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Ficus tikoua Bur. May Have Resistance to Invasive Plant *Alternanthera philoxeroides*

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Abstract: The interaction between native and invasive plant species plays a crucial role in understanding invasion mechanisms and developing control strategies. This study investigated the differences in photosynthetic and fluorescence parameters, gene expression profiles, and rhizosphere soil microbial composition between the native species *Ficus tikoua* Bur. and the invasive species *Alternanthera philoxeroides*. For the first time, it was demonstrated that that *F. tikoua* Bur. may enhance photoinhibition ability and the expression of genes involved in carbon metabolism, protein processing in the endoplasmic reticulum, and plant-pathogen interactions to maintain growth. Additionally, it recruits beneficial microbes, *e.g. Actinomarina* (Bacteria), *Nitrosotenuis* (Archaea) and *Laccaria* (fungi), to suppress pathogens, thereby preserving soil DMSP and carbon cycling pathways to resist invasion by *A. philoxeroides*. These findings provide novel insights into the mechanisms underlying plant invasion and suggest potential strategies for invasive plant management. Further research is needed to investigate this relationship and determine the mechanisms underlying the potential resistance of *F. tikoua* Bur. to plant invasion.

Keywords: *F. tikoua* Bur.; *A. philoxeroides*; Plant invasion; RNA-seq; Rhizosphere microbial

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Introduction

Alternanthera philoxeroides is a perennial herb that belongs to the *Amaranthaceae* family. It is native to South America but was introduced to China in the early 20th century (Mao *et al*.,2011). Unfortunately, this plant has now spread to all parts of the country and causes great harm to the ecosystem due to its difficulty to control. *A. philoxeroide*s is a very adaptable plant that can grow in a variety of habitats, including dry, wet, and saline soil. It can also tolerate a wide range of temperatures and light conditions (Xu *et al*., 2012). This makes it a very difficult plant to control as it grows rapidly and can outcompete native plants for resources. Additionally, it produces a lot of seeds, which can be spread by wind and water, making it very easy for *A. philoxeroides* to spread and invade new areas (Liu *et al*., 2020). The invasive plant has massively negative impacts on the ecosystem. It reduces the diversity of native plants, alters the structure of the vegetation community, and changes the flow of nutrients and water (Zheng *et al*., 2015). It can also harbor pests and diseases. To control *A. philoxeroides*, there are a number of measures that can be employed, including mechanical removal, chemical control, and biological control, and so on (Wang *et al*., 2021). Despite the huge challenges from controlling *A. philoxeroides*, a combination of methods is more likely to be effective compared to a single method.

Biological control is a more environmentally friendly option for controlling *A. philoxeroides*. Introducing natural enemies of the plant, such as insects or diseases, can help with this. However, it can be challenging to implement biological control and may take a long time to be effective. In a study by Fu *et al*., (2013), the population performance, photosynthesis, leaf nitrogen concentration, and constitutive and induced resistances of invasive plant, *A.philoxeroides*, and its native congener, *A. sessilis*, were investigated in the presence of three population densities of the grasshopper, *Atractomorpha sinensis*. Fan *et al*., (2016) revealed that native generalist herbivores prefer the invasive plant to the natives because of the higher leaf nitrogen content. Wei *et al*., (2016) implemented greenhouse experiments to examine the interaction between a native defoliating beetle, *Cassida piperata*, and a native root-knot nematode, *Meloidogyne incognita*, on the invasive alligator weed, *A.philoxeroides*. Natural habitats and disturbance are two prominent environmental factors affecting the invasion process of exotic invaders (Wang et al., 2017). Another study by Wang *et al*., (2018) explored the phenotypic responses of clonal populations of the invasive plant *A. philoxeroides* and three co-occurring non-invasive clonal species, i.e., *A. sessilis*, *Myriophyllum aquaticum* and *Jussiaea repen*s, to different levels of fluctuating flood disturbances using a mesocosm experiment. While empirical tests on the topic have been strongly biased toward examining constitutive resistance, less is known about the evolution of induced resistance and tolerance. Liu *et al*., (2020) found that warming can directly affect the leaf chemistry of invasive plant *A. philoxeroides* and its native counterpart *A. sessilis*, and these changes can indirectly impact herbivorous insects associated with these plants.

The study by Wang *et al*., (2021) where they conducted a greenhouse experiment to observe the effects of Cd (0, 10, 30, 60, and 100 mg kg) and inter-specific competition on the reproductive capabilities of the invasive plant *A. philoxeroides* and its native congener *A. sessilis*. Their findings indicated that *A. philoxeroides* exhibits greater plasticity in root mass ratio as a positive response to both heavy metal contamination and inter-specific competition in comparison to its native congener *A. sessilis*. To gain in-depth understandings on how biological invasion affects the natural recovery potential of native vegetation (Liu *et. al*., 2020) focus on the soil seed banks of reed (*Phragmites australis*) communities with different degrees of *A. philoxeroides* invasion. Hu *et al*., (2023) conducted an experiment to investigate the latitudinal and longitudinal allelopathic patterns of *A. philoxeroides* in China, finding that the allelopathic effects increased with latitude and decreased with longitude, and were determined by soluble protein (SP), free amino acids (FAA), plant polyphenol (PP), phosphorus (P) and potassium (K) contents of leaf, suggesting potential control strategies for reducing the component contents in the plant tissues. Recently, Gao *et al*.,(2023) proposed that aboveground herbivory can facilitate the invasion of *A. philoxeroides* via both intra- and interspecific aboveground-belowground interactions based on multiyear field surveys and a 2-years plant-soil feedbacks (PSF) experiment. However, the interaction between native plants and invasive plants was not given much attention. In this work, we found that the native plants of *Ficus tikoua* Bur. may have natural resistance to the alien invasive plant of *A. philoxeroides*. The differences in photosynthetic physiology, gene expression, and rhizosphere microorganisms were investigated to provide a new approach for exploring ecological control of invasive plants(*A. philoxeroides*).

Materials and Methods

In 2018, test plots measuring approximately 100 m^2 were established at Guiyang University to plant *Ficus tikoua* Bur. Five years later, in 2023, the invasive alien species *A.philoxeroides* was observed growing at the edges of the plots. However, a dense planting of *F. tikoua* Bur. within the plots appeared to prevent any encroachment of *A. philoxeroides* into their interior space. The dense growth of *F. tikoua* Bur. may have created an effective barrier that restricted the inward spread of the invasive plant from the boundaries of the plots. Hence, in order to investigate the interaction effect between the two species, various aspects were examined, including photosynthetic parameters, fluorescence parameters, gene expression profiles, and microorganism diversity in rhizosphere soils.

The portable photosynthesis system (LI-6800, USA) was used to detected photosynthetic parameters and chlorophyll fluorescence parameters. At 9:00 am on sunny days, photosynthetic parameters (photosynthetic rate (A), transpiration rate (E), intercellular CO_2 concentration (Ci), stomatal conductance to water vapor (gsw), boundary layer conductance to water vapor (gbw), total conductivity to CO_2 (gtc), total conductivity to water vapor (gtw), water vapor pressure deficit based on leaf temperature calculation (VPD), leaf temperature (T), relative humidity of leaf chamber (RHcham)) of *F. tikoua* Bur. and *A.philoxeroides* leaves were measured. Water use efficiency (WUE) is defined as the ratio of A to E. The stomatal limitation (LS) was calculated as (Ca- Ci)/Ca, Ca is defined as atmospheric CO_2 concentration.

And their chlorophyll fluorescence parameters, including fluorescence origin (F_0), fluorescence maximum (Fm), steady-state fluorescence under light (Fs), maximum fluorescence under light (Fm'), minimum fluorescence under light (F_0'), were determined at 9:00 pm. Based on the data, the following indexes can be calculated: potential PSII activity (Fv/F0), the maximum quantum yield of PSII (Fv/Fm), effective PSII photochemical quantum yield (Fv'/Fm'), actual PSII photochemical quantum yield (ΦPSII), electron transport rate (ETR), photochemical quenching coefficient (qP), and non-photochemical quenching coefficient (NPQ), Non-regulatory energy dissipation $[Y(NO)]=1/[NPQ+1+qP \times F_0'/Fs \times (Fm/F_0-1)]$, regulatory energy dissipation $[Y(NPQ)]=1-\Phi PS\,II-1/[NPQ+1+qP \times F_0'/Fs \times (Fm/F_0-1)]$(Demmig-Adams *et al.*, 1996).

The distribution ratio of light energy absorbed by PS II is calculated according to the method of Demmig-Adams *et al.*, (1996), namely, fraction of excitation energy that is utilized for photochemistry $(P)=qP \times Fv'/Fm' \times 100\%$, fraction of absorbed light that is dissipated thermally $(D) = (1-Fv'/Fm') \times 100\%$, fraction of excitation energy not dissipated in the antennae that cannot be utilized for photochemistry $(Ex) = (1-qP) \times Fv'/Fm' \times 100\%$.

Leaf samples and rhizosphere soil were collected, and transported on dry ice to Majorbio Company for RNA sequencing and Metagenomic sequencing, respectively. Besides, the surface area of leaves were determined through WinRHIZOTM (pro LA2400, Canada).

Results and Discussion

The difference of photosynthetic parameters between F. tikoua Bur. and A. philoxeroides

The E, gsw, VPD, gbw, and gtw of *F. tikou*a Bur. was found significantly reduced through it has higher surface area of leaves compared to that of *A. philoxeroide*s leaves (SFigure 1). Even the three parameters, E, gsw and VPD, exhibited the same trend during both observation periods. Conversely, the LS, T, gbw, and RHcham of *F. tikou*a Bur. demonstrated a significant increase compared with that of *A. philoxeroides* leaves (Table 1). These trends were consistent across both observation periods. According to Faqruhar and sharkeyt (1982), the main reason for the decrease in leaf photosynthetic rate is determined to be either stomatal or non-stomatal factors, depending on change of *Ci* and LS values. Hence, this results suggested that stomatal factors are the main reason for affecting the photosynthetic rate of *F. tikou*a Bur. Leaf, which was also reflected in the decline of gsw. The invasive ability of *A. philoxeroide*s might be attributed to the higher gas exchange parameters, such as E and gsw. Research has shown that the main environmental factors affecting net photosynthetic rate of *F. tikoua* Bur. are photosynthetically active radiation and atmospheric relative humidity. Net photosynthetic rate was positively correlated with atmospheric relative humidity, and negatively correlated with intercellular CO_2 concentration and stomatal conductance (Oku *et al.*, 2014), which is also in agreement with our study. Thereby, the relationship between environmental factors and stomatal limitation in *F. tikoua Bur.* is needed into further investigation.

