Effects of Mikania Sesquiterpene Lactones on Soil Microbes

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Abstract

Background and aims Allelopathy is frequently invoked as being important for successful invasion by non-native plants. Yet, the effects of specific phytochemicals of invasive plants on soil microbes remain unexplored.

Methods Here we used manipulative experiments and next generation sequencing (NGS) approaches to investigate how the sesquiterpene lactones (STLs) of invasive *Mikania micrantha* influence soil microbial communities and nutrients.

Results We found *Mikania* STLs to significantly increase the regulation of soil microbial activity (i.e. increased CO_2 concentrations). Using the specific STL, dihydromikanolide, we found available soil nutrients to increase in the presence of this phytochemical and that bacterial richness increased while fungal richness decreased. The presence of dihydromikanolide also increased the abundance of beneficial soil bacteria and fungi associated with nutrient cycling and supply, while simultaneously lowering pathogen abundance. Clustering analysis found bacterial functional groups, such as those involved in carbon, nitrogen, phosphorus, and sulfur metabolism, to be similar in experimentally-treated dihydromikanolide soils and *Mikania*-invaded soils collected from the field, but significantly higher than those in uninvaded soils. This suggests that *M. micrantha* can enhance certain bacterial functional groups via its phytochemicals. Soil fungi, on the other hand, appeared to be less sensitive to dihydromikanolide than bacteria.

Conclusions We conclude *Mikania* STLs, and in particular dihydromikanolide, may be key factors in determining soil microbial structure and function and may contribute to the invasion success of the species. Our findings provided a new perspective for understanding the effects of invasive plants on soil microbial communities via their impacts through phytochemicals.

Introduction

Invasive plants often change soil (a)biotic conditions, leading to altered soil microbial community diversity, structure and function (Kourtev et al. 2002; Kueffer et al. 2008; Liu et al. 2020; Prescott and Zukswert 2016; Sun et al. 2019; Zhao et al. 2019). These impacts are mediated primarily through two pathways. First, invasive plants may produce more litter which differs in chemistry from that of native species (Ehrenfeld 2003; Jo et al. 2017; Kueffer et al. 2008; Prescott and Zukswert 2016). Second, invasive species release novel phytochemicals into soils as root exudates or foliar excretions that can directly interact with soil microbes (Callaway et al. 2008; Hierro and Callaway 2003; Morris et al. 2016; Zhang et al. 2009). While the impacts of invasive plants on soil microbial community structure and function have attracted a lot of attention recently (Keet et al. 2021; Song et al. 2015; Xiao et al. 2014; Yang et al. 2020; Zhang et al. 2019), it is generally not well-understood what the contribution of these two main pathways are to changes in soil microbial communities under invasion.

Metabolites produced by some plants can also influence how plants interact with each other. The synthesis of phytotoxic chemicals by invasive plant species can disrupt the growth of native plants (Seigler 1996). For example, some phytochemicals may have antifungal activities that inhibit plant pathogens and/or mutualists (Lankau 2012; Yang et al. 2016; Zheng et al. 2018). Changes in soil microbial communities in response to invasion may also impact soil nutrients (Becerra et al. 2018; Czaban et al. 2018). Ultimately, these changes may lead to nutrient cycles that enhance the performance of invasive plants through positive plant-soil feedbacks (Inderjit et al. 2009; Lankau 2012; Morris et al. 2016). For example, invasive spotted knapweed *Centaurea maculosa* in North America modifies soil microbial communities to its own benefit (Callaway et al. 2004). Similarly, we found that invasive *Mikania micrantha* enrich soils for microbes that participate in nitrogen cycling in China, causing positive plant-soil feedbacks by increasing soil nitrogen availability (Liu et al. 2020). Yet, few studies have documented the effects of specific phytochemicals produced by invasive plants on soil microbial communities and on soil nutrients.

M. micrantha is also known as 'mile-a-minute' weed. The species is native to the tropical regions of South America, Central America and the Caribbean, and is invasive in many tropical regions of Asia and the Pacific (Zhang et al. 2004; Manrique et al. 2011). The invasive success of *M. micrantha* is partly due to its strong allelopathic effects on native plants and soil microbes (Chen et al. 2009; Kaur and Malhotra 2012; Wu et al. 2009). Various phytochemicals, such as sesquiterpene lactones (Huang et al. 2008; Piyasena and Dharmaratne 2013) and phenolic compounds (Xu et al. 2013) have been isolated from different *M. micrantha* tissues. Our latest work found sesquiterpene lactones (STLs) to mainly accumulate in the species' leaves and flowers, and that these phytochemicals are released into the surrounding soil through litter (Liu et al. 2020). However, very little is known about the effects of these STLs on soil microbes and how they relate to the invasion success of *M. micrantha*. In this study, we use manipulative experiments and next generation sequencing (NGS) approaches to investigate how *Mikania* STLs influence soil microbial communities and nutrients. We hypothesized that soil nutrients would increase in the presence of *Mikania* STLs and therefore that soil bacterial diversity would increase. We also hypothesized that fungal diversity will decrease as *Mikania* STLs are known to have antifungal properties.

Materials And Methods

STLs manipulation experiment

We collected soils from two sites: a *M. micrantha* monoculture (i.e. invaded soils, hereafter IS) and a neighboring uninvaded area (i.e. uninvaded soils, hereafter US), located in a dry riverbed of Liuxi River, Guangzhou City, Guangdong Province, China (23º28′37.99″ N, 113º28′41.49″ E). Five independent replicates of each soil type were randomly sampled from a 30 m × 30 m field plot using the five-point sampling method (Jin et al. 2015). All collected samples were transported back to the laboratory as soon as possible under cooled conditions. Soils were loosened, sifted through a 1.0-mm sieve and stored at

4°C. Soils were weighed (equivalent of 30 g dry soil) and placed into sterile 100 mL incubation bottles and left at room temperature for one week.

We added 4.5 mL of five different *Mikania* STLs (20 mg L^{-1}; anhydroscandenolide, deoxymikanolide, dihydromichaelide, scandenolide and 3-epi-dihydroscandenolide) separately to US. Therefore, the final concentration of each STL per soil was approximately 3 mg kg^{-1}. We also kept untreated US and IS soils as negative and positive controls, respectively. Soil moisture was adjusted with sterilized water to 70% of soil water holding capacity and bottles sealed using rubber plugs. Each treatment had five replicates, leading to 35 samples in total (25 US with STL addition, 5 US and 5 IS). All bottles were placed in an incubator (RXZ intelligent, Ningbo Jiangnan Instrument Factory) for 12 days (28°C, dark condition, 65% humidity) and rubber plugs opened for 30 min every two days to balance air. Gas in incubation bottles was extracted with a 50 mL syringe and collected in 100 mL aluminum foil air sampling bags (Shanghai Haocheng Technology Co., Ltd.). At the end of the experiment, a subset of each soil sample was stored at 4°C to determine soil nutrient contents and a subset at -80°C for DNA extraction for NGS.

Quantification of *Mikania* STLs and CO_2 concentration

Initial *Mikania* STLs content of IS was immediately analyzed by UPLC-MS (Waters, Milford, MA, USA) after collection in the field. Analyses were performed on an ACQUITY™ UHPLC system couple with a triple-quadrupole Xevo TQD mass spectrometer. An ACQUITY UPLC® BEH C18 column (2.1 mm × 50 mm, 1.7 μm) was employed and the column temperature maintained at 40°C. The gradient elution with acetonitrile containing 0.1% formic acid (A) and water containing 0.1% formic acid (B), was performed as follows: 0–1.0 min, 20% A; 1.0–3.0 min, 20–60% A; 3.0–6.0 min, 60–95% A; 6.0–8.0 min, 95% A; 8.0-8.5 min, 95 – 20% A; 8.5–10.0 min, 20% A. The flow rate was set at 0.3 mL min^{-1}. The auto-sampler was conditioned at 22°C and the injection volume of solution was 2 μL for all analyses. Mass spectrometric detection was performed on Xevo-TQD equipped with an electrospray ionization source (ESI). The capillary voltages were set to 3.0 and 2.22 kV at positive and negative modes, respectively, and the source temperature maintained at 150°C. The collision gas was Ar, and N_2 was used for desolvation at 400°C and cone gas at a flow rate of 700 L h^{-1}, the cone gas set to 50 L h^{-1}. Compounds for multiple reaction monitoring (MRM) were optimized in negative mode, the dwell time being 0.025 s (Table S1). Mass spectrometry and selected ion recording (SIR) were also used in relative quantitative analysis in both positive and negative ions measures.

