statistical significance of differences was conducted by *t*-distribution tests. *, $p < 0.05$.

regions, whether or not the invasive plant formed nodules to facilitate biological N-fixation (Liao et al., 2008). In coastal Australia, greater litter input and more than a two-fold increase of litterfall N content was measured in *Chrysanthemoides monilifera* invaded regions, which led to two to five times higher soil N contents in invaded areas compared to native areas (Lindsay and French, 2005). Our results similarly found that *A. adenophora* invasion resulted in higher litter accumulation, ammonium and nitrate in bulk and rhizosphere soils. In addition, our GeoChip data suggested that *A. adenophora* invasion might improve the microbial potential for labile carbon degradation, thereby leading to faster rates of litter decomposition. Greater inputs of nitrogen to the soil could result from this enhanced labile carbon degradation potential, although mineralization of carbon and nitrogen have been shown to be decoupled in some cases (Prescott and Zukswert, 2016). These results verify the capacity of invasive plants to alter the nutrient status in soils to fundamentally change ecosystem functioning (Liao et al., 2008).

Nitrogen fixation processes associated with nitrogen-fixing plant invasion have long been studied (Vitousek, 1990; Vitousek and Walker, 1989), and many of the world's most aggressive wildland weeds are nitrogen-fixing trees and shrubs (Richardson et al., 2000). Especially in nutrient-poor ecosystems, nitrogen-fixing symbionts are principally responsible for the acquisition of limiting nutrients (Rout and Chrzanowski, 2009; Van Der Heijden et al., 2008b). The role of N cycling in exotic plants that do not form N-fixing associations, such as *A. adenophora*, is less understood. These exotics can also potentially acquire nitrogen via free-living microbes. Coinciding with significantly higher nitrogen fixation rates, the relative abundance of *nifH* genes was significantly higher in our study, suggesting that *A. adenophora* presence potentially induced higher N-fixation rates by accompanying higher abundance of functional microbes. In particular, the higher abundance of N-fixing communities under *A. adenophora* invasion could indicate a superb aptitude of this species to shape both bulk and rhizosphere soils, likely through root exudates and litter inputs.

Higher nitrogen availabilities in nitrogen additive experiments have been shown to suppress nitrogen fixation (Dynarski and Houlton, 2018). However, soil bulk density, exchangeable potassium, and soil aggregate stability were also reported to be significant drivers of nitrogen fixation and nitrification in field studies (Ciccolini et al., 2016; Pérez Brandan et al., 2019). The abundance of *nifH* genes has been positively correlated with soil NH_4^+-N content in previous work (Pérez Brandan et al., 2019), which was consistent with the results in our study. In addition, it was recently shown that nitrogen fixation is negatively correlated with the NO_3^-/NH_4^+ ratio (Wang et al., 2019). In our study, NO_3^-/NH_4^+ ratio was 0.72 in the *A. adenophora* rhizosphere soil and 1.43 in the rhizosphere soil of non-invaded plants. This, along with altered soil properties from increased litter carbon inputs, might contribute to the higher nitrogen fixation rate we found in the *A. adenophora* rhizosphere soil.

Table 3
Correlations between nitrogen cycling genes and nitrogen contents in soils as determined by Mantel test.