Table 1. The photosynthetic parameters of *F. tikoua* Bur. and *A. philoxeroides* leaves.

Time	Parameters	*F. tikoua* Bur.	*A. philoxeroides*
Oct.16th	E($\times 10^{-2}$molm^{-2}s^{-1})	0.49±0.00bB	0.63±0.35aA
	A(μmolm^{-2}s^{-1})	11.10±2.49aA	11.99±0.40aA
	Ci(μmolmol^{-1})	321.63±2.03aA	324.38±9.01aA
	LS	20.80±0.32aA	19.24±2.21aA
	WUE(μmolmmol^{-1})	2.66±0.01aA	2.39±0.49aA
	gsw($\times 10^{3}$molm^{-2}s^{-1})	289.88±2.44bA	319.96±23.34aA
	gbw($\times 10^{3}$molm^{-2}s^{-1})	2834.21±0.14aA	2831.93±1.78bA
	gtw($\times 10^{3}$molm^{-2}s^{-1})	278.15±7.89aA	286.29±20.10aA
	gtc($\times 10^{3}$molm^{-2}s^{-1})	175.21±5.01aA	180.38±13.34aA

	T (°C)	28.41±0.19aA	27.53±0.08bB
	VPDKpa)	1.51±0.02bB	1.92±0.06aA
	RHcham(%)	59.87±0.06aA	47.17±0.80bA
Oct.30th	E(×10^{-2}molm^{-2}s^{-1})	1.10±0.03bA	1.30±0.20aA
	A(μmolm^{-2}s^{-1})	16.32±0.2aA	16.65±0.95aA
	Ci(μmolmol^{-1})	313.23±2.41aA	325.15±10.46aA
	LS	21.52±0.60aA	17.20±2.14bA
	WUE(μmolmmol^{-1})	1.31±0.20aA	1.38±0.13aA
	gsw(×10^{3}molm^{-2}s^{-1})	385.53±6.78bA	453.74±20.80aA
	gbw(×10^{3}molm^{-2}s^{-1})	2842.71±0.67aA	2840.81±0.89bA
	gtw(×10^{3}molm^{-2}s^{-1})	364.97±13.50bA	416.94±17.57aA
	gtc(×10^{3}molm^{-2}s^{-1})	230.45±8.62bA	263.65±11.24aA
	T (°C)	30.42±0.09aA	30.13±0.07bA
	VPDleaf(Kpa)	2.65±0.02bA	2.85±0.06aA
	RHcham(%)	35.74±0.11aA	34.60±0.16bB

The difference of chlorophyll fluorescence parameters between F. tikoua Bur. and A. philoxeroides

From table 2, the F$_0$, ΦPSII, ETR, and NPQ in *F. tikoua* Bur. were remarkable higher than that in *A. philoxeroides*. However, a significant decline of Fv/Fm, Fv/F$_0$, [Y(NO)], and Fv'/Fm' were observed in *F. tikoua* Bur. compered with *A. philoxeroides*. The increased ΦPSII and ETR suggest *F. tikoua* Bur. can maintain higher the actual photochemical reaction efficiency in PS II in comparison with *A. philoxeroides.* This can be due to the partial closure of the PSII reaction center under light conditions, as indicated by ΦPSII, which is known to be associated with downstream electron transfer, capture efficiency, and openness of the reaction center excitation energy (Weis and Berry, 1988). Additionally, ETR reflects the rate of photosynthetic energy transfer under actual light intensity conditions (Wu, 2017). The rise in F$_0$ can also be considered as a form of heat dissipation, the increment of F$_0$ and NPQ implied that *F. tikoua* Bur. consumed excessive light energy by increased heat dissipation to avoid damage caused by environmental stress (Stirbet *et al*., 2018).

Table 2. The fluorescence parameters of *F. tikoua* Bur. and *A. philoxeroides* leaves.

Time	Parameter	*F. tikoua* Bur.	*A. philoxeroides*
Oct.16th	F0	252.87±2.60 aA	211.32±1.74 bA
	Fm	1318.64±83.09 aA	1275.75±44.33 aA
	Fv/Fm	0.81±0.01 bA	0.83±0.01 aA
	Fv/F0	4.21±1.24 bA	5.04±1.56 aA
	ΦPSII	0.36±0.03 aA	0.28±0.02 bA
	ETR(μmolm^{-2} s^{-1})	151.99±10.55 aA	117.82±7.95 bA
	NPQ	1.56±0.11 aA	1.38±1.78 bA
	Fv'/Fm'	0.62±0.01 bA	0.71±0.03 aA
	qP	0.58±0.04 aA	0.53±0.17 aA
	[Y(NO)]	0.25±0.01bA	0.31±0.00aA
	[Y(NPQ)]	0.37±0.01aA	0.40±0.04aA
Oct.30th	F0	243.12±11.72 aA	166.01±48.07 bA
	Fm	1251.25±117.48 aA	1090.54±5.28 aA

Fv/Fm	0.81±0.01 bA	0.84±0.01 aA
Fv/F0	4.15±1.25 bA	5.57 ±1.22 aA
ΦPSII	0.41±0.01 aA	0.25±0.02 bA
ETR(μmolm^{-2} s^{-1})	166.50±4.43 aA	106.10±6.83 bA
NPQ	1.79±0.25 aA	1.54±0.26 bA
Fv'/Fm'	0.63±0.00 bA	0.68±0.00 aA
qP	0.62±0.01 aA	0.60±0.07 aA
[Y(NO)]	0.21±0.02bA	0.30±0.00aA
[Y(NPQ)]	0.38±0.01aA	0.31±0.03aA

The decreased Fv/Fm, Fv'/Fm' and Fv/F$_0$ indicated that a decline in the overall performance of PS II in *F. tikoua* Bur. compared to *A. philoxeroides*, which was related to alterations in the ultrastructure of chloroplasts. It notwithstanding that maximum quantum yield of PSII (Fv/Fm) in *F. tikoua* Bur. was markedly reduced compared to *A. philoxeroides*, the value is also exhibited within the normal range (from 0.8 to 0.84) (You *et al*., 2013), suggesting that *F. tikoua* Bur. alleviated invasive plants against competitive stress as maintaining photosynthesis activity. As the report of zhang *et al*., (2016), *Bidens pilosa*, a invasive plant, was responsible for inhibiting photosynthesis in native species through allelopathic effects. The gametophytes exposed to *B. pilos*a had decreased fluorescence parameters in comparison with the control, except for non-photochemical quenching. NPQ is mainly divided into non-regulatory energy dissipation Y (NO) and regulatory energy dissipation Y (NPQ). Y(NO) refers to the process by which excess energy absorbed by plants during photosynthesis is dissipated as heat rather than being used for growth or other metabolic processes, which is a negative evaluation index of light damage (Guidi *et al*., 2019). The noticeably lower Y(NO) in *F. tikoua* Bur. compared to *A. philoxeroides* means that it is able to efficiently utilize the absorbed light energy for photosynthesis and minimize energy wastage through non-regulated processes (Xia *et al*., 2023). Therefore, *F. tikoua* Bur. *F. tikoua* Bur. exhibits enhanced adaptability to varying light conditions, which could potentially be attributed to its ability to mitigate photoinhibition through increasing heat dissipation (NPQ).

Dissipation of light energy absorbed by leaves of F. tikoua Bur. and A. philoxeroides

The distribution of light energy absorbed by leaves is shown in Table 3. Heat dissipation (D) and photochemical reaction (P) dissipation in leaves of *F. tikoua* Bur. were observed dramatically higher than that of *A. philoxeroides*. while dissipation of non-chemical reaction energy (Ex) of *F. tikoua* Bur. was much lower than that of *A. philoxeroides*. It suggested that the dissipation of residual light energy of *F. tikoua* Bur. was mainly based on the pathway of D or P dissipation, and that of *A. philoxeroides* was involved in the dissipation of Ex. The increment in Ex can prevent excessive reduction of PSII and the electron transport chain, thereby avoiding excessive destruction of the photosynthetic system caused by excess light energy (Tie *et al*., 2020). This adaptation is associated with the invasiveness of *A. philoxeroides* as a plant.

Table 3. Dissipation of light energy absorbed by leaves of *F. tikoua* Bur. and *A. philoxeroides*.

Time	Parameter	*F. tikoua* Bur.	*A. philoxeroides*
Oct.16th	P %	34.09±1.72aA	26.63±3.75aA
	D %	38.44±0.75aA	31.85±0.86bA
	Ex %	27.47±2.48bA	41.51±2.88aA
Oct.30th	P %	38.80±0.03aA	23.98±1.75bA
	D %	37.03±0.02aA	31.36±0.36bA

Ex %	24.17±0.06bA	44.65±1.39aA

Differences in gene expression profiles between F. tikoua Bur. and A. philoxeroides

According to RNA-seq, a total of 316,197,552 clean reads with lengths of 150 base pairs (bp) were obtained from the six samples (A1, A2, A3, F1, F2 and F3) (STable 1). All clean bases with 41.73% to 45.38% GC content were deposited in The National Center for Biotechnology Information (NCBI), which you can obtain by using the accession number (PRJNA1056514). Principal component analysis (PCA) and correlation showed a good biological repeatability (SFigure 2) and sample dispersion (SFigure 3), suggesting that this data are available for subsequent bioinformatics analyses.