After 12 days of incubation, CO_2 concentration was measured in each bottle using an Agilent 7890B Gas Chromatograph (Agilent Technology, USA), in which hydrogen flame ionization detector (FID) was used for detection, the temperature of which was set at 250°C, the temperature of the separation column set at 55°C, and the carrier gas was high-purity N_2.

The influence of dihydromikanolide on soil nutrients and soil acidity

Because dihydromikanolide was the STL with the highest concentration in IS that caused the largest increase in CO_2 concentration (see Results section), we chose it to compare the effects of lactones addition (i.e. dihydromikanolide soils, hereafter referred to as DS) on soil nutrients and microbial communities with US and IS.

Subsets of fresh soil from dihydromikanolide treatments and controls were immediately extracted with 50 mL 2 mol L^{-1} KCl to determine ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) concentrations by Continuous Flow Analyzer (Proxima, Alliance instruments, France). Subsets of each fresh soil sample was also air-dried at room temperature. A subset of the air-dried soil was sieved through a 0.15-mm mesh and finally used for total N measurement by combustion using a TOC analyzer (LI-8100A, Elementar company, Germany), available phosphorus (AP) by using spectrophotometer (UV-2000, Shimadzu, Kyoto, Japan). A second subset of the air-dried soils was sieved through a 2-mm mesh and used for available potassium (AK) measurements by using flame atomic absorption spectrometry (Z-5300, Polarized Zeeman Atomic, Absorption Spectrophotometer). Soil pH was measured using electrode pH meter (ST3100, Ohaus Instrument (Changzhou) Co., Ltd.), in a 1:5 (w/v) soil-water suspension.

Effects of dihydromikanolide on soil microbial community diversity and structure

Total genomic DNA was extracted from dihydromikanolide-treated and control soil samples (three independent samples for each soil type; total n = 9) using the Fast DNA SPIN extraction kit (MP Biomedicals, Santa Ana, CA, USA). The quantity and quality of extracted DNA were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and samples stored at -20^{o}C until further analysis. We amplified the V3-V4 variable region of the 16S rRNA of bacteria using the primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). We used the primers ITS5F (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS1R (5'-GCTGCGTTCTTCATCGATGC-3') to amplify and sequence fungal communities. Each 25.0 μL PCR reaction included DNA 2.0 μL template DNA, 5.0 μL 5 x Reaction Buffer, 0.25 μL Q5 high-fidelity DNA polymerase, 5.0 μL 5 x High GC Buffer, 2.0 μL dNTPs (10 mM), 1.0 μL of the forward and reverse primer (10 μM), and 8.75 μL ddH_2O. Both gene regions followed the following thermal cycle for PCR amplification: initial denaturation at 98^{o}C for 30 s, followed by 25–27 cycles of denaturation at 98^{o}C for 15 s, annealing at 50^{o}C for 30 s, extension at 72^{o}C for 30 s, final extension at 72^{o}C for 5 min. PCR amplicons were purified with Agencourt AMPure Beads (Beckman Coulter, Indianapolis, IN) and quantified using the PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA).

PCR products were sequenced by Pair-end 2 × 300 bp using the Illumina Miseq platform at Shanghai Personalbio Biotechnology Co., Ltd (Shanghai, China). Low-quality sequences (< 150 bp in length, average Phred scores < 20, containing mononucleotide repeats of > 8 bp) were filtered out (Chen and Jiang 2014; Gill et al. 2006). FLASH was used to assemble paired-end reads (Magoč and Salzberg 2011). After chimera detection, the remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at 97% DNA sequence similarity using the UCLUST algorithm (Edgar 2010; Navarro-Noya et al. 2013). OTUs accounting for less than 0.001% of all sequence reads were discarded. Representative

sequences of all OTUs were aligned and annotated against the Greengenes database (Release 13.8) for bacterial OTUs and the UNITE database (Release 7.0) for fungal OTUs. The QIIME software (Version 1.8.0) was used to draw rarefaction curves and to calculate diversity indexes of samples, including Chao1, ACE, Shannon and Simpson's. Cluster analyses and heat maps of the top 50 genera were drawn in QIIME. Venn diagrams were drawn in the R statistical environment. Linear discriminant analysis of effect size (LEfSe) was used to identify indicator taxa (biomarkers) in different soil samples. This approach combines linear discriminant analysis with non-parametric Kruskal-Wallis and Wilcoxon rank sum tests to screen for key biomarkers (Segata et al. 2011). By submitting the relative abundance matrix (at genus level) on the galaxy online analysis platform (http://huttenhower.sph.harvard.edu/galaxy/), LEfSe can automatically analyze the composition of each classification level and visualize the results. We used partial least squares discriminant analyses (PLS-DA) based on species abundance matrix and sample to analyze species classification levels. PLS-DA is a supervised pattern recognition method based on partial least squares regression models, and it reorders samples in a new low dimensional coordinate system by searching for the maximum covariance of the species richness matrix and the given sample distribution or grouping information. Since PLS-DA can reduce the influence of multicollinearity among variables, it is more suitable for the study of complex microbial community datasets. We used the PICRUSt software (Langille et al. 2013) to predict gene function present in our soil bacterial communities. Fungal sequencing reads were associated with putative ecological functions using FUNGuild tools (Nguyen et al. 2016).

Statistical analysis

All statistical analyses were done using SPSS 11.0 software (SPSS Inc., USA). *Mikania* STL concentration, CO_2 concentration, soil pH and nutrient contents and soil microbial diversity indices were individually analyzed using one-way ANOVAs, followed by Duncan-test at $P < 0.05$. The origin 8.5 software was used to visualize data.

Results

Changes of *Mikania* STL content and CO_2 concentration

The presence of all *Mikania* STLs was confirmed in IS: dihydromichaelide (76.50 ug kg^{-1}), scandenolide (35.36 ug kg^{-1}), anhydroscandenolide (19.33 ug kg^{-1}), 3-epi-dihydroscandenolid (15.19 ug kg^{-1}) and deoxymikanolide (7.34 ug kg^{-1}) (Fig. 1-a).

CO_2 concentrations gradually increased in the all soils over the first three days of incubation (Fig. 1-b). Compared with US, the concentrations of CO_2 released from soils treated with *Mikania* STLs increased by 113.08% (anhydroscandenolide), 102.86% (deoxymikanolide), 108.05% (dihydromichaelide), 57.61% (scandenolide) and 52.85% (3-epi-dihydroscandenolid), by the end of the experiment. Amongst of them, soils treated with anhydroscandenolide and dihydromichaelide released the highest amount of CO_2.

Changes in soil nutrients and soil acidity in response to dihydromikanolide

Total N and NO_3^--N contents were significantly reduced, and the content of NH_4^+-N significantly increased, in DS compared with US (Fig. 2). On average TN and NO_3^--N content decreased by 3.69% and 46.55% respectively, while NH_4^+-N content increased by 58.79%. The addition of dihydromikanolide to US also increased AP and AK compared with US only and on average AP and AK content increased by 18.24% and 14.77% in DS, respectively. There was no significant difference in soil nutrients between DS and IS (Fig. 2), but there is a significant difference in the pH value between DS (4.78 ± 0.15) and IS (5.48 ± 0.11) ($P < 0.05$).