Gene	TN		AN		NO_3-N		$NH4^+$-N	
	Rhizosphere	*Bulk*	*Rhizosphere*	*Bulk*	*Rhizosphere*	*Bulk*	*Rhizosphere*	*Bulk*
nifH	0.06	0.15	0.2.	0.21	0.06	0.12	0.37*	0.35*
amoA-Arc	−0.02	−0.02	0.19.	0.19	0.03	0.05	0.33*	0.56***
amoA-Bac	0.16	0.1	0.41**	0.18	0.24.	0.14	0.28.	0.54**
hao	−0.12	0.08	−0.16	−0.05	−0.27	0.17	−0.02	0.03
gdh	0.25.	0.1	0.25	0.39*	−0.01	0.26.	0.12	0.52***
ureC	0.25	0.04	0.37*	0.35*	0.05	0.28.	0.16	0.47**
narG	0.03	−0.01	0.27*	0.25.	0.07	0.19	0.41*	0.4*
nirS	0.21	0.1	0.23	0.31.	0.12	0.23	0.33*	0.44**
nirK-D	0.04	0.02	0.11	0.24	−0.03	0.16	0.33.	0.41**
nirK-N	−0.1	0.04	−0.18	0.11	−0.04	0.05	0.22	0.22
norB	0.25	0.07	0.34.	0.13	0.04	0.01	0.02	0.18
nosZ	0.13	0.08	0.43**	0.23	0.28*	0.14	0.46*	0.54**
NirB	0.1	0.24.	−0.17	−0.03	−0.06	−0.03	0.08	0.39*
hzo	0.03	−0.14	−0.02	−0.07	−0.1	0.06	0.28.	0.21
napA	−0.1	0.11	0.2.	0.45**	0.04	0.37*	0.26.	0.49**
nasA	0.11	0.03	0.32.	0.08	0.16	−0.05	0.1	0.28.
nirA	−0.28	−0.08	0.22*	0.29.	0.25*	0.13	0.48*	0.49**
nrfA	0.14	0.04	0.39**	0.3*	0.26*	0.27.	0.51*	0.48**

***$p < 0.001$. **$p < 0.01$. *$p < 0.05$. '.'$p < 0.1$.

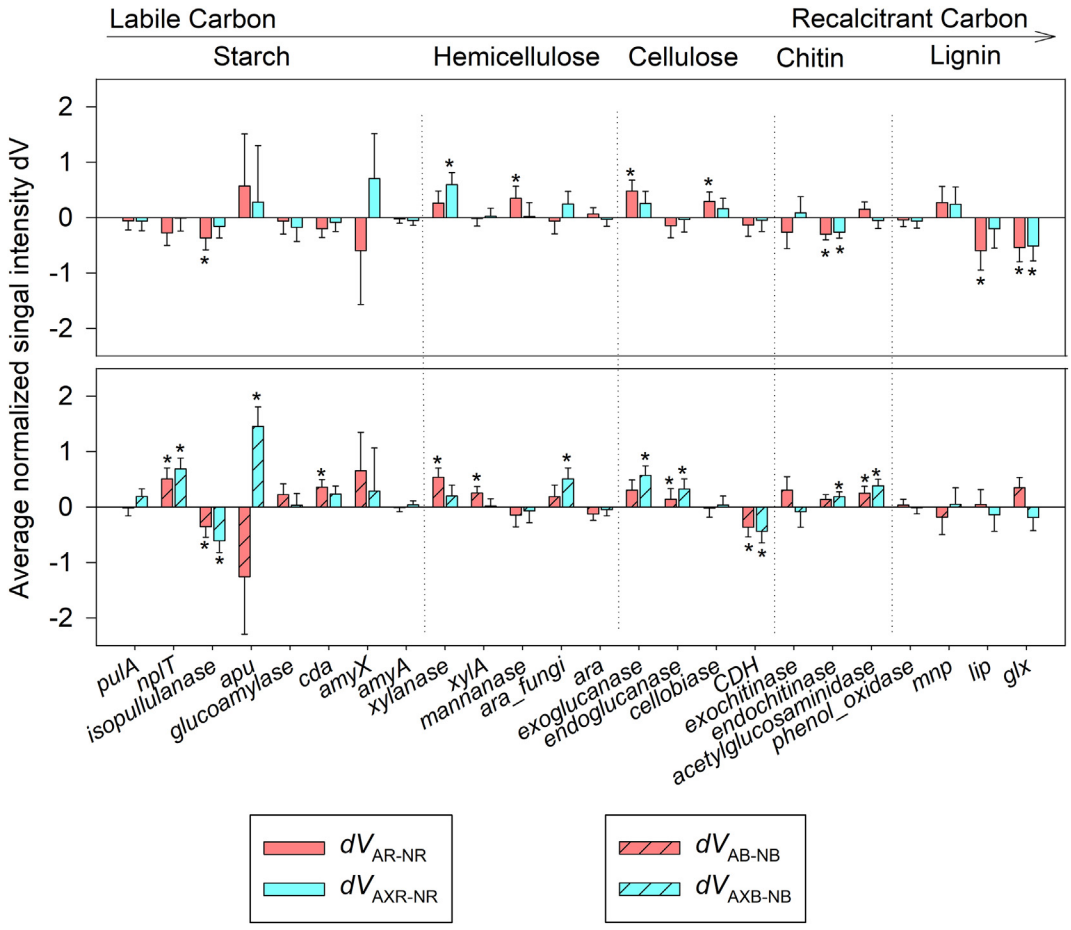

Fig. 3. Normalized average signal intensity differential values (dV) of key genes involved in carbon degradation processes. Genes are arranged in order from most labile to most recalcitrant carbon. Soil samples were compared separately at rhizosphere and bulk levels. $dV_{AR\text{-}NR}$ indicates the differential values between rhizosphere monoculture *A. adenophora* soils and rhizosphere non-invaded plant soils. $dV_{AXR\text{-}NR}$ indicates the differential values between rhizosphere mixture-plant soils and rhizosphere non-invaded plant soils. $dV_{AB\text{-}NB}$ indicate the differential values between bulk monoculture *A. adenophora* soils and bulk non-invaded plant soils. $dV_{AXB\text{-}NB}$ indicate the differential values between bulk mixture-plant soils and bulk non-invaded plant soils. All data are presented as mean ± standard error, $n = 3$. Statistical significance of differences was conducted by *t*-distribution tests. *, $p < 0.05$.

Table 4
Correlations between plant or soil geochemical properties and soil microbial communities as determined by Mantel test.

		16S rRNA gene			GeoChip		
		All	*Rhizosphere*	*Bulk*	*All*	*Rhizosphere*	*Bulk*
Plant properties	Richness	0.03	0.13.	−0.06	0.21*	0.51**	0.31*
	Coverage of *A. adenophora* (%)	0	0.12.	−0.07	0.26**	0.68***	0.43**
	Height (m)	0	0.18.	−0.04	0.28***	0.61**	0.41*
	Aboveground Biomass (g/m^2)	0.01	0.26*	−0.1	0.1	0.29.	0.25
	Belowground Biomass (g/m^2)	−0.01	0.27.	−0.09	0.06	0.19	0.2
	Litter (g/m^2)	0	0.23.	−0.1	0.08	0.2.	0.23
	Shannon's Index (*H′*)	0.04	0.14.	−0.08	0.13.	0.42*	0.19
	Simpson's Index (*1/D*)	0.05	0.08	−0.01	0.07	0.33*	0.05
	Pielou's Evenness	0	0.12	−0.11	0.16*	0.44*	0.24.
	Simpson's Evenness	−0.07	−0.1	0.01	0.22*	0.34*	0.36.
Soil properties	pH	0.72***	0.61***	0.71***	−0.08	0.02	−0.11
	M (%)	0.23**	0.25.	0.24*	0.03	0.09	−0.09
	C_{org} (g/kg)	0.32**	0.03	0.23.	0.22*	−0.05	0.35.
	TP (g/kg)	0.09	0.31*	−0.13	−0.05	−0.1	0.06
	TK (g/kg)	−0.04	0.06	0.03	−0.06	−0.03	0
	TN (g/kg)	0.42***	0.04	0.36**	0.04	0.13	0.07
	AP (mg/kg)	−0.04	0.07	−0.18	0.04	0.07	−0.04
	AK (mg/kg)	0.31**	0.02	0.17	−0.04	0.35*	−0.09
	AN (mg/kg)	0.25**	0.33.	−0.1	0.13.	0.33**	0.24
	NO_3-N (mg/kg)	0.27**	0.46**	−0.05	0.03	0.12	0.19
	NH_4-N (mg/kg)	0.04	0.26*	−0.18	0.17*	0.36*	0.45**

M: Moisture; C_{org}: Organic carbon; TN: Total N; TP: Total P; TK: Total K; AN: Available N; AP: Available P; AK: Available K.
All, all soil; Rhizosphere, rhizosphere soil; Bulk, bulk soil. Significant values are indicated as follows: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; '.', $p < 0.1$.

Potential nitrification rates are often higher in soils of exotic species compared with soils of native plants (Ehrenfeld et al., 2001; Hawkes et al., 2005; Kourtev et al., 2003; Parker and Schimel, 2010). In this study, we also detected higher nitrification rates after *A. adenophora* invasion. The higher abundances of genes involved in nitrification in the invaded bulk soils indicated that *A. adenophora* uptake and litter input may influence differences in nitrification potentials. More so, it is likely that the higher rates of nitrification were attributed to the higher concentrations of NH_4^+ in *A. adenophora*-dominated soils, which were two times higher than adjacent non-invaded patches. It is expected that increased nitrification rates would couple with higher denitrification rates (Parker and Schimel, 2010), yet *A. adenophora*-invaded soil did not have higher denitrification rates. This lack of change may be due to the high *napA*/*nrfA* gene-associated dissimilatory nitrogen reduction process.

Our study revealed that invasive *A. adenophora* not only had significantly higher nitrogen fixation rates, potential ammonification rates, and potential nitrification rates, but also influenced the related functional gene abundances. These observed effects suggest a possible invasive mechanism involving functional microbes. The nitrogen functional groups served to accumulate available ammonium in *A. adenophora* soil. Taking these results and the significant correlation between ammonium with nitrogen functional genes into consideration, the exotic plant *A. adenophora* might be designated as an ammonium-preferring species that acquires nutrients with the help of self-selected or self-stimulated microbial communities. Alternatively, *A. adenophora* may more-easily invade soils with these preferable microbial community traits.

5. Conclusions

In this study, we demonstrate superior material productivity of invasive plant *A. adenophora*, suggesting its strong competitive capacity for nutrient resource allocation. We also show that not only were the rhizosphere communities shaped by *A. adenophora* roots and exudates, but also that the bulk soil communities in *A. adenophora* soils differed from those in non-invaded plant soils. Nitrogen cycle-related communities potentially adjusted soil N species for the direct benefit of the invasive plant. In addition, the microbial decomposer communities associated with invaders potentially degraded labile carbon. Most studies addressing plant species invasiveness have exhibited accelerated litter decomposition under the invading plants. Thus, exotics may have an additional beneficial trait of supporting microbial communities that return a supply of preferred plant available nutrients. These mechanisms provide vital information for the development of mitigation strategies and prediction of invasiveness and invasibility.

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Authors' contributions

This study was conceived and led by Fanghao Wan and Jizhong Zhou. Xiaofei Lu and Qiao Li carried out GeoChip experiments and environmental measurements. Mengxin Zhao and Xiaofei Lu performed the analytical work. Mengxin Zhao, Xiaofei Lu and Yunfeng Yang wrote the manuscript. Lauren Hale, Wanxue Liu and Jianying Guo revised the manuscript. All authors discussed the results and their implications and commented on the manuscript as it progressed. All authors approved the final version to be published and agreed to be accountable for the aspects of the work.

Conflict of interest

The authors declare no conflict of interest.

Data accessibility

Both MiSeq sequencing and GeoChip 4.0 data is available online (http://www.ncbi.nlm.nih.gov/). The accession number of GeoChip 4.0 data is GSE114498 and the accession number of MiSeq data is SRP145570.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.04.330.

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