In total, 73,881 differentially expressed genes (DEGs) were identified in the comparison group of *A. philoxeroides* vs *F. tikoua* Bur. (A vs F), including 44,100 up-regulated and 29,781 down-regulated DEGs. The high number of genes exhibiting differential expression between the two species may be attributable to substantial systematic variations in their biological classification. This suggested that the observed disparities in gene expression might not exclusively mirror genuine biological dissimilarities between this species, but could also be influenced by intrinsic genetic backgrounds. As a result, the shared genes between the two samples were exceptionally rare (SFigure 4). And then, 83 significantly enriched GO terms (corrected P-value <0.05) were identified through GO enrichment analysis (Figure 1), among them most DEGs were involved in transferase activity (7,822 of 36,363, molecular function), catalytic activity (17,547 DEGs, molecular function) integral component of membrane (10,423 DEGs, cellular component), response to stimulus (4,159 DEGs, biological process), protein modification process (4,290 DEGs biological process), and macromolecule modification (4,538 DEGs, biological process). Furthermore, 18 significantly pathways of Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment (corrected P-value <0.05) were obtained and shown in Figure 2, of them, lots of DEGs linked to carbon metabolism (854 DEGs, metabolism), protein processing in endoplasmic reticulum (609 DEGs, genetic information processing), plant-pathogen interaction (448 DEGs, organismal systems), implying this pathway may be involved in interactions between *F. tikou*a Bur. and *A. philoxeroides.* In line with the findings of Xu *et al*., (2019), most DEGs and their corresponding miRNA targets associated with metabolism, response to stimulus, catalytic activity, circadian rhythm-plant, protein processing in endoplasmic reticulum and plant-pathogen interaction may provide biochemical and ecological advantages that facilitated the successful invasion of hexaploid cytotypes of *Solidago canadensis* L. Manoharan *et al*., (2019) proposed that plant hormones and their cross-talk signaling could enhance the resistance of *A. philoxeroides* to pathogens, thereby facilitating its invasion. However, our findings indicated that *F. tikoua* Bur. has the potential to alter the gene expression patterns of *A. philoxeroides*, potentially leading to a reduction in its invasive capabilities. Stress-induced modifications of the genome are considered a crucial strategy for invasive plants to adapt to novel environments (Prentis *et al*., 2008). These modifications can involve changes in gene expression, DNA methylation patterns, or even structural alterations to the genome itself. Hence, the comprehension of how gene expression variations affect plant invasion is essential for clarifying the fundamental mechanisms underlying this process.

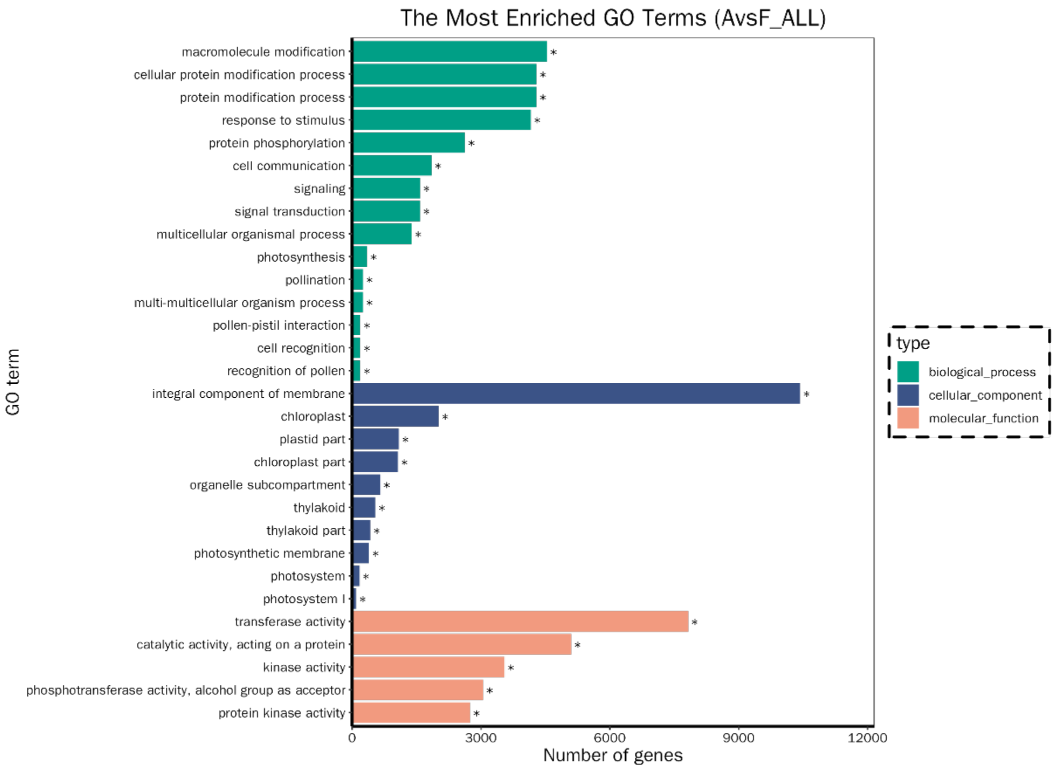


Figure 1. GO classification. A:*A. philoxeroides*, F:*F. tikoua* Bur.

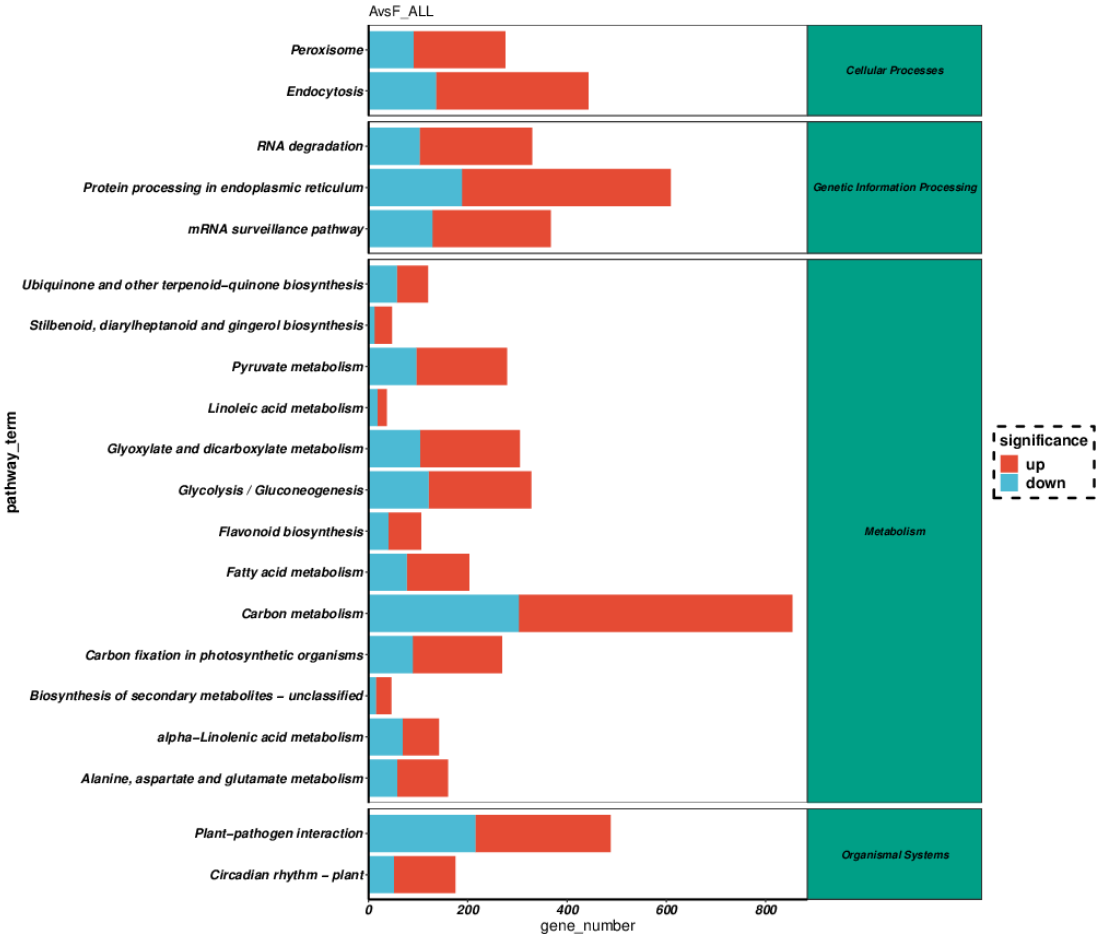
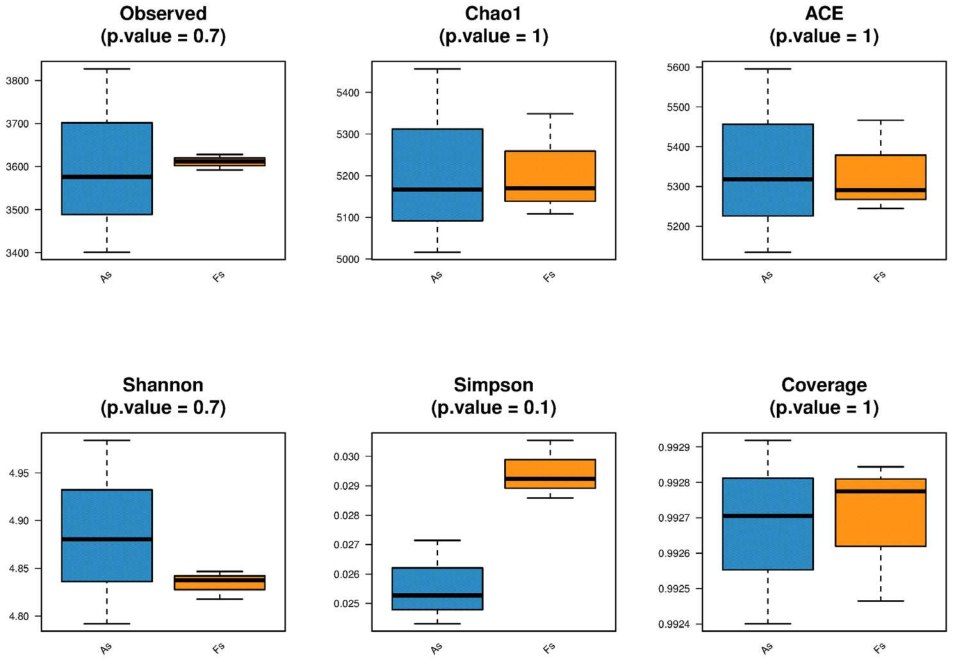


Figure 2. KEGG enrichment analysis.A:*A. philoxeroides*, F:*F. tikoua* Bur.