Microbial alpha-diversity responses to dihydromikanolide

Rarefaction curves for bacteria and fungi plateaued in all three soils types (US, DS and IS) indicated that our sequencing depth was reasonable (Fig. S1).

Estimates of alpha diversities showed Chao1 and ACE indices for bacteria to be significantly higher in DS and IS compared with US, while the Chao1 index for fungi in DS was significantly lower than in IS and US (Table 1). Simpson and Shannon indexes of the bacterial and fungal were not significantly different among the three soil types (Table 1).

Table 1
Alpha-diversity indices of bacteria and fungi in three soil types (mean ± SD, n = 3).

Microbes	Index	US	DS	IS
Bacteria	Chao1	2741.00 ± 78.43c	4110.66 ± 246.28a	3449.24 ± 511.33b
	ACE	2741.00 ± 78.43c	4356.51 ± 195.45a	3606.77 ± 546.93b
	Simpson	0.998 ± 0.001a	0.997 ± 0.001a	0.998 ± 0.000a
	Shannon	10.27 ± 0.21a	10.17 ± 0.35a	10.39 ± 0.089a
Fungi	Chao1	913.46 ± 97.57ab	740.33 ± 38.39b	940.28 ± 128.43a
	ACE	916.02 ± 94.90a	746.77 ± 38.42a	947.40 ± 134.05a
	Simpson	0.99 ± 0.00a	0.98 ± 0.02a	0.99 ± 0.00a
	Shannon	7.95 ± 0.06a	7.27 ± 0.69a	7.87 ± 0.12a

IS - invaded soil; US - uninvaded soil; DS - uninvaded soil with dihydromikanolide (Dih) addition. Data with different letters in the same line indicate significant differences (Duncan-test, $P < 0.05$).

Bacterial and fungal community composition in response to dihydromikanolide

The number of shared bacterial OTUs between the three soil types was 2170. US and IS only shared 458 OTUs. Invaded soils harbored the highest number of unique bacterial OTUs (2089) of all soils. 591 fungal OTUs, accounting for 17.75% of all OTUs, were shared between the three soil types. US and DS had

similar numbers of unique OTUs (US, 624; DS, 631) and IS the highest number of unique OTUs (770) (Fig. 3). The fungi:bacteria ratio was 0.34, 0.28 and 0.34 in US, DS and IS, respectively (Fig. 3).

The number of soil bacterial and fungal OTUs that could be classified to phylum, class, order, family and genus levels is provided in Table 2. Our results showed that the addition of dihydromikanolide led to bacterial diversity across various taxonomic levels that was similar to IS but significantly higher than in US. However, fungal diversity in DS was not significantly different from that of US, and only significantly lower than that present in IS at the phylum-level.

Table 2
Comparisons of bacterial and fungal OTU abundances at different taxonomic levels between different soil types (mean ± SD, n = 3).

	Taxonomic level	US	DS	IS
Bacteria	Phylum	2740.33 ± 78.24b	3253.00 ± 213.60a	3138.00 ± 145.75a
	Class	2700.00 ± 80.89b	3206.67 ± 215.24a	3100.67 ± 141.18a
	Order	2415.00 ± 15.13b	2877.33 ± 119.63a	2810.00 ± 160.25a
	Family	1817.67 ± 31.01b	2247.67 ± 116.12a	2271.00 ± 120.13a
	Genus	1031.00 ± 101.34b	1373.33 ± 74.10a	1427.33 ± 103.39a
Fungi	Phylum	594.67 ± 63.22ab	530.33 ± 81.73b	677.33 ± 40.50a
	Class	555.00 ± 58.51a	492.67 ± 87.92a	623.00 ± 33.72a
	Order	550.67 ± 59.00a	483.67 ± 78.77a	606.67 ± 34.53a
	Family	458.67 ± 47.26a	399.67 ± 59.14a	480.67 ± 28.92a
	Genus	450.00 ± 47.09a	398.33 ± 61.44a	488.00 ± 32.60a

IS - invaded soil; US - uninvaded soil; DS - uninvaded soil with dihydromikanolide (Dih) addition. Data with different letters in the same line indicate significant differences (Duncan-test, $P < 0.05$).

Eight bacterial phyla had relative abundances > 1% across soil types, namely Proteobacteria, Acidobacteria, Chloroflexi, Actinobacteria, Gemmatimonadetes, Planctomycetes, Firmicutes and Verrucomicrobia, accounting for more than 95% of the total bacterial community (Fig. 4-a). Among these, Proteobacteria had the highest relative abundance, accounting for between 23.19%-29.27% of all OTUs, followed by Acidobacteria, accounting for between 22.71%-27.61% of all OTUs. Across different soil types, the relative abundance of Proteobacteria was significantly higher in DS and IS compared with US, while the relative abundance of Chloroflexi and Gemmatimonadetes was significantly lower in these two soils compared with US ($P < 0.05$). Compared with US, the relative abundance of Firmicutes in DS was significantly higher, while that of Acidobacteria was significantly lower ($P < 0.05$).

We identified four fungal phyla with relative abundances > 1% in all soils, namely Ascomycota, Basidiomycota, Mortierellomycota and Rozellomycota, accounting for between 55.09%-68.58% of taxa

present in our fungal communities (Fig. 4-b). Among them, Ascomycota had the highest relative abundance, followed by Basidiomycota. The relative abundance of Ascomycota and Mortierellomycota was significantly higher in DS and IS compared with US; while the relative abundance of Basidiomycota was slightly lower in these two soils than in US ($P < 0.05$). The relative abundance of Rozellomycota in IS was significantly lower than in US ($P < 0.05$).

Indicator taxa analyses

We found apparent different indicators among soil types. LEfSe analyses identified AD3, *Nitrospira*, Nitrospirales, Nitrospirae and Nitrospiraceae as key bacterial groups (indicator taxa or bioindicators) in US, while in DS Firmicutes, Ruminococcacea, Caproiciproducens, Lachnospiraceae, *Caldicoprobacter* and Caldicoprobacteraceae were identified as indicator taxa. The indicator bacterial taxa in IS included Alpha-proteobacteria, Xanthobacteraceae, *Bradyrhizobium*, *Aerobacter* and Microbacteriaceae (Fig. 5). On the other hand, indicator fungal groups in US were Spegazzini, *Aplosporella*, Pleosporales_fam_incertae_sedis, Aplosporellaceae and Pseudorobillarda, while those in DS were Ustilaginomycetes, Ustilaginales, Anthracoideaceae and Anthracoidea. The bioindicator fungal groups in IS were *Staphylotrichum*, Phaeosphaeriaceae and Dermateaceae (Fig. 5).

Bacterial and fungal community structure and function in response to dihydromikanolide

PLS-DA indicated that differences in the structure of bacterial and fungal communities were mainly driven by soil type (Fig. 6). In all instances, community structure between DS and US was more similar than between any one of them and IS (Fig. 6). Genus-level clustering and heat map analyses of bacteria and fungi similarly found DS and US to cluster together, and separately from IS (Fig. 7).

Our PICRUSt analysis found bacterial functional groups in DS and IS to cluster, which was apparently different from those in US (Fig. 8). Moreover, most functions in carbon, nitrogen, phosphorus, and sulfur metabolisms in DS and IS were significantly ($P < 0.05$) higher than those in US (Table 3). On the other hand, FUNGuild analysis found fungal functional groups of DS to mainly cluster with US, and to be different from IS (Fig. 8).

Table 3
Differences in metabolic functions of bacterial OTUs between different soil types (mean ± SD, n = 3).

Metabolic function		US	DS	IS
Carbohydrate metabolism	Carbohydrate digestion and absorption	546 ± 108b	1721 ± 686a	993 ± 75ab
	Carbon fixation in photosynthetic organisms	121161 ± 6410b	165063 ± 22459a	159823 ± 12174a
	Carbon fixation pathways in prokaryotes	298626 ± 15402b	391058 ± 49629a	384572 ± 26498a
	Butanoate metabolism	273201 ± 12705b	359759 ± 37409a	370353 ± 18335a
	Citrate cycle (TCA cycle)	224143 ± 12617b	292198 ± 36350a	288918 ± 18933a
	Fructose and mannose metabolism	162658 ± 12666b	213731 ± 29291a	207804 ± 19534a
	Galactose metabolism	173592 ± 25489a	212133 ± 32087a	212987 ± 27623a
	Glycolysis / Gluconeogenesis	289563 ± 16829b	380980 ± 49516a	371955 ± 25637a
	Glyoxylate and dicarboxylate metabolism	199931 ± 9198b	275789 ± 36609a	274437 ± 20340a
	Pyruvate metabolism	269841 ± 12614b	362601 ± 43180a	356131 ± 20840a
	Starch and sucrose metabolism	247407 ± 34973a	305741 ± 47227a	306108 ± 39554a
	Lipid biosynthesis proteins	237138 ± 12963b	310619 ± 37744a	312414 ± 23686a
	Lipoic acid metabolism	19476 ± 1554b	25399 ± 3849a	24428 ± 1922ab
	Lipopolysaccharide biosynthesis	128069 ± 17251a	155998 ± 20443a	155137 ± 24057a
Nitrogen metabolism	Nitrogen metabolism	204223 ± 14111b	274306 ± 43020a	271119 ± 22714a
	Nitrotoluene degradation	34291 ± 1206b	43463 ± 3439a	40628 ± 2949a
	Non-homologous end-joining	28948 ± 3464b	37212 ± 6381ab	40726 ± 3676a

IS - invaded soil; US - uninvaded soil; DS - uninvaded soil with dihydromikanolide (Dih) addition. Data with different letters in the same line indicate significant differences (Duncan-test, $P < 0.05$).

Metabolic function		US	DS	IS
Phosphorus metabolism	Phosphatidylinositol signaling system	24264 ± 1044b	32629 ± 3847a	30854 ± 2095a
	Phosphonate and phosphinate metabolism	8007 ± 1224b	13015 ± 1621a	12721 ± 675a
	Phosphotransferase system (PTS)	11781 ± 2677b	18265 ± 2790a	15649 ± 607ab
Sulfur metabolism	Sulfur metabolism	98381 ± 8410b	126731 ± 17638a	127981 ± 13010a
	Sulfur relay system	98231 ± 5442b	127807 ± 16300a	124111 ± 9646a
	Synthesis and degradation of ketone bodies	41836 ± 2154b	54114 ± 5206a	59881 ± 2887a
	Systemic lupus erythematosus	181 ± 6a	286 ± 76a	273 ± 51a

IS - invaded soil; US - uninvaded soil; DS - uninvaded soil with dihydromikanolide (Dih) addition. Data with different letters in the same line indicate significant differences (Duncan-test, $P < 0.05$).

Discussion

Soil microbes not only play important roles in how soil ecosystems react to the abiotic changes caused by invasive plants (Rout and Callaway 2009), but also in the allelopathic effects of invasive plants on native species (Inderjit and van der Putten 2010; Uddin et al. 2017). Here we show that *Mikania* STLs significantly altered both soil CO_2 release and nutrient availability. These findings suggest that secondary metabolites exuded by this invasive vine into soils can enhance soil microbial activity to promote soil nutrient mineralization. Our focus on the specific *Mikania* STL, dihydromikanolide, provided important insights of how phytochemicals impacts these processes.

We found that dihydromikanolide to significantly increase soil bacterial richness, but to have insignificant effects on bacterial Shannon or Simpson diversity. Bacterial OTU richness in dihydromikanolide-treated soils was similar to that in *Mikania*-invaded soils collected in the field. On the other hand, we found richness of fungi to decrease in the presence of dihydromikanolide, compared with US and IS, indicating a negative/inhibitory effect of this phytochemical on soil fungi. There are potentially many reasons for this observation. For example, bacteria was more sensitive to soil pH than fungi (Rousk et al. 2010), which was significantly influenced by dihydromikanolide addition in our study. However, the opposite has been found, where plant invasion led to decreased bacterial diversity or increased soil fungal diversity (Lorenzo et al. 2013; Si et al. 2013). Obviously, the effects of invasive plants on soil microbial diversity may depend on the species-specific phytochemicals or the specific environmental contexts under which they operate.

Previous studies have changed soil nutrients following plant invasion to impact soil fungal:bacterial (F:B) ratios (e.g., Perkins and Nowak 2013). Moreover, ecosystems with high F:B ratios typically have higher carbon storage and slow soil organic matter (SOM) turnover (Malik et al. 2016; Soares and Rousk 2019). In our study, the F:B ratio in DS was lower than that in US, accompanied by higher CO_2 release in DS, implying that *M. micrantha* may weaken soil carbon storage and enhance SOM turnover through its phytochemicals. Surprisingly, the F:B ratio in DS was also lower than that in IS, suggesting that these changes may develop over time. Therefore, we speculate that, while *M. micrantha* may initially interfere with the soil micro-environment by releasing phytochemicals, more stable F:B ratios may emerge after successful colonization. Another reason for these differences may be because the micro-environment in IS has been influenced by *M. micrantha* roots for longer, and by more phytochemicals, than that of DS.

The changes in bacterial and fungal communities we observed might be related to certain dominant microbial groups occupying unique niches (Fig. 4). Compared with uninvaded soils, the relative abundance of Proteobacteria in dihydromikanolide and *Mikania*-invaded soils was significantly higher, while the relative abundance of Chloroflexi and Gemmatimonadetes was significantly lower. Moreover, Firmicutes in dihydromikanolide soils increased significantly, while Acidobacteria decreased (Fig. 4-a). Similar effects have been observed for the secondary metabolite astragalin produced by the invasive plant *Flaveria bidentis*, with the relative abundance of soil Proteobacteria increasing when astragalin is added exogenously to soils (Zhang et al. 2016). Acidobacteria are oligotrophic and their ability to compete with eutrophic bacteria is relatively weak in high-nutrient environments (Pascault et al. 2013). Therefore, *M. micrantha* may enhance immediate nutrient availability by secreting phytochemicals that reduce oligotrophic bacterial groups during the early stages of colonization, which is consistent with the characteristics of *M. micrantha* growing in high-nutrient soil environments (Zhang et al. 2004).

With regard to fungi, the relative abundance of Ascomycota and Mortierellomycota in dihydromikanolide and invaded soils in this study was significantly higher compared with uninvaded soils, whereas the opposite was true for the relative abundance of Basidiomycota (Fig. 4-b). Ascomycota is known to promote the transformation of soil organic matter (Egidi et al. 2019; Hanson et al. 2008). Mortierellomycota possesses a variety of plant growth-promoting characteristics, such as phosphate solubilization, nitrogen supply and enhanced disease resistance (Liao et al. 2013; Zhang et al. 2011). These general characteristics are consistent with our results showing that NH_4^+-N and AP contents increase after the addition of dihydromikanolide. (Fig. 2). By contrast, Basidiomycota is a major fungal phylum that includes important plant and animal pathogens (Martinez et al. 2004), some with high virulence and wide host ranges (Olson and Stenlid 2001; Zhang and Zhang 2015). Therefore, *M. micrantha* may increase the abundance of beneficial fungi and reduce the number of soil pathogens through some of its phytochemicals, thereby changing soil microbial communities in ways that promotes its own growth (e.g. Blumenthal et al. 2009; Lankau 2011) and negatively impacts co-occurring natives (e.g. Mangla et al. 2008; Stinson et al. 2006).

It is well-known that invasive plants can stimulate certain soil functional microbes and their relevant activities (Chen et al. 2009; Chen et al. 2020; Liu et al. 2020). For example, soil ammonia-oxidizers can be

dramatically influenced by leaf exudates of invasive plants, in turn impacting soil nitrogen transformation and availability (Chen et al. 2020). Previous work has also found leave extracts from *M. micrantha* to enhance soil nitrification rates and higher ammonium (NH_4^+-N) and nitrate soil nitrogen (NO_3^--N) levels (Chen et al. 2009). Our previous work has also found the relative abundances of microbes carrying nitrogen cycling genes, ammonium-oxidizing bacteria, phosphorus- solubilizing, and potassium-solubilizing bacteria to be significantly higher in *M. micrantha*-invaded soils compared with uninvaded or control soils (Liu et al. 2020). As a result, these enriched microbial functional groups promote utilization of the available nitrogen, particularly ammonium nitrogen, phosphorus, and potassium by *M. micrantha*. In agreement with these findings, we found the amount of certain bacterial functional groups, such as those involved in in carbon, nitrogen, phosphorus, and sulfur metabolism, to be similar between dihydromikanolide-treated soils and invaded soils, but significantly higher than in uninvaded soils, suggesting that *M. micrantha* can enhance bacterial functional groups via phytochemicals.

Conclusion

In general, the addition of *Mikania* STLs enhanced soil microbial activity and increased the availability of soil nutrients. Our DNA barcoding results indicate that soil microorganisms were stimulated by *Mikania* STLs, of which bacteria were more sensitive to dihydromikanolide than fungi. These changes were associated with higher bacterial richness and functional diversity that appears to accelerate the release of available nutrients. These effects may contribute the ease by which *M. micrantha* colonize new habitats. These findings provide a new perspective for understanding the phytochemical effects of invasive plants on soil microbial communities and how these may impact invasiveness.

Declarations

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Author contributions WL and MZ designed the experiment. HY performed the experiments and analyzed the data. HY, JLR, MZ and WL wrote the first draft of the manuscript.

Compliance with ethical standards

Competing interests The authors declare no competing interests.

Data availability The raw sequences of bacteria and fungi were deposited in the NCBI Sequence Read Archive under accession number SRP219405 and SRP219476, respectively.

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Figures

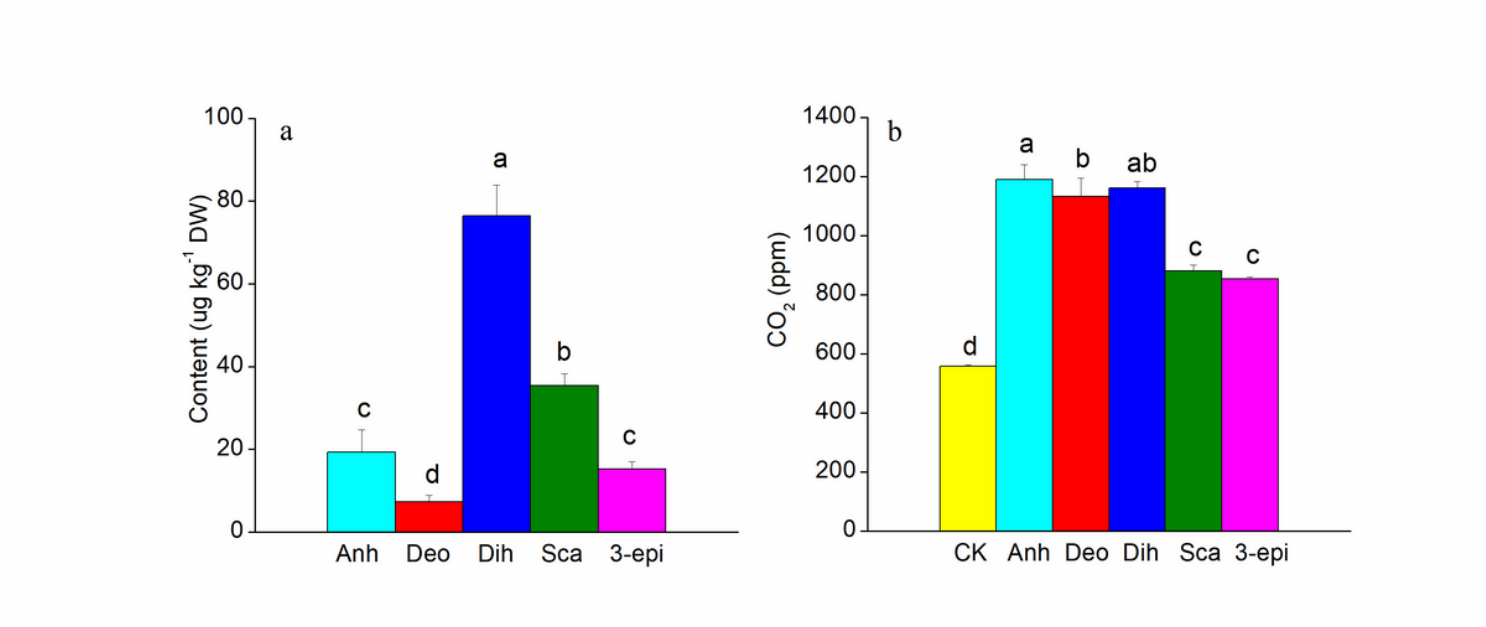

Figure 1

Initial concentrations of five Mikania STLs in invaded soils (a) and changes of CO2 concentration over time after the addition of these Mikania STLs to soils and in control soils (b) (mean ± SD, n=5). Different letters above bars indicate significant differences (Duncan-test, P < 0.05). Anh – anhydroscandenolide addition; Deo – deoxymikanolide addition; Dih – dihydromikanolide addition; Sca – scandenolide addition; 3-epi - 3-epi-dihydroscandenolide addition; IS - invaded soil; US - uninvaded soil.

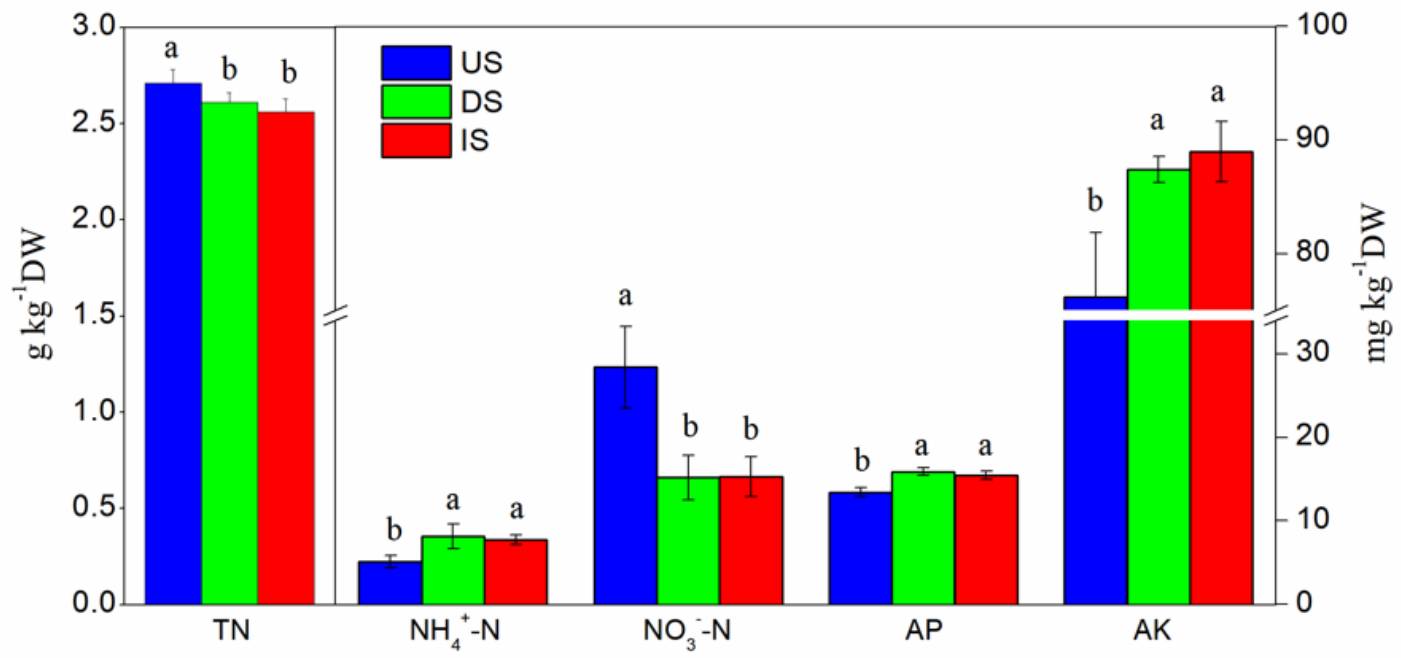

Figure 2

Changes of soil nutrients in uninvaded soils with the addition of dihydromikanolide (Dih) after 12 days of incubation (mean ± SD, n=5). Different letters above bars indicate significant differences (Duncan-test, $P < 0.05$). IS - invaded soil; US - uninvaded soil; DS - uninvaded soil with dihydromikanolide addition; TN-total nitrogen; NH4+-N-ammonium nitrogen; NO3–N-nitrate nitrogen; AP-available phosphorus; AK-available potassium.

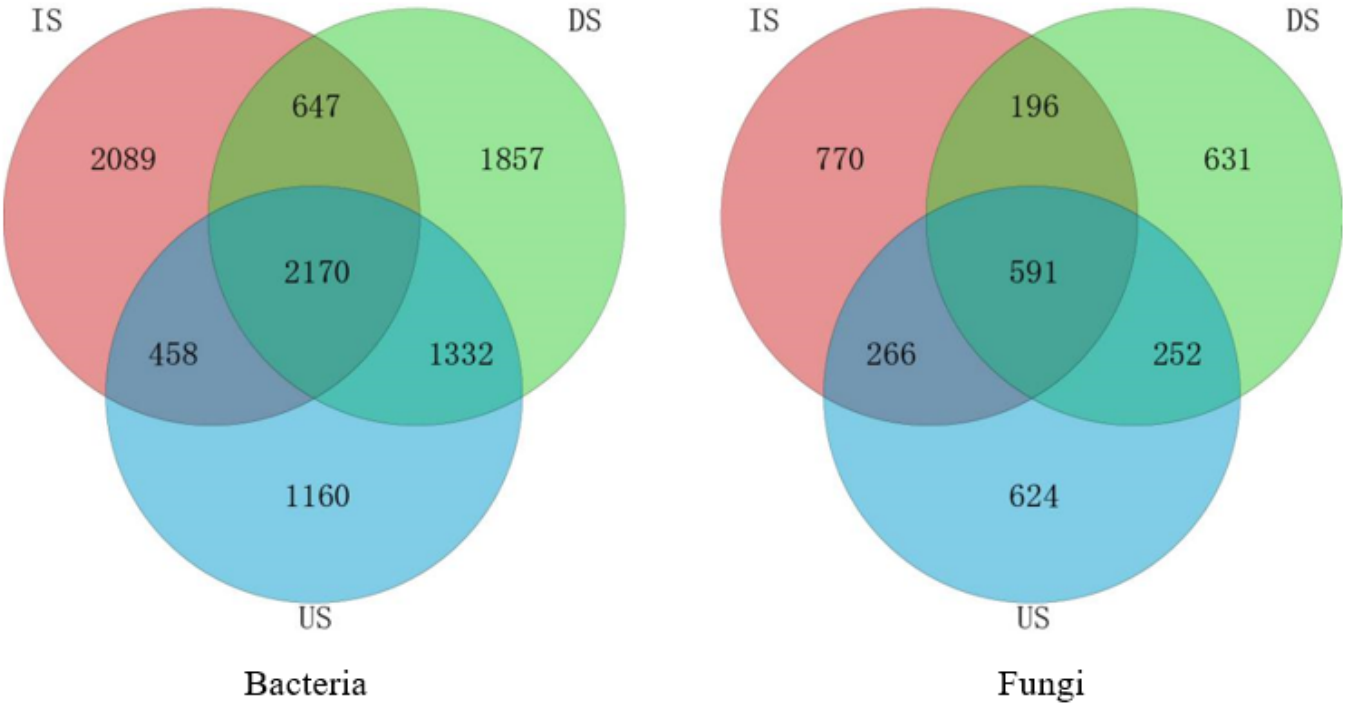

Figure 3

Venn diagrams of soil bacteria (left) and fungi (right) in three soils at the OTU level (n=3 for each soil type). IS - invaded soil; US -uninvaded soil; DS - uninvaded soil with dihydromikanolide (Dih) addition.

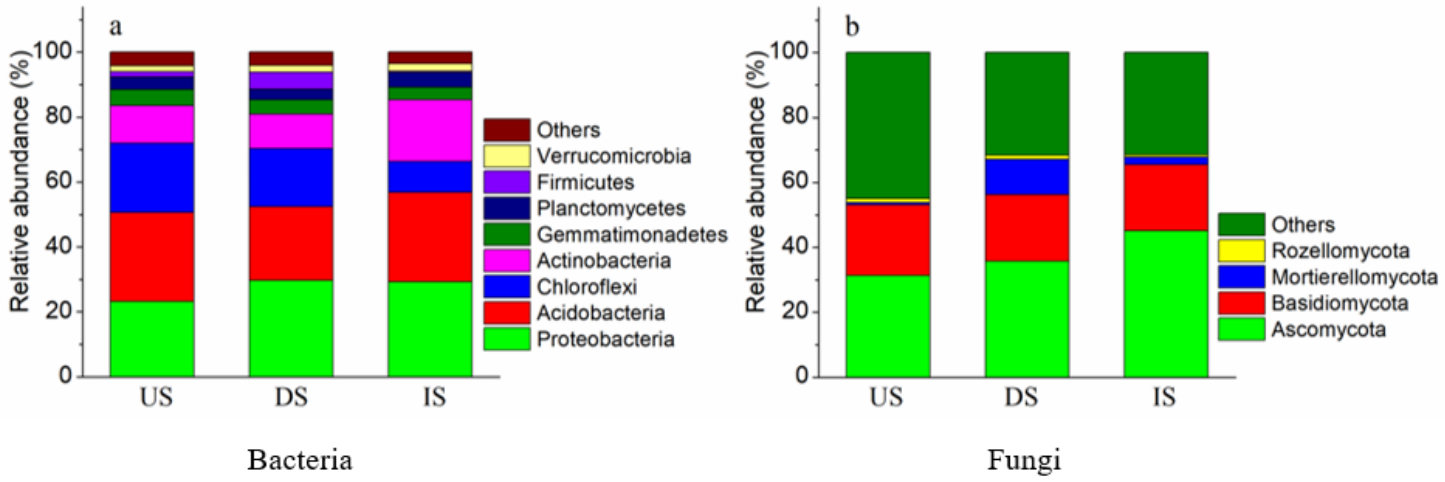

Figure 4

Microbial composition of bacteria (a) and fungi (b) in different soil types at the Phylum level (n=3 for each soil type). IS - invaded soil; US - uninvaded soil; DS - uninvaded soil with dihydromikanolide addition.

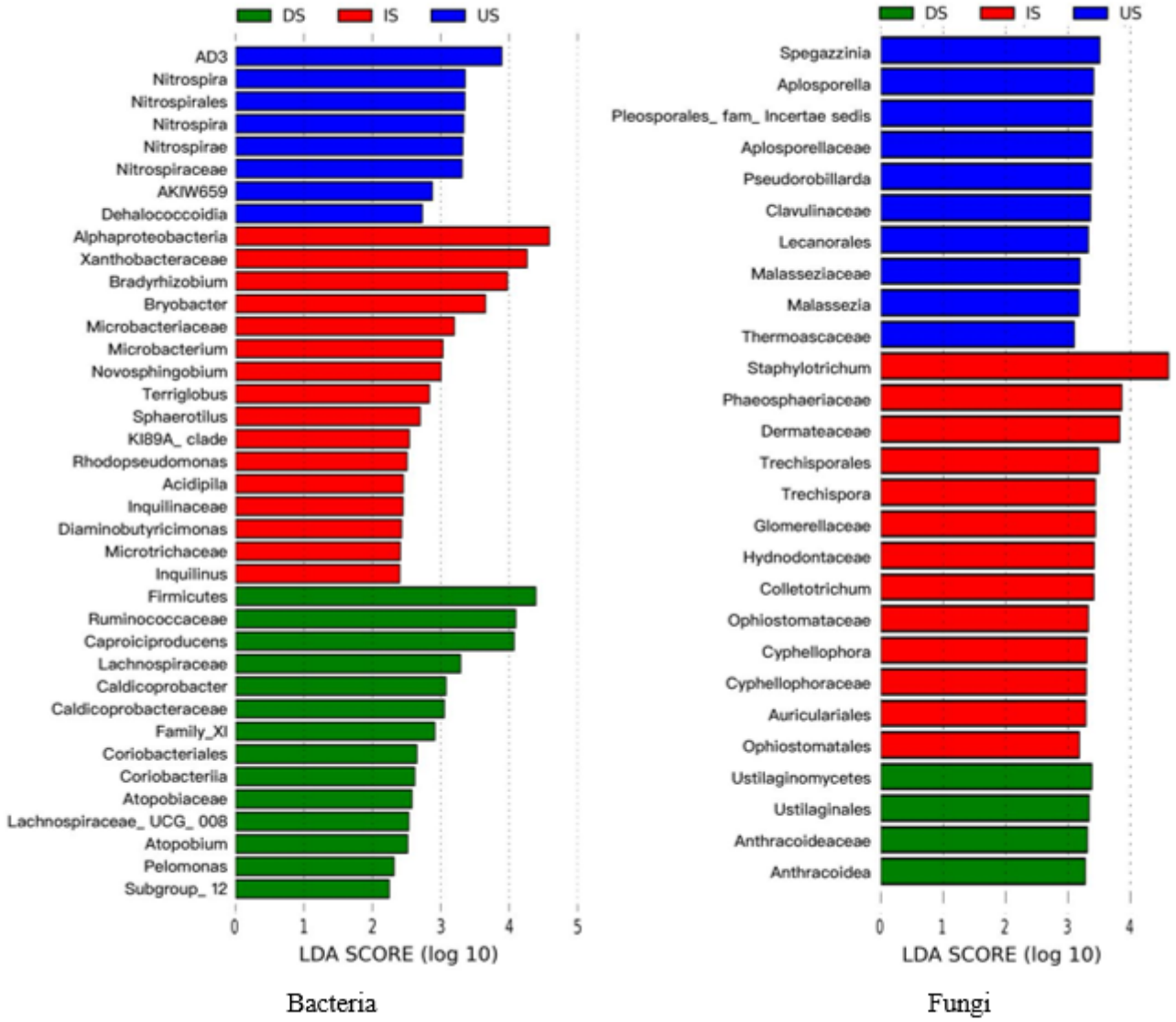

Figure 5

Key community members (indicator taxa) of soil bacteria (left) and fungi (right) in different soil types identified by linear discriminant analysis effect size (LEfSe) analysis (n=3 for each soil type). IS - invaded soil; US - uninvaded soil; DS - uninvaded soil with dihydromikanolide (Dih) addition. Vertical axes display indicator taxa in each soil type (different bar colors indicate the grouping of samples with higher abundance corresponding to the taxon: blue-uninvaded soil; red-invaded soil; green-uninvaded soil with dihydromikanolide addition) and horizontal axes provide their LDA scores (higher scores correspond to more important indicator taxa).

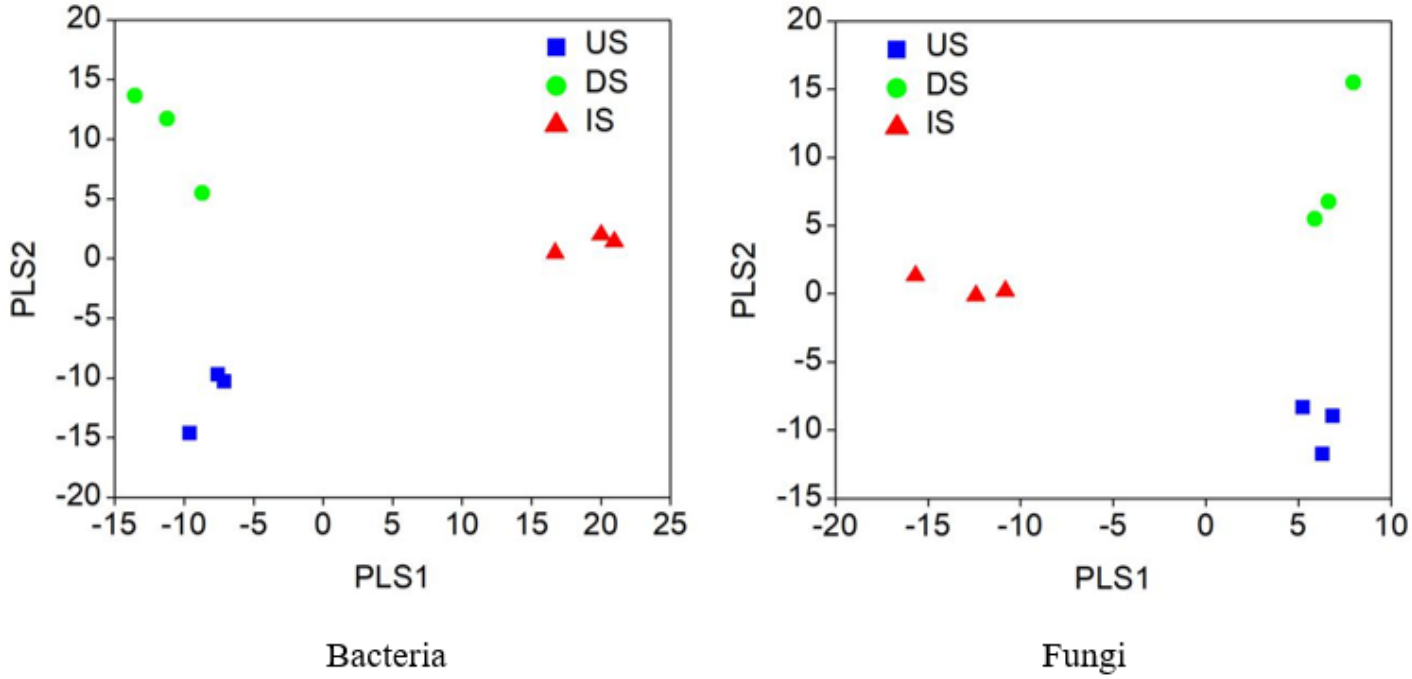

Figure 6

Partial least squares discriminant analysis (PLS-DA) of soil bacteria (left) and fungi (right) (n=3 for each soil type). IS - invaded soil; US - uninvaded soil; DS - uninvaded soil with dihydromikanolide (Dih) addition.

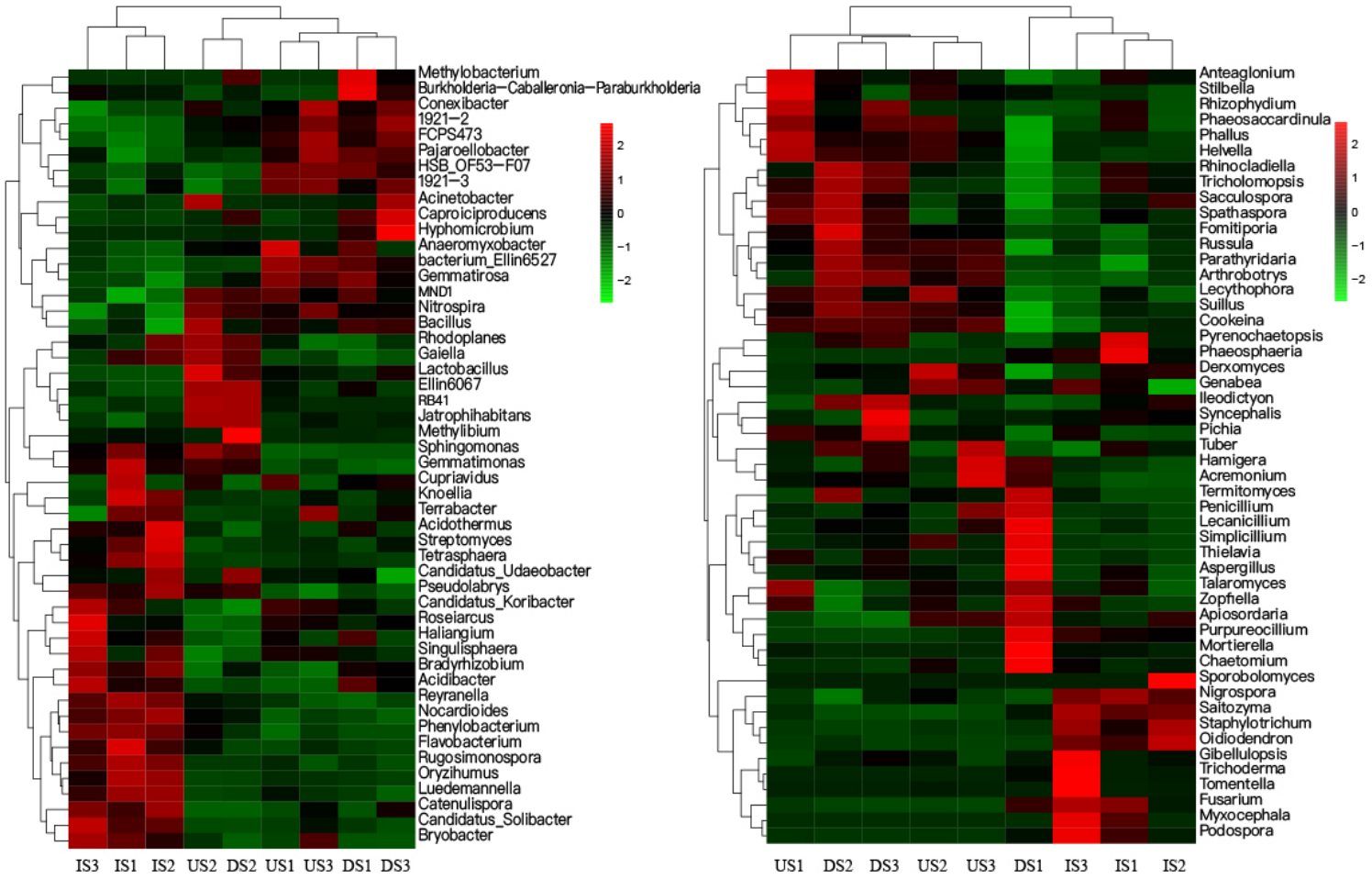

Figure 7

Heatmap of community composition of soil bacteria (left) and fungi (right) at genus level combined with cluster analysis across different soil types (n=3 for each soil type). IS - invaded soil; US - uninvaded soil; DS - uninvaded soil with dihydromikanolide (Dih) addition.

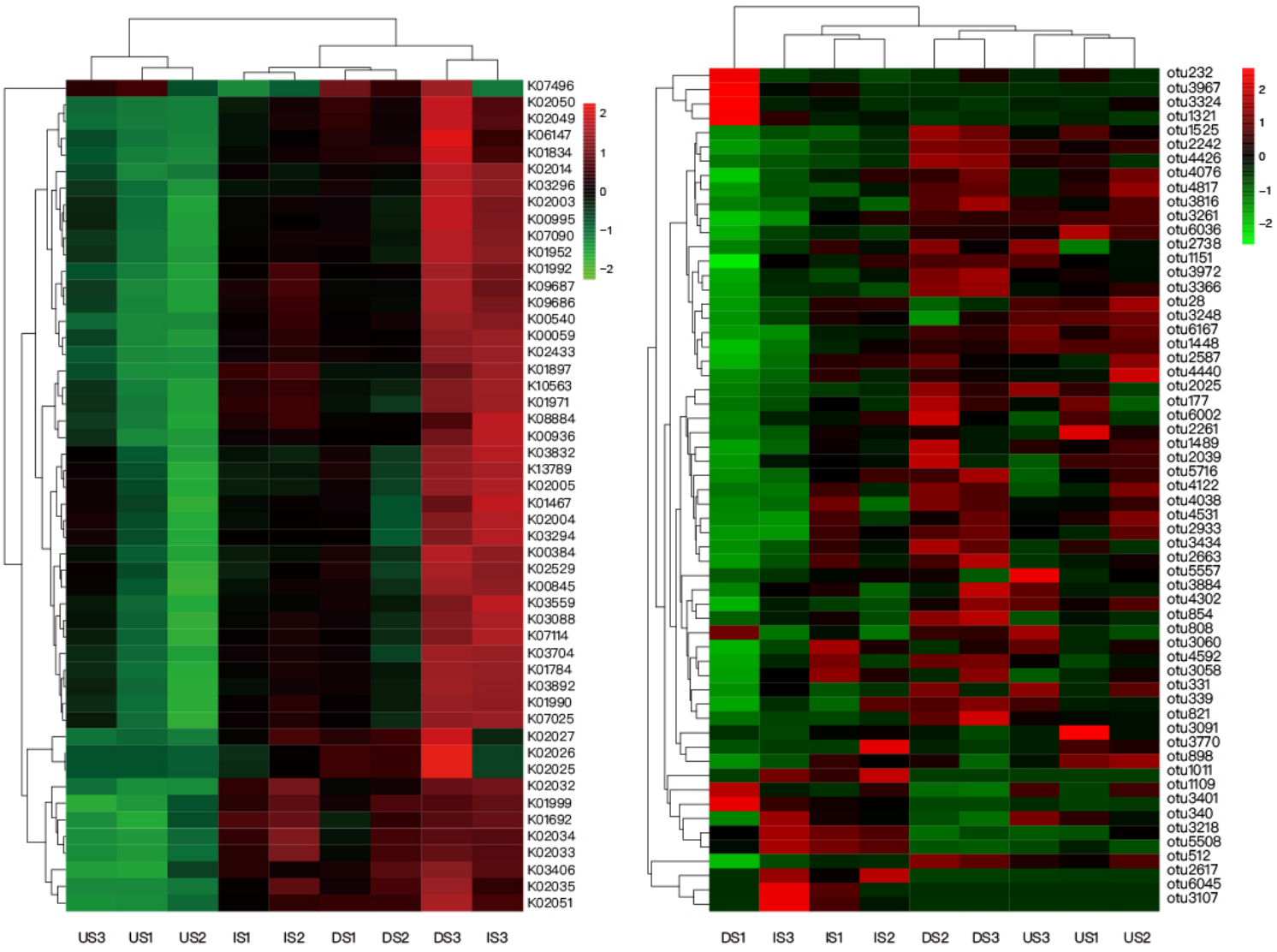

Figure 8

Heatmap analysis of functional groups composition of bacteria (left) and fungi (right) with cluster analysis found in three soil types (n=3 for each soil type). IS - invaded soil; US - uninvaded soil; DS - uninvaded soil with dihydromikanolide (Dih) addition.

Supplementary Files

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- 0208SupplementaryInformation.docx