Effect of *Ageratina adenophora* invasion on the composition and diversity of soil microbiome

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In the present study, high throughput 16S rRNA gene sequencing was used to investigate soil invaded by the aggressive weed *Ageratina adenophora* to determine its effect on the species composition, distribution, and biodiversity of the bacterial communities. Soil samples from 12 micro-sites containing a monoculture of *A. adenophora* plants, mixtures of *A. adenophora* and different native plant species, and native species alone were studied. We found that the invasion of this weed resulted in a selection of bacteria belonging to phyla *Acidobacteria* and *Verrucomicrobia* and the lack of bacteria belonging to *Actinobacteria* and *Planctomycetes*, but did not affect significantly the percentage abundances of members of other phyla. A similar bacterial population selection was also observed at genus or subgroup levels. The NO₃⁻-N level was an important factor affecting soil bacterial communities and contributed to the dominance of *A. adenophora*. However, the numbers of total bacterial species, and the diversity and structure of soil bacterial microbiome did not (P > 0.05) change significantly following invasion by this weed.

Key Words: *Ageratina adenophora*; high throughput sequencing; undersurface soil microbiome; weed invasion

Introduction

*Ageratina adenophora* (Sprengel) (Synonym: *Eupatorium adenophorum* Sprengel), a notorious weed originat-
2014). Consequently, the levels of available P, K, NO$_3^-$, NH$_4^+$ and organic C in the affected region are substantially higher than those in non-invaded soils. However, most of these data come from using culture-dependent microbiological techniques. It is well known that less than 0.1% of soil (Hill et al., 2000) and 10% of rhizosphere bacteria (Hirsch et al., 2010) can be recovered as pure cultures. The limitation of these culture-dependent techniques is such that meaningful information about the diversity and population composition of the soil microbiome located in close proximity to A. adenophora is still lacking. Furthermore, it remains unclear to what extent invasion of A. adenophora may affect the native soil microbiome. We believe this information is an essential first step in understanding interactions between A. adenophora and its immediate soil microbiome, before proposing new measures to control this weed.

In recent years, as a result of the accelerated advances in sequencing technology, metagenomic analysis has been increasingly used in the study of the interaction of soil-plant-microorganisms. Soil metagenomics of the 16S rRNA gene and/or the ITS1 region, using next generation sequencing technologies (Niedringshaus et al., 2011), has revealed an unsurpassed diversity of soil microbiota (Nesme et al., 2016). Soil high-throughput sequencing, combined with soil physicochemical analysis, has been successfully used to investigate the effects of soil pH (Zhalnina et al., 2014), plant type (Prober et al., 2014) and crop rotation (de Quadros et al., 2012), on soil microbial diversity and composition. In this study, we have investigated the composition and diversity of the microbiome in soil invaded by A. adenophora using 16S rRNA gene high throughput sequencing to assess the possible influence of the invasion of A. adenophora on the composition and diversity of the soil microbiome. Also, the key factors controlling the soil bacterial microbiota invaded by A. adenophora have been examined using redundancy analysis.

Materials and Methods

**Site description and sampling.** The study site was situated in a foothill of Maomao Qing, Xishan, Kunming (24°55′29″ N, 102°36′36″ E), where the invasion of A. adenophora began in 1987. It had an obvious gradient of A. adenophora as monoculture, a mixture of A. adenophora (40–50% coverage) and native species Elsholtzia ciliata, Artemisia indica and Setaria plicata, and native species only. The foothill has an area of ca. 400 x 400 m$^2$ with a mean altitude of 2170 m (from 2150 to 2190 m) without tree coverage. The study site has a precipitation of 975 mm (from 937–1058 mm). To investigate the effects of A. adenophora’s invasion on the soil microbiome, four micro-site samples were collected in May 2015 from each zone, with A. adenophora as a monoculture, a mixture of A. adenophora and native species Elsholtzia ciliata, Artemisia indica and Setaria plicata, and native species only. The sample micro-sites were spaced by at least 2 m intervals and located at the same altitude. In total, twelve soil samples were collected. Before soil collection, all surface vegetation and litter layers were removed with a shovel, which was thoroughly cleaned with 70% ethanol after each sampling. At each micro-site, soil samples were collected at a depth of 0–10 cm and 10–20 cm. The collected soil samples were wrapped in autoclaved foil, placed into sterile Whirl-Pak sample bags (Nasco, Fort Atkinson, WI, USA), and transported to the laboratory within 1 h. About a 500 g subsample of each soil sample was homogenized (medium treatment for 1 min) in a Waring heavy-duty blender (Lab-Biogen, Kunming, China) and passed through a 2-mm sieve. A 250 g subsample collected at a 0–10 cm depth was mixed with the same amount of subsample collected at a 10–20 cm depth at the same micro-site (by the same blender, medium treatment for 1 min) and the mixture was used for physicochemical analysis.

**Physicochemical analysis of soil samples.** Soil organic matter composition was determined using the K$_2$Cr$_2$O$_7-H_2$SO$_4$ oxidation method (Nelson and Sommers, 1996). The total phosphorus nitrogen was measured using the Kjeldahl method. Soil inorganic N was extracted with 2 mol KCl, and concentrations of NO$_3^-$-N and NH$_4^+$-N in the KCl extracts were determined with hydrazine sulfate colorimetry and indophenol blue colorimetry, respectively (Mulvaney, 1996). The total phosphorus and available phosphorus were determined using the colorimetric Moblue-method (Kuo, 1996). The pH value (1:2.5 solution of soil to water) was measured using a pH meter (Mettler-Toledo International Inc., China).

**DNA extraction, PCR amplification and illumina sequencing.** Soil DNA was extracted using a PowerSoil DNA Isolation Kit (Ambiosci, Shenzhen, China) according to the manufacturer’s protocol. The same amount of DNA extracted from a 0–10 cm and 10–20 cm depth from the same micro-site was mixed, and the mixture was used for downstream analyses. The crude DNA was purified on 0.8% (wt/vol) low melting point agarose gel in a TAE buffer to obtain genomic DNA. The DNA bands were excised, extracted with QIAquick Gel Extraction Kits (Qiagen Inc., Mississauga, ON, Canada), and then used as the template for PCR amplification. The V4 hypervariable region of bacterial 16S rRNA gene (Biddle et al., 2008) was amplified using 2 × KAPA HiFi HotStart ReadyMix (Shanghai Dobio, Shanghai, China) with the primer set: 341F (CCTACGGGNGGCWGCAG) (Muyzer et al., 1993) and 785R (GACTACHVGGGTATCTAATCC) (Lee et al., 1993) with the following PCR conditions: initial denaturation at 94°C for 3 min followed by 5 cycles of denaturing at 94°C for 10 s, annealing at 55°C for 15 s and extension at 72°C for 30 s before a final extension at 72°C for 5 min. The 16S rRNA gene applicons obtained were attached after cleanup with AMPure XP beads to dual indices and Illumina sequencing adapters using the Nextera XT Index Kit (Illumina China, Shanghai) with the following PCR (also prepared with 2 × KAPA HiFi HotStart ReadyMix) condition: initial denaturation at 95°C for 30 s followed by 8 cycles of denaturing at 95°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s.
before a final extension at 72°C for 5 min. The PCR was done in triplicate for each sample and PCR products were again cleaned up with AMPure XP beads, quantified using an Agilent Technologies 2100 Bioanalyzer, normalized and pooled. Then, the 16S rRNA gene applicons obtained were diluted to 4 nM with 10 mM Tris (pH 8.5) before being denatured with NaOH and sequenced (with 5% PhiX as an internal control) on a MiSeq IIllumina Platform (PE250) at Genergy Biotechnology (Shanghai, China).

Phylogenetic analyses. The V4 applicons of 16S rRNA genes were pair-end assembled and checked using the software Flash (Magoc and Salzberg, 2011) to ensure that their sequences perfectly matched in the index sequences, had no more than one 1 mismatch error present in the forward primer sequences, and the trimmed sequences were longer than 200 bp. Trimmed high-quality sequences were uploaded into QIIME (Caporaso et al., 2010) to perform OTU clustering through the ‘pick_otus_through_otu_table.py’ pipeline using the 97% identity threshold (3% dissimilarity levels), and to carry out alpha diversity analyses. The OTUs grouped were annotated by using RDP classifier (Cole et al., 2014). Any OTU represented by £3 sequences was removed. The 16S rRNA amplicon sequences have been deposited in the NCBI Sequence Read Archive under the following accession number SAMN04251321: Agtykh2015 (Taxld: 410658). Biodiversity indices, including the Chol index, Sharron index, coverage ratios, and Venn figures, were calculated with Mothur (Schloss et al., 2009) by following the procedures provided, applying a 97% identity threshold. Phylogenetic trees showing distance and OTU abundances of the different taxa were constructed with MEGAN4 (Huson et al., 2011) following the procedures recommended by the provider. A heatmap for showing the clustering of sequences and OTU abundances for individual genera was calculated and constructed with the software Cluster 3.0 (de Hoon et al., 2004).

Redundancy analysis. Redundancy analysis (RDA) was carried out to investigate the relationships between environmental variables and bacterial community. To test whether RDA analysis was appropriate for the dataset, the data were first tested for normality. Detrended correspondence analysis (DCA) was used first to determine the character of variability in the assemblages using the following criterion: if the length of the first gradient was greater than 3 standard deviations (SD) we assumed a unimodal variation, and a length less than 3 SD indicates a linear

### Table 1. physiochemical characterization of soil invaded by Ageratina adenophora at different degrees.

| Site description | pH | Organic C (mg Kg$^{-1}$) | NH$_4^+$-N (mg Kg$^{-1}$) | NO$_3^-$-N (mg Kg$^{-1}$) | Total N (%) | P (mg Kg$^{-1}$) | K (mg Kg$^{-1}$) |
|------------------|----|--------------------------|---------------------------|---------------------------|-------------|----------------|----------------|
| Monoculture 1    | 5.04 | 78.1                     | 15.92                     | 5.42                      | 0.28        | 7.7            | 278            |
| Monoculture 2    | 5.28 | 73.6                     | 14.85                     | 6.10                      | 0.24        | 7.8            | 295            |
| Monoculture 3    | 5.49 | 81.5                     | 20.33                     | 4.28                      | 0.20        | 7.4            | 250            |
| Monoculture 4    | 5.42 | 67.0                     | 17.34                     | 5.99                      | 0.28        | 6.5            | 258            |
| Mixed-species 1  | 5.83 | 50.7                     | 14.09                     | 5.31                      | 0.20        | 6.7            | 299            |
| Mixed-species 2  | 6.02 | 52.7                     | 12.57                     | 5.11                      | 0.25        | 6.1            | 241            |
| Mixed-species 3  | 6.33 | 55.0                     | 24.78                     | 4.34                      | 0.21        | 5.6            | 237            |
| Mixed-species 4  | 5.62 | 62.2                     | 23.04                     | 3.41                      | 0.29        | 7.5            | 214            |
| Native-species 1 | 6.18 | 47.6                     | 17.96                     | 3.12                      | 0.27        | 5.4            | 251            |
| Native-species 2 | 5.28 | 50.5                     | 20.71                     | 2.27                      | 0.36        | 5.6            | 246            |
| Native-species 3 | 5.99 | 51.4                     | 18.06                     | 3.65                      | 0.41        | 6.8            | 243            |
| Native-species 4 | 6.73 | 57.7                     | 18.24                     | 3.83                      | 0.22        | 5.5            | 279            |

### Table 2. Biodiversity index and similarity of bacterial communities in soils invaded by Ageratina adenophora at different degrees.

| Sample description | Number of raw sequences | Number of HQ* sequences | Number of OTUs | Chol index | Shannon index | Coverage |
|--------------------|-------------------------|-------------------------|----------------|------------|---------------|----------|
| Monoculture 1      | 26,860                  | 26,598                  | 3,670          | 5,814      | 6.573         | 0.92     |
| Monoculture 2      | 30,728                  | 30,449                  | 3,749          | 6,045      | 5.932         | 0.93     |
| Monoculture 3      | 30,019                  | 29,794                  | 4,208          | 6,970      | 6.491         | 0.94     |
| Monoculture 4      | 23,196                  | 22,978                  | 2,992          | 5,144      | 5.613         | 0.92     |
| Mixed-species 1    | 31,840                  | 31,473                  | 3,157          | 4,991      | 5.530         | 0.94     |
| Mixed-species 2    | 25,944                  | 25,787                  | 3,663          | 6,393      | 6.372         | 0.91     |
| Mixed-species 3    | 36,396                  | 36,164                  | 4,843          | 7,606      | 6.478         | 0.91     |
| Mixed-species 4    | 35,036                  | 34,868                  | 4,545          | 6,737      | 6.894         | 0.93     |
| Native-species 1   | 33,067                  | 32,939                  | 4,263          | 5,928      | 6.944         | 0.93     |
| Native-species 2   | 24,313                  | 24,169                  | 3,771          | 6,105      | 6.557         | 0.91     |
| Native-species 3   | 24,966                  | 24,808                  | 3,870          | 6,109      | 7.073         | 0.91     |
| Native-species 4   | 26,744                  | 26,589                  | 3,379          | 5,189      | 6.640         | 0.93     |

*HQ represents high quality.

*a,b,c represent the soil micro-sites with three aboveground vegetation types: a Ageratina adenophora as monoculture, b a mixture of Ageratina adenophora and native plant species, and c only native species.*
variation. The length of the first gradient calculated for the bacterial communities was 0.7 SD, indicating a linear variation and justifying redundancy analysis. Statistical calculations were performed using CANOCO for Windows 4.5 software. The significance of the relationship was assessed using Monte Carlo permutation tests provided in the software. The significance of the first ordination and canonical axes was assessed in permutation tests with 999 unrestricted Monte Carlo permutations ($P < 0.05$).

**Statistical analysis.** Statistical analyses were carried out using a Student $t$-test, and, when necessary, normalization of data to be compared was carried out in SPSS (version 16.0). The significance was declared at $P < 0.05$.

**Results**

**Soil physiochemical properties**

The invasion of *A. adenophora* affected the physiochemical characters (Table 1) of the colonized soil. The pH values of micro-sites with a monoculture of *A. adenophora* (abbreviated as monoculture micro-sites hereafter) were lower ($P = 0.01$) than those occupied by native species (abbreviated as native-species micro-sites hereafter). The former contained more organic C than both the latter ($P = 0.001$) and the micro-sites occupied by mixtures of *A. adenophora* and native species (abbreviated as mixture micro-sites hereafter) ($P = 0.002$). Soil NO$_3^-$ and available phosphorus levels at the monoculture micro-sites were higher than those at the native-species micro-sites ($P$ values of 0.01 and 0.01, respectively). No significant differences ($P > 0.05$) in soil NH$_4^+$, available K and total N were found between these three sites.

**Effect of invasion of *A. adenophora* on biodiversity and composition of soil bacterial microbiome**

16S rRNA amplicon sequences (Table 2) retrieved from the 12 micro-sites colonised with the three vegetation types (four replicates for each) were used to investigate any effect the invasion of *A. adenophora* might have on the composition of each local soil microbiome. High quality sequence reads of 22,978 to 36,164 were obtained from individual samples taken from these 12 micro-sites. Phylogenetic analyses showed they belonged to between 4452 and 4843 OTUs. Chao1 analyses (Table 2) estimated the numbers of OTUs of bacterial communities in soil with *A. adenophora* affected the relative abundances of members of the phyla Acidobacteria (22.4%), Proteobacteria (14.6%), Verrucomicrobia (9.3%), Actinobacteria (6.2%), Bacteroidetes (6.1%), Planctomycetes (3.7%), Gemmatinonadetes (0.8%), Chloroflexi (0.43%), Firmicutes (0.40%), OD1 (0.21%), Chlamydiae (0.20%), TM7 (<0.01%), Nitrospira (<0.01%), WS3 (<0.01%), OP10 (<0.01%), BRC1 (<0.01%), Cyanobacteria (<0.01%) and Spirochaetes (<0.01%). Unclassifiable bacteria contributed an average of 33.6% to the total bacterial OTUs. The orders and families identified for each of the main phyla, their relative abundances, and their phylogenetic relationship are shown in Fig. S1.

**Invasion of *A. adenophora*** affected the relative abundances of members of these phyla. Thus, bacterial communities at the monoculture micro-site soils contained higher abundances of members of Acidobacteria [34.7($\pm$6.2)% cf. 22.0($\pm$4.6)%], Proteobacteria [26.9($\pm$10.0)% cf. 11.4($\pm$6.4)%], Verrucomicrobia [26.9($\pm$10.0)% cf. 11.4($\pm$6.4)%], Planctomycetes [1.3($\pm$0.3)% cf. 3.1($\pm$0.7)%], Bacteroidetes [3.0($\pm$2.7)% cf. 11.68($\pm$7.7)%], and Nitrospira (<0.01%) were found between the microbial communities at the different vegetation sites.
Impacts of this invasion on the soil microbiome are also apparent at the genus and group levels. Of the OTUs identified for each microbiome at the 12 micro-sites (Table 2), 29–81% could be classified reliably at the genus level. These belong to 159 genera, and their distribution and relative abundances in the different bacterial communities are shown in Fig. S2. Of these, 34 genera are assessed to be relatively abundant, with each accounting for at least an average of 0.25% of the total OTUs detected per micro-site, and they are listed in Table S1.

OTU community distributions varied in soil samples taken from the different vegetation types. Thus, percentage abundance values of Bradyrhizobium at the A. adenophora sites were higher than those in soils at the native-species sites [7.8±1.3% cf. 2.8±0.7%, P = 0.03] and mixed-species sites [7.8±1.3% cf. 5.3±0.7%, P = 0.01]. Similarly, percentage abundance values of Singulisphaera [2.0±0.6% cf. 0.6±0.3%, P = 0.01], Phenylbacterium [2.1±1.9% cf. 0.7±0.2%, P = 0.01], Prosthecococcos [0.6±0.3% cf. 0.2±0.1%, P = 0.05] and Kedonobacter [2.9±4.0% cf. 0.2±0.1%, P = 0.05], at the A. adenophora sites also exceeded those at the native-species sites. In contrast, the A. adenophora soil communities contained fewer members of Nocardioides [0.8±0.2% cf. 7.3±2.1%, P = 0.001], Terrimonas [1.0±0.5% cf. 4.8±3.5%, P = 0.05], Flavobacterium [0.1±0.01% cf. 4.1±3.9%, P = 0.05], Soliubrobacter [0.3±0.2% cf. 3.1±1.1%, P = 0.001], Pirellula [0.4±0.3% cf. 2.6±0.8%, P = 0.001], Steroidobacter [0.3±0.2% cf. 1.1±0.1%, P = 0.02], Adhaeribacter [0.1±0.01% cf. 1.7±0.9%, P = 0.001], Pseudonocardia [0.3±0.1% cf. 1.3±1.0%, P = 0.01], Nitrospira [0.2±0.2% cf. 0.8±0.3%, P = 0.02], Lysobacter [0.1±0.03% cf. 0.7±0.2%, P = 0.001], Marmorica [0.1±0.09% cf. 0.6±0.4%, P = 0.03], and Arthrobacter [0.2±0.1% cf. 0.5±0.2%, P = 0.001], than those at the native-species micro-sites. However, the percentage values of members of the genera Opitutus, Gemmatimonas, Kribbella, Conexibacter, Mycobacterium, Mucilaginibacter, Gemma, Planctomyces, Varioroxar, Zavarzinella, Sphingosinicella, Chitinophaga and Solitae were not significantly different in soil samples taken at the 3 different vegetation sites.

Acidobacteria and Verrucomicrobia members were highly abundant in all examined soil samples. In total, 2,404 acidobacterial OTUs were identified belonging to subgroups GP1–GP7, GP10, GP11, GP13, GP15–GP18, GP20, GP22 and GP25. Of these, subgroups GP1–GP6 were relatively abundant, with each accounting for an average of at least 0.26% of the total OTUs per micro-site. The communities at the A. adenophora micro-sites contained more members of GP1 [7.7±0.8% cf. 1.9±0.5%, P = 0.001], GP2 [4.4±1.2% cf. 0.6±0.4%, P < 0.001], GP3 [1.5±0.2% cf. 0.6±0.2%, P < 0.001] and GP6 [7.7±0.8% cf. 1.9±0.5%, P < 0.001], but fewer members of GP4 [2.0±0.2% cf. 2.6±0.4%, P = 0.04] and GP5 [0.5±0.1% cf. 0.7±0.1%, P = 0.04], than those at the native-species micro-sites. A total of 882 verrucomicrobial OTUs were retrieved. Most belonged to unclassified Spartobacteria_genera_incertae_sedis (359 OTUs) and subdivision 3_incertae_sedis members (343 OTUs). Communities at the A. adenophora micro-sites

Fig. 2. Phylum composition of bacterial communities at 12 soil micro-sites invaded by A. adenophora at different densities.

Monoculture, Mixed-species and Native-species represent surface vegetation with A. adenophora as a monoculture, a mixture of A. adenophora and native plant species, and only native plant species, respectively.
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contained more Spartobacteria_genera_incertae_sedis species than those at the native-species micro-sites [2.3(±0.4)\% cf. 1.4(±0.2)\%, P = 0.03], but about the same abundances of Subdivision 3_incertae_sedis species [1.7(±0.4)\% cf. 1.7(±0.2)\%, P = 0.6].

Clustering analyses showed that no clear patterns (Fig. S2) could be recognized for the communities at the four micro-sites with the same vegetation type, or among the 12 communities in soil colonised by the different vegetation types. Thus, differences in vegetation type did not appear to influence significantly the structures of their soil microbiomes.

**Relationship between environmental factors and composition of bacterial communities**

RDA was applied to reveal the relationship between the bacterial community and environmental factors. The environmental factors in the first two RDA axes, respectively, explained 85.3\% and 7.1\% of the total variance on bacterial community (Fig. 3). Soil NO_3^-N (P = 0.0010, F = 5.41, 999 Monte Carlo permutations) significantly correlated with the abundance of bacterial communities, indicating that it was the important determinant to bacterial community. Soil pH had a tendency (P = 0.0910, F = 1.83, 999 Monte Carlo permutations) to contribute to the composition of bacterial communities, while soil available P and K, TN, TOC, NH_4^+-N and temperature did not show any significant community-environment relationship with P values of 0.1680, 0.7100, 0.2910, 0.2330, and 0.4550, respectively, showing a lesser importance in affecting the composition of bacterial communities.

**Discussion**

In this study, we have used high-throughput 16S rRNA amplicon sequencing to investigate the composition and
diversity of soil bacterial communities invaded by the aggressive weed *A. adenophora* at different population densities. Our study has focused on the potential influences of such an invasion on the localised soil microbiome by taking bulk soil samples under different vegetation types (see Section “Materials and Methods” for more detail) but not strictly rhizosphere samples.

We were able to show that members of the *Acidobacteria*, *Proteobacteria*, *Verrucomicrobia*, *Actinobacteria*, *Bacteroidetes* and *Planctomycetes* were dominant in all the soil samples examined. This was not unexpected as these bacteria are considered to be core components of the soil microbiome (Janssen, 2006). However, the data presented here clearly show that this invasion appeared to result in the favourable selection of bacteria belonging to the phyla *Acidobacteria* and *Verrucomicrobia*, and the exclusion of bacteria belonging to the phyla *Actinobacteria* and *Planctomycetes*. Rhizosphere selection of *Acidobacteria* and *Verrucomicrobia* by *A. adenophora* has been reported (de Rocha et al., 2013). *Acidobacteria* have been attributed an important role in the soil C cycle due to their ability to degrade cellulose and lignin (Ward et al., 2009) and *Verrucomicrobia* may play an important role in methanol transformation (Fierer, 2015). Their selection in bulk soil below *A. adenophora*, found in the current study, indicates that invasion of this weed significantly modified soil microbiota close to its rhizosphere, which could be mainly due to plant litters produced by the fast growth of *A. adenophora*.

We also noticed that *A. adenophora*’s invasion selected bacterial species from α-Proteobacterial genera *Bradyrhizobium*, *Phenylobacterium* and *Prosthecomicrobium*, *Planctomycetes* genus *Singularisphaera*, and *Chloroflexus* genus *Ktedonobacter*. However, except in the case of the genus *Bradyrhizobium* in which *B. japonicum* (100% match in 16S rRNA sequence), a nitrogen-fixing bacterium when living either free in the soil or endosymbiotically in infected host cells of the central nodule tissue (Preisig et al., 1993; Saito et al., 1998), accounted for 1.2% of the total bacteria (data not shown) at the monoculture sites, thus implying a role in nitrogen fixation, it is impossible to explain the enrichment of members from other bacterial genera by invasion. 

In conclusion, the high-throughput sequencing conducted in this study has provided sufficient molecular evidence to support that the enrichment of select soil functional bacterial groups play a vital role in the successful establishment and eventual dominance of *A. adenophora*. Furthermore, higher levels of NO$_3$-N were also linked to the dominance of *A. adenophora*. However, the invasion of *A. adenophora* did not constitute a significant change in the structure of the underlying soil’s bacterial microbiome.

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Supplementary Materials

Supplementary figures and table are available in our J-STAGE site (http://www.jstage.jst.go.jp/browse/jgam).

References

Bajpai, D. and Inderjit (2013) Impact of nitrogen availability and soil communities on biomass accumulation of an invasive species. *Agron. Plants*, 1–27.

Biddle, J. F., Fitz-Gibbon, S., Schuster, S. C., Brenchley, J. E., and House, C. H. (2008) Metagenomic signatures of the Peru margin subseafloor biosphere show a genetically distinct environment. *Proc. Natl. Acad. Sci. USA*, **105**, 10583–10588.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D. et al. (2010) QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods*, **7**, 335–336.

Cole, J. R., Wang, Q., Fish, J. A., Chai, B., McGarrell, D. et al. (2014) Ribosomal Database Project: Data and tools for high-throughput *rRNA* analysis. *Nucleic Acids Res.*, **42**, D633–D642.

da Rocha, U. N., Plougge, C. M., George, I., van Elsas, J. D., and van Overbeek, L. S. (2013) The rhizosphere selects for particular groups of *Acidobacteria* and *Verrucomicrobia*. *PloS ONE*, **8**, e82443.

da Hoon, M. J. L., Inoto, S., Nolan, J., and Miyano, S. (2004) Open source clustering software. *Bioinformatics*, **20**, 1453–1454.

de Quadros, P. D., Zhalina, K., Davis-Richardson, A., Fagen, J. R., Drew, J. et al. (2012) The effect of tillage system and crop rotation on soil microbial diversity and composition in a subtropical acrisol. *Diversity*, **4**, 375–395.

Fierer, N. (2015) *Verrucomicrobia* and their role in soil methanol consumption. *B21J-02 Microbial Controls of Biogeochemical Cycling I, Presented at 2015 Fall Meeting, AGU, San Francisco, CA, 14–18 Dec.