Differences in rhizosphere microbial abundance between F. tikoua Bur. and A. philoxeroides

Rhizosphere soil microorganisms from *F. tikoua* Bur. and *A. philoxeroides* were subjected to metagenomic sequencing in accordance with standard operational procedures using the Illumina platform, generating paired-end reads of 150 bp. The raw sequencing data have been deposited in the NCBI database under accession number PRJNA1056649. The alpha diversity (Observed, Shannon, Simpson, Chao1, ACE, and Coverage) of both soil bacterial and archaeal communities did not differ significantly between the two samples, as illustrated in Figure 3. It should be noted that a very small amount of viruses and fungal communities (SFigure 5) were detected, while the most were unassigned in this study, which is similar to previous findings (wang *et al*., 2022 and Chen *et al*., 2021). Additionally, unique soil bacterial communities were separated based on principal coordinate analysis (PCoA) (Figure 4). Then, the Adonis test revealed no significant difference in the species composition of soil bacterial, archaeal, and fungal communities (STable 2) between the two samples.The antagonistic interactions between *F. tikoua* Bur. and *A. philoxeroides* may partly account for the neutral effect on alpha diversity of soil microorganisms. As the study of Gibbons *et al*., (2017), when multiple invasive plants coexist, certain species may exhibit preferences for specific microbial taxa while inhibiting others, leading to a lack of significant changes in the overall microbial community. Li *et al*., (2022) reported that the invasion of *A. philoxeroides* decreased soil microbiome beta-diversity while increasing alpha-diversity. Invasion-present soils had a more intricate and robust network structure compared to invasion-absent soils, characterized by an increased number of keystone species, decreased modules, and enhanced co-occurring associations. Alterations to the soil environment can be conceptualized as niche construction, thereby facilitating the establishment and proliferation of invasive species (Stefanowicz *et al*., 2019). However, upon encountering *F. tikoua* Bur., the invasive plant *A. philoxeroides* did not induce substantial alterations in the soil microecological diversity, suggesting the potential resilience of native species against *A. philoxeroides* invasion.



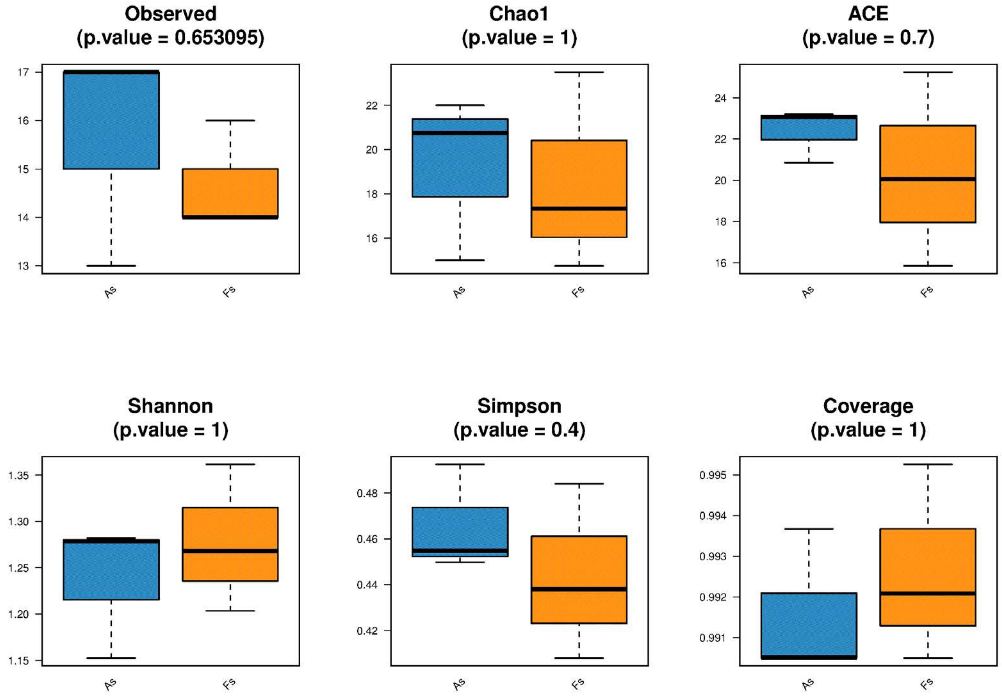
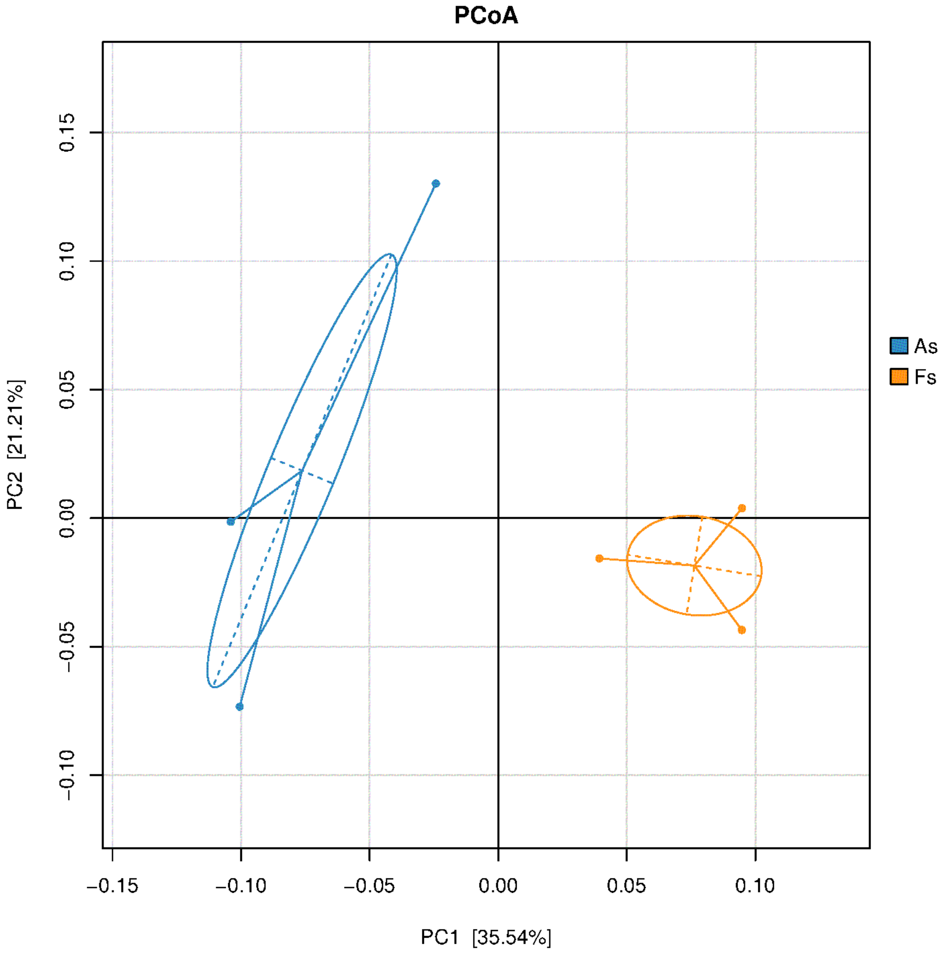
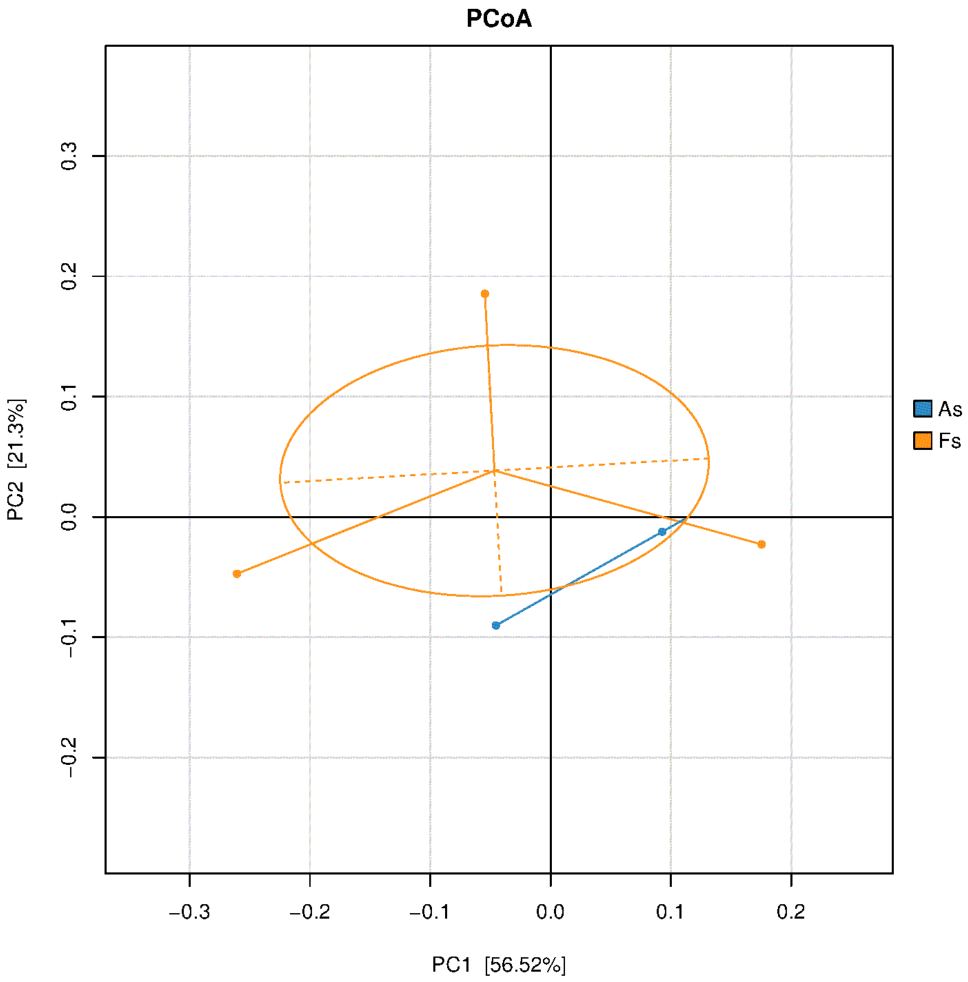
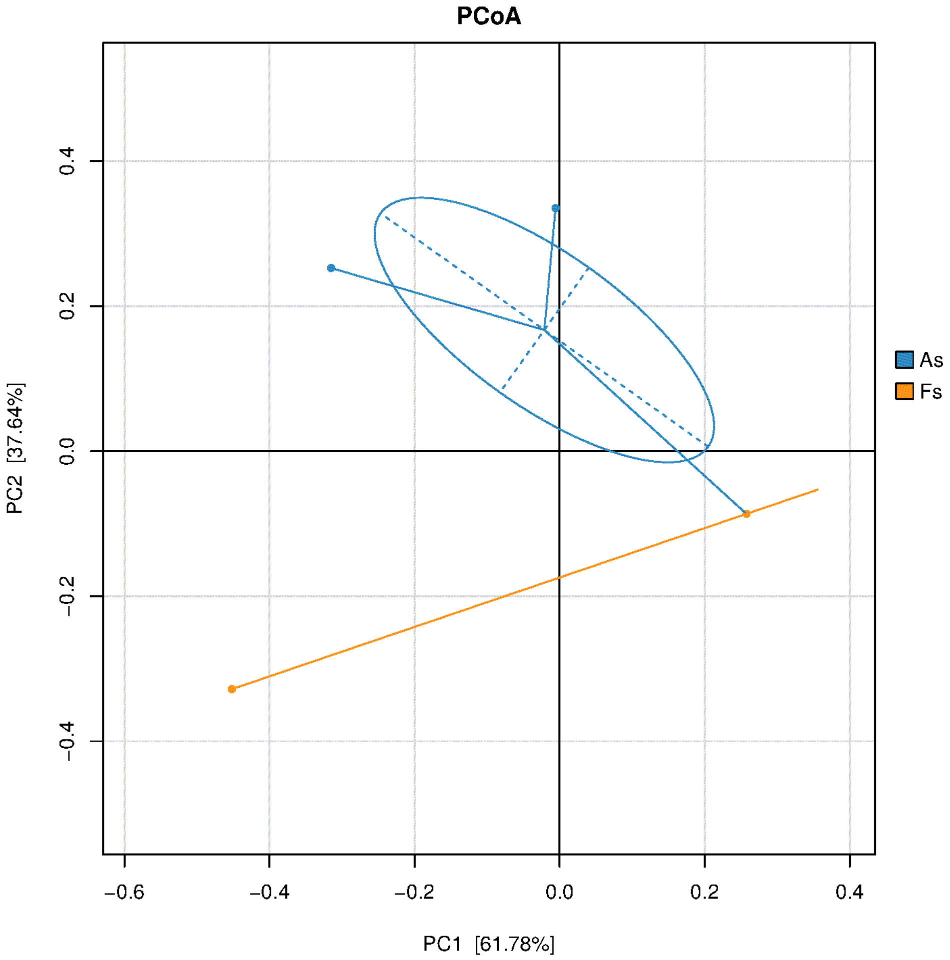


Figure 3. Alpha diversity index (Observed, Shannon, Simpson, Chao1, ACE, and Coverage) of soil bacterial (A) and archaeal (B) communities.


PCoA
PC2 [21.21%]
PC1 [35.54%]
0.15
0.10
0.05
0.00
−0.05
−0.10
−0.15
−0.10
−0.05
0.00
0.05
0.10
As
Fs


PCoA
PC1 [56.52%]
PC2 [21.3%]
−0.3
−0.2
−0.1
0.0
0.1
0.2
0.3
As
Fs


PCoA
PC2 [37.64%]
PC1 [61.78%]
0.4
0.2
0.0
−0.2
−0.4
−0.6
−0.4
−0.2
0.0
0.2
0.4
As
Fs

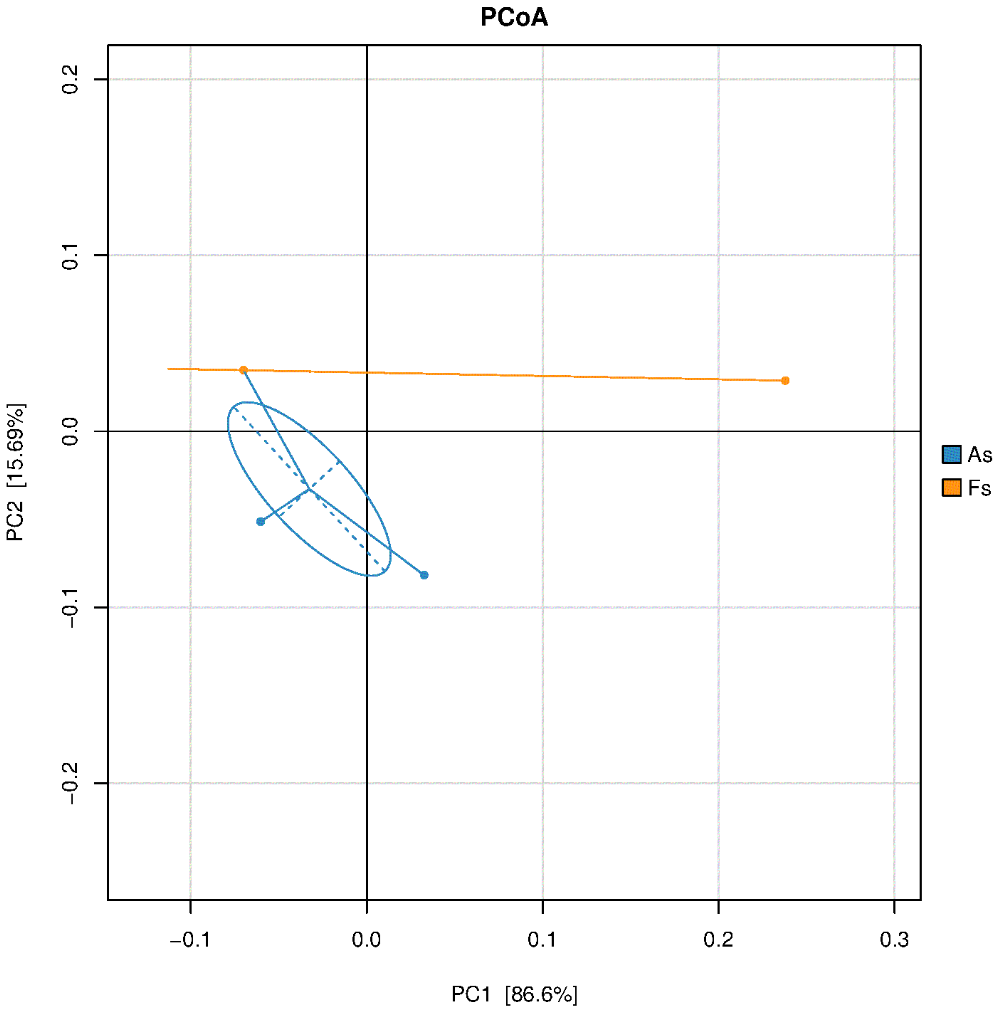
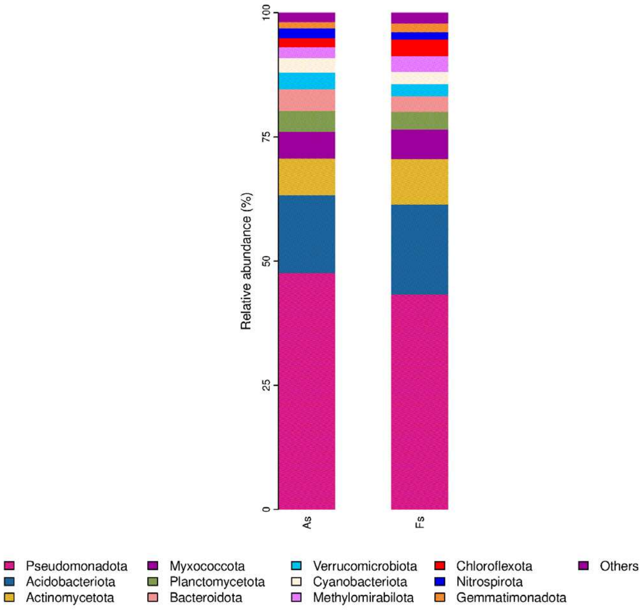
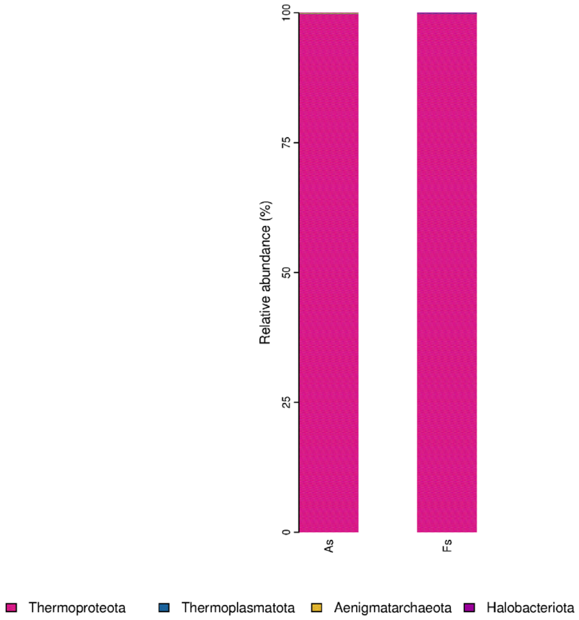
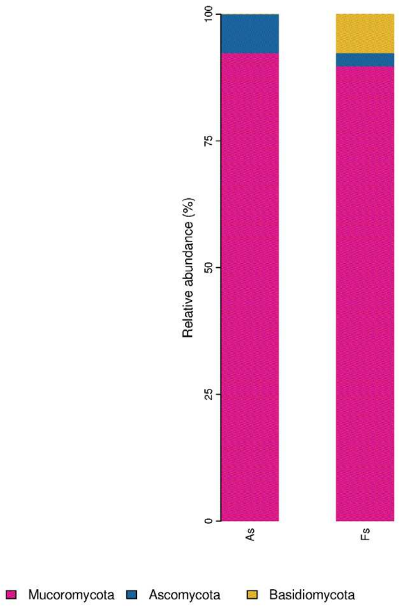


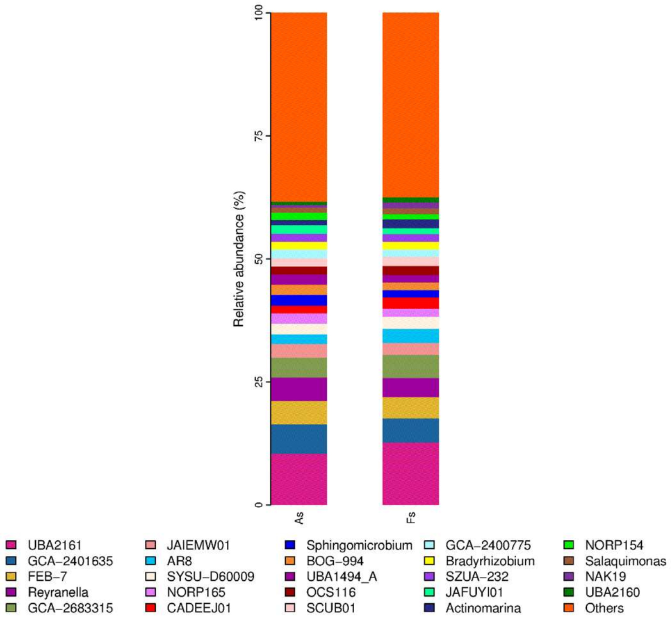
Figure 4. PCoA result of soil bacterial communities.

Moreover, bacterial community exhibited the most pronounced difference between rhizosphere soil microorganisms of *A. philoxeroides* (As) and *F. tikoua* Bur. (Fs) in comparison to the archaeal, viral, and fungal communities, as revealed by non-metric multidimensional scaling (NMDS) analysis (SFigure 6). This finding suggested that invasive plants had a significant impact on the structure and function of soil microbial communities, consistent with previous observations (Gioria *et al*., 2014). Regarding soil bacterial taxa, most were observed to be shared in the two samples (SFigure 7). In light of the numerous significant differences observed between the two samples, Pseudomonadota emerged as the most abundant phylum (P-value <0.05), followed by Acidobacteriota and Planctomycetota (Figure 5A). And *UBA2161*, *GCA-2401635*, and *Reyranella*, were the three top abundant with P-value <0.05 in the 25 genera taxon level (Figure 5D). In terms of soil Archaea taxa, the thermoproteota phylum was the most abundant. However, the differences between the As and Fs were not found to be statistically significant (P > 0.05) (Figure 5B). And only *Nitrosotenuis* and *PWEA01* (Figure 5E) was observed to have prominent abundances at the genus taxon level. As regards soil fungi taxa, only Basidiomycota (Figure 5C) was a prominent abundant phylum, and at the genus taxon level, *Rhizophagus*, *Psilocybe*, and *Laccaria* (Figure 5F) exhibited a dramatic abundance in the group of As vs Fs. According to enhanced soil-mediated invasion self-reinforcement upon herbivory hypothesis (Gao *et al*., 2023), the changes in community structure of plant rhizosphere microbiome play an important role in promoting *A. philoxeroides* invasion. Nevertheless, our study has demonstrated that *Actinomarina* (Bacteria), *Nitrosotenuis* (Archaea) and *Laccaria* (fungi) were significantly up-regulated in the Fs compared to As, which may be attributed to the resistance of *F. tikoua* Bur. to invasive plants *A. philoxeroides*. The work of Wang *et al*., (2022) demonstrated that the relative abundances of Actinobacteriota were negatively associated with the relative abundance of the invasive plants *Sesbania cannabina* and *Talinum paniculatum*. Actinobacteriota, as plant growth-promoting rhizobacteria, play a significant role in rhizosphere nutrient cycling, act as biocontrol agents against pathogenic fungi, and promote plant growth through phosphate solubilization, secondary metabolite production, and antimicrobial synthesis. *Candidatus Nitrosotenuis* was found to closely correlate with the regulation of ecological functions under saline stress in various types of Cd-contaminated soils from the North China Plain (Wang *et al*., 2019). Quan *et al*., (2023) identified two species of *Laccaria* can protect the host tress root system to assist *Pinus densiflora* against heavy metal toxicity.






Relative abundance (%)
100
75
50
25
0
As
Fs
Mucoromycota
Ascomycota
Basidiomycota


Relative abundance (%)
100
75
50
25
0
As
Fs
UBA2161
GCA−2401635
FEB−7
Reyranella
GCA−2683315
JAIEMW01
AR8
SYSU−D60009
NORP165
CADEEJ01
Sphingomicrobium
BOG−994
UBA1494_A
OCS116
SCUB01
GCA−2400775
Bradyrhizobium
SZUA−232
JAFUYI01
Actinomarina
NORP154
Salaquimonas
NAK19
UBA2160
Others

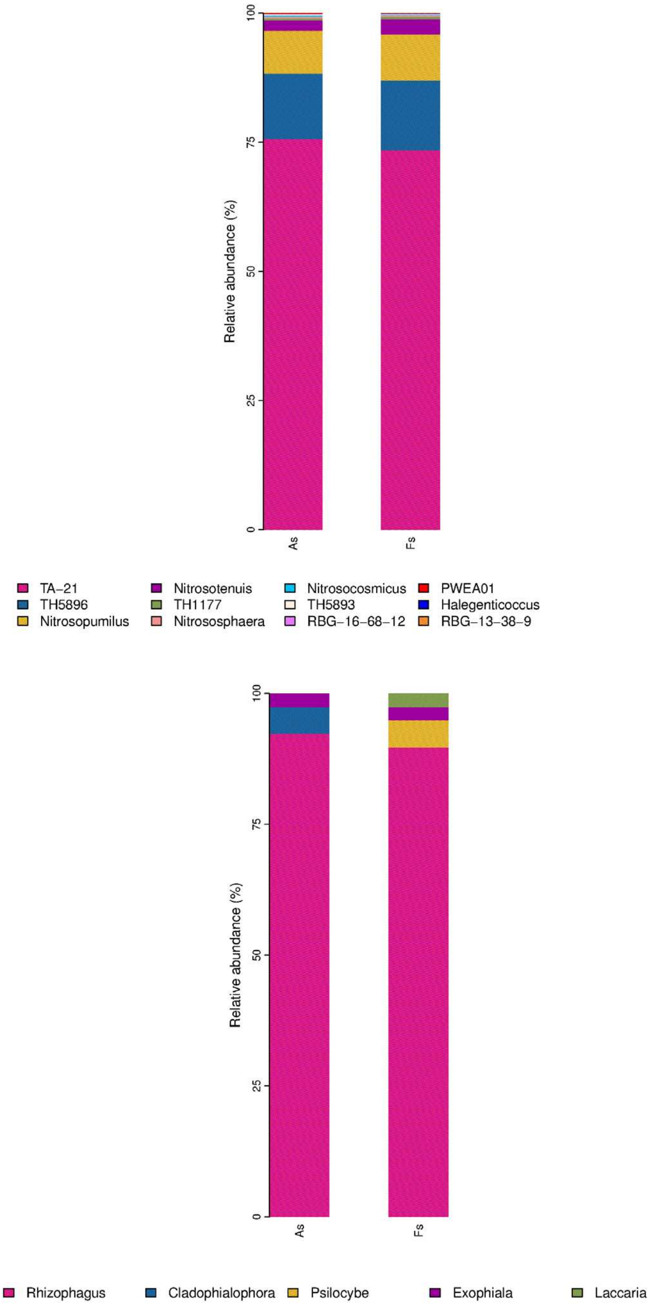


Figure 5. Relative abundance of the most abundant phyla of soil fungi bacteria(A), archaea (B) and fungi(C), and the most abundant genera of soil bacteria (D), archaea (E) and fungi (F) in As vs Fs.

Intriguingly, virulence factor test (SFigure 8) indicated that the relative abundance of microbes involved in nutritional/metabolic factor, metabolic adaptation, antiphagocytosis were improved significantly in Fs compared with As. This further suggested that the resistance of *F. tikoua* Bur. to invasive plants might be associated with soil microbiota-mediated nutrient regulation and antibiotic production. Furthermore, comparative analysis against the Comprehensive Antibiotic Resistance Database (CARD) revealed a higher abundance of tetracycline antibiotics and tetracycline antibiotic drug classes in As compared to Fs, while glycopeptide antibiotics were less abundant (SFigure 9A). This observation is corroborated by the increased abundance of antiphagocytosis in As, suggesting that invasive plants possess a remarkable ability to adapt to novel environments by promoting the synthesis of antibiotics, thereby conferring resistance against pathogens. In accordance with the studies on *M. micrantha* (Yin *et al*., 2020), it has demonstrated that specific microbes in the rhizosphere play a significant role in nutrient acquisition and pathogen suppression, thereby enhancing the plant's adaptation and invasiveness in diverse environments. In terms of resistance mechanisms, antibiotic efflux was significantly enhanced in As compared to Fs (SFigure 9B). Additionally, by aligning to the structured ARG reference database (SARG), the increased type of vancomycin (SFigure 9C) and regular mechanism (SFigure 9D) were identified in Fs compared to As. However, the type of trimethoprim, multidrug, and the mechanism in efflux pump, antibiotic target replacement were enhanced in As, suggesting that native plants and invasive plants have employed distinct mechanisms to resist pathogen damage by recruiting different microorganisms to sustain their growth.

Comparative analysis of Clusters of Orthologous Groups (COG) pathways revealed significant differences in pantothenate/CoA biosynthesis, photosystem II, archaeal ribosomal proteins, lipid A biosynthesis, A/V-type ATP synthase, and folate biosynthesis between As and Fs (Figure 6). Additionally, KEGG analysis identified a specific translation-related pathway ($p<0.05$) with significantly decreased *Luteitalea* functional contribution in Fs compared to As (SFigure 10), potentially contributing to the adaptability of the microbial community. Through DiTing analysis, this determined microbial communities involved in the cycling pathways were identified. Dimethylsulfoniopropionate (DMSP) cycle pathways (Figure 7A) and carbon cycle pathways (Figure 7B) were dominant in the Fs, which may be attributed to the increased activity of enzymes related to carbon metabolism, such as rhamnogalacturonase, endo-xylogalacturonan hydrolase, polygalacturonase, alacturonan alpha-1,2-galacturonohydrolase, exo-polygalacturonase, and others, as revealed by aligning against the Carbohydrate-Active enZYmes Database (CAZy) (Figure 8A). In particular, the abundance of cellulose and fucose substrate in As were remarkable higher than that in Fs, which can be explained as the the intricate regulatory network of glycometabolism (Figure 8B). While nitrogen cycle pathways (Figure 7C) and sulfur cycles pathways (Figure 7D) were more pronounced in the As. This could be associated with the augment in the abundance of *Sphingomicrobium, Luteitalea, Reyranella, Mitsuaria, Rhizobacter* in the As (SFigure 11), which are involved in the nitrogen or sulfur cycle. As the previous research by Sun *et* al., (2020), they demonstrated that the invasive plant *A. philoxeroides* outperforms native species under flooding conditions with high nitrogen levels. Similarly, the metabolites of *Mikania micrantha* promoted its growth and invasive adaptation by enriching the microbial community involved in nitrogen cycling pathways, thereby enhancing nitrogen availability (Liu *et al.*, 2020). This suggested that *M. micrantha* can manipulate the soil microbial community to improve its access to nitrogen, a crucial nutrient for plant growth. Invasive plants can alter the soil microbial community near their roots by recruiting different soil microbes, which is a potential mechanism for them to influence nutrient cycling. Microorganisms promote host plant growth and development through various mechanisms, such as nitrogen fixation, indoleacetic acid production, and iron carrier production (Li *et al.*, 2023). However, our results indicated that the DMSP and carbon cycle pathways of *F. tikoua* Bur. were not negatively affected by invasive plants, which may contribute to its resistance to invasion.

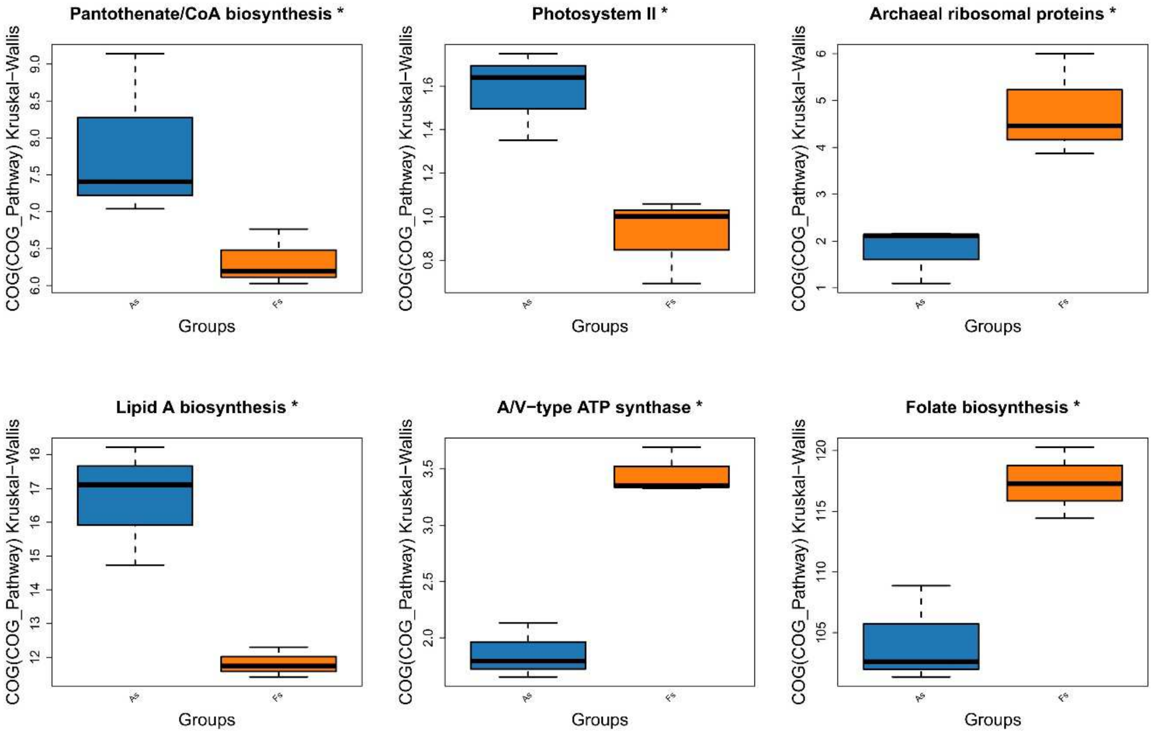


Figure 6. GOG pathway in As vs Fs.

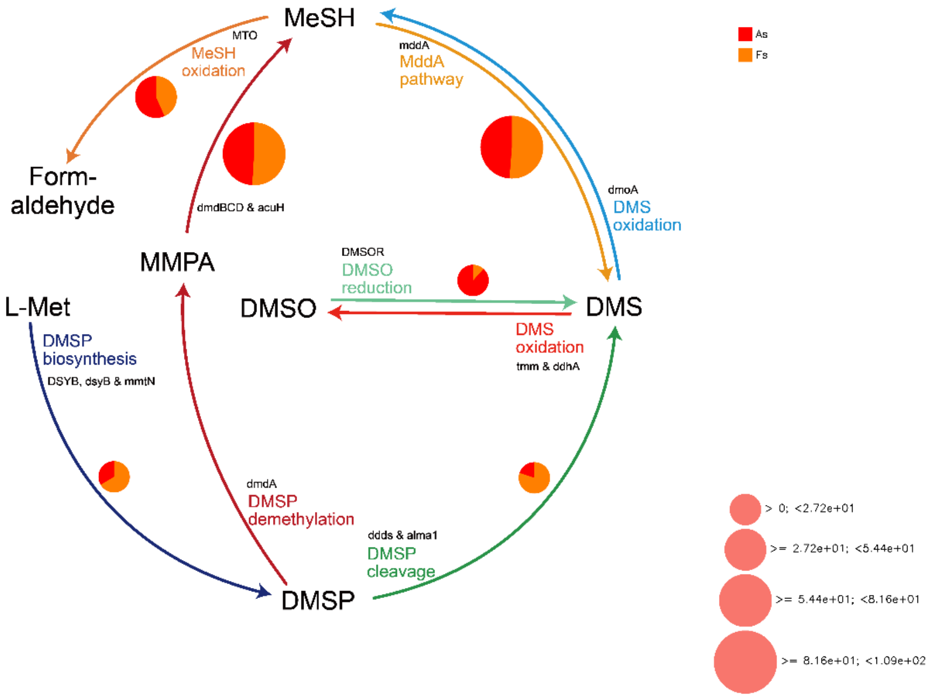


Figure. Relative abundances of the pathways involved in the DMSP cycle. The pie chart indicates the relative abundance of each pathway in each metagenomic sample. The size of pie charts represent the total relative abundance of each pathway.

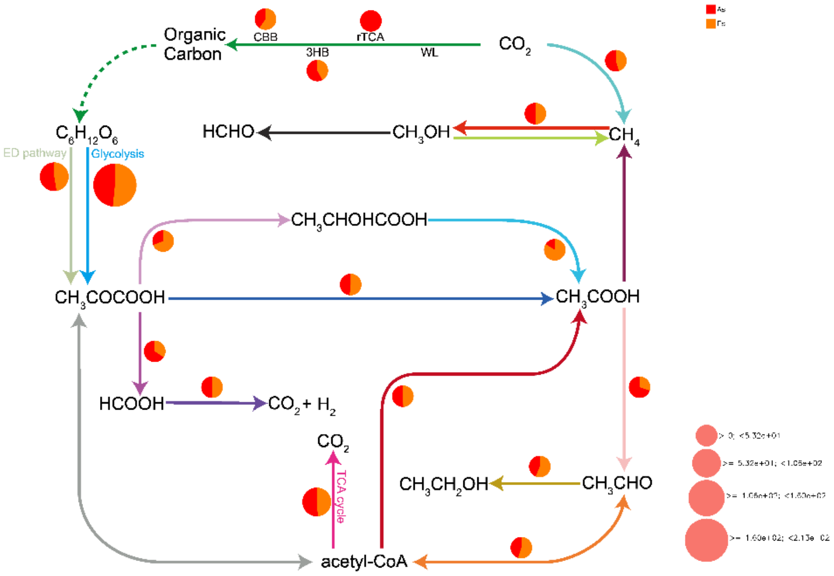


Figure. Relative abundances of the pathways involved in the carbon cycle. The pie chart indicates the relative abundance of each pathway in each metagenomic sample . The size of pie charts represent the total relative abundance of each pathway. CBB, Calvin Benson Bassham cycle; rTCA, reductive citric acid cycle; WL, Wood–Ljungdahl pathway; 3HB, 3-hydroxypropionate bicycle; DFC, dicarboxylate-hydroxybutyrate cycle.

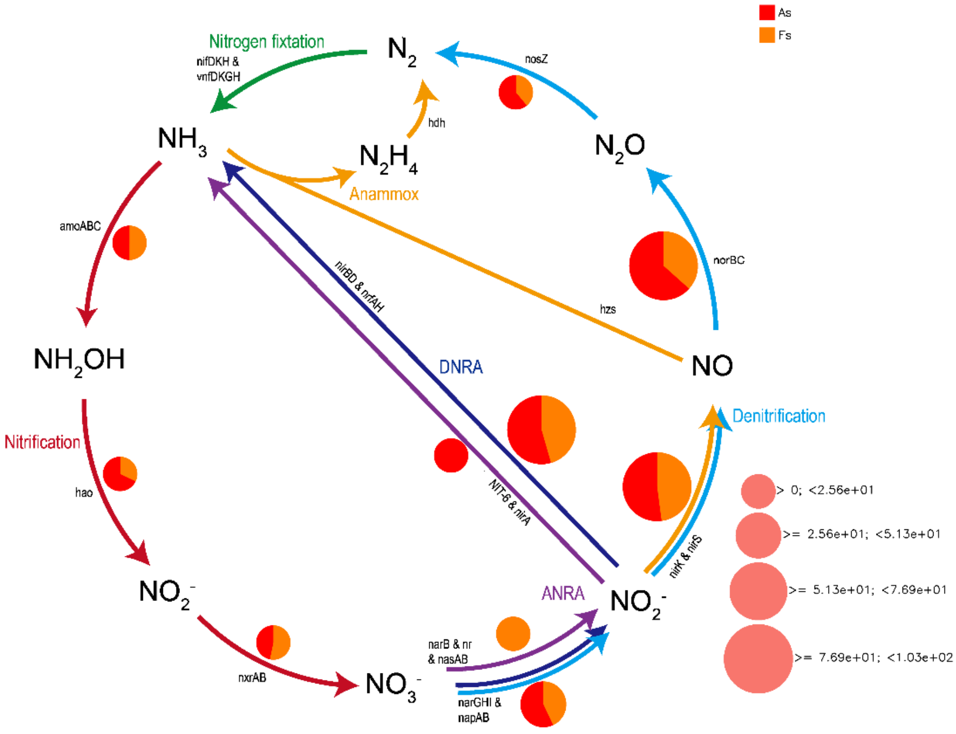


Figure. Relative abundances of the pathways involved in the nitrogen cycle. The pie chart indicates the relative abundance of each pathway in each metagenomic sample . The size of pie charts represent the total relative abundance of each pathway. ANRA, assimilatory nitrate reduction to ammonium; DNRA, Dissimilatory nitrate reduction to ammonium; Anammox, anaerobic ammonium oxidation.

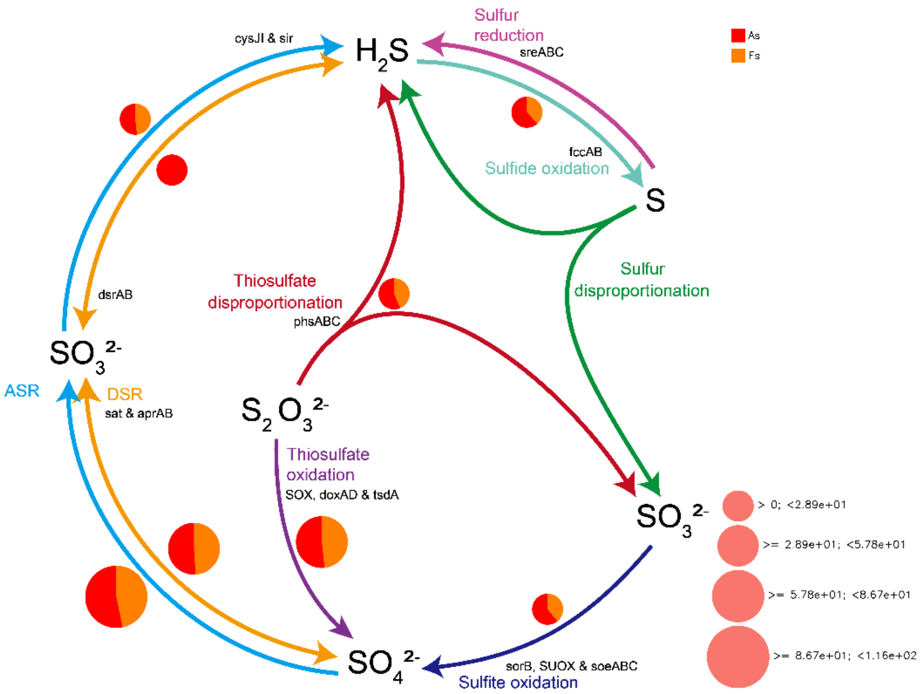
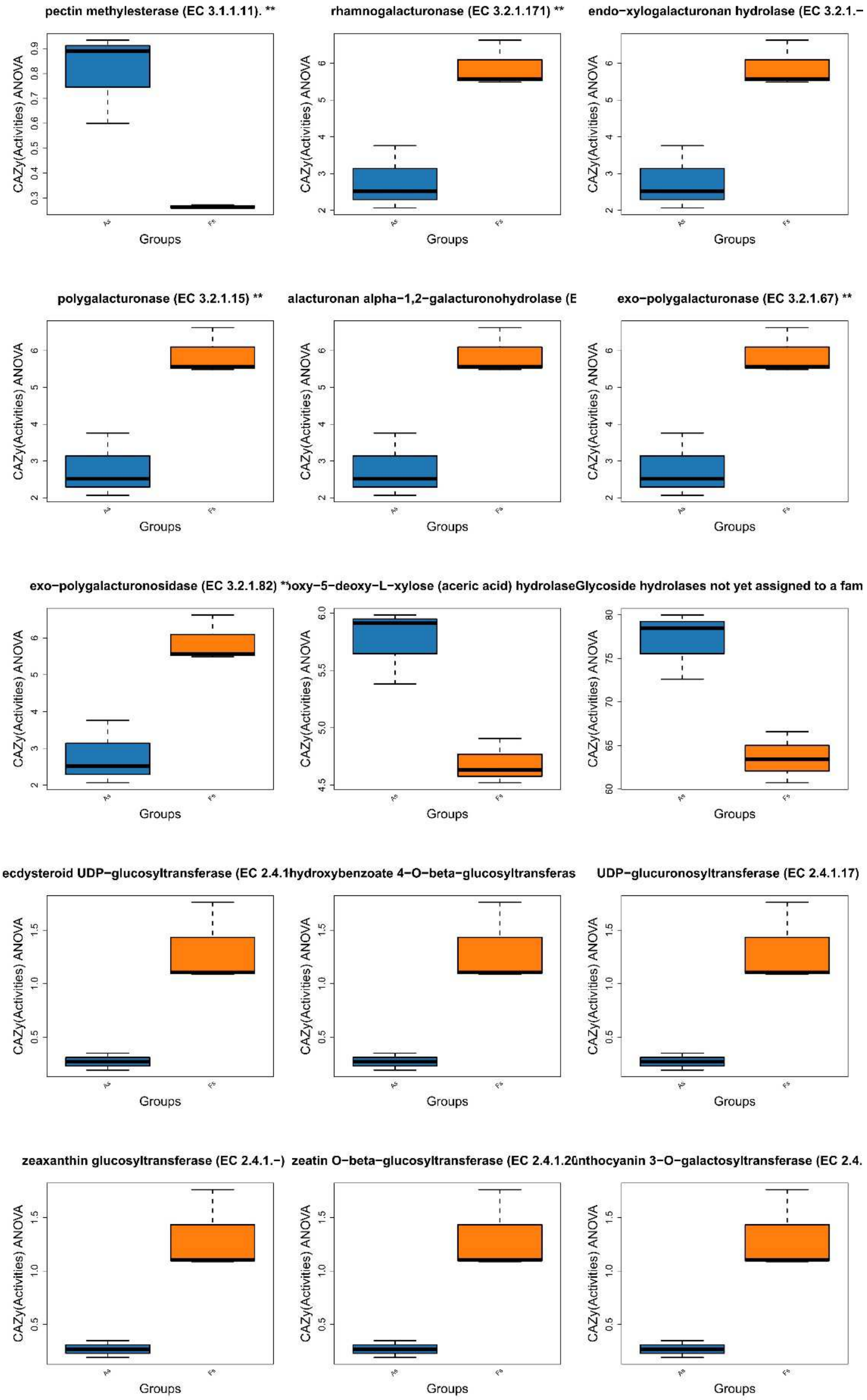


Figure. Relative abundances of the pathways involved in the sulfur cycle. The pie chart indicates the relative abundance of each pathway in each metagenomic sample . The size of pie charts represent the total relative abundance of each pathway. ASR, assimilatory sulfate reduction; DSR, dissimilatory sulfate reduction.

Figure 7. The cycling pathway in As vs Fs. A: DMSP cycle pathways, B: carbon cycle pathways, C: nitrogen cycle pathways, D: sulfur cycles pathways.


pectin methylesterase (EC 3.1.1.11). **
rhamnogalacturonase (EC 3.2.1.171) **
endo−xylogalacturonan hydrolase (EC 3.2.1.−
polygalacturonase (EC 3.2.1.15) **
alacturonan alpha−1,2−galacturonohydrolase (E
exo−polygalacturonase (EC 3.2.1.67) **
exo−polygalacturonosidase (EC 3.2.1.82) *
*oxy−5−deoxy−L−xylose (aceric acid) hydrolase
Glycoside hydrolases not yet assigned to a fam
ecdysteroid UDP−glucosyltransferase (EC 2.4.1
hydroxybenzoate 4−O−beta−glucosyltransferas
UDP−glucuronosyltransferase (EC 2.4.1.17)
zeaxanthin glucosyltransferase (EC 2.4.1.−)
zeatin O−beta−glucosyltransferase (EC 2.4.1.20
nthocyanin 3−O−galactosyltransferase (EC 2.4.
CAZy(Activities) ANOVA
Groups
As
Fs
0.3
0.4
0.5
0.6
0.7
0.8
0.9
2
3
4
5
6
4.5
5.0
5.5
6.0
60
65
70
75
80
0.5
1.0
1.5

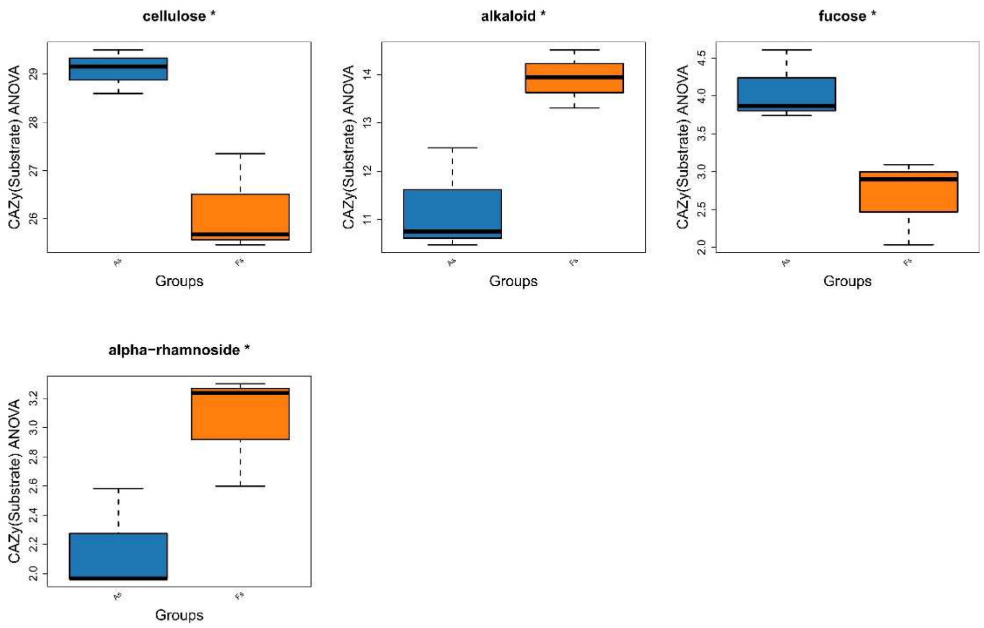


Figure 8. The abundance of active enzymes (A) and their corresponding substrates (B) in the enzyme base on CAZy database.

Although little is known about the potential role that rhizosphere soil microbial communities play in facilitating or resisting the spread of invasive species into native plant communities. It has been documented that invasive plants can have major effects on microbial decomposition in soil. The study of Bell *et al*., (2015) suggested that plants can alter their rhizosphere microbiomes through influencing nutrient availability. Putten *et al*., (2007) reported that exotic plant invasion may alter underground microbial communities, and invasion-induced changes of soil biota may also affect the interaction between invasive plants and resident native species. Native plants that are associated with a limited group of microbial symbionts may have an increased likelihood of being impacted by a harmful invader that disrupts local mutualisms. Therefore, the establishment of exotic invasive plants can be hindered by native plants that form associations with a diverse array of effective microbes, especially dominant microorganisms. This is because native plants that rely on symbionts are more likely to outcompete invasive plants for resources.

The significance of clonal integration in invasiveness of *A. philoxeroides* in heterogeneous environments was highlighted by the study of You et al.,(2014). However, *F. tikoua* Bur. exhibits a unique characteristic with its enclosed inflorescence known as hypanthodium. This inflorescence depends on particular insect pollinators that provide nourishment and shelter for their reproduction. Successful pollination requires a morphological match between the *F. tikoua* Bur. and their pollinators (Zhang *et al*., 2020). The size and shape of the pollinators' bodies are correlated with the size of the inflorescence, and specific volatile organic compounds (VOCs) attract these obligatory pollinators (Chen *et al*., 2016). This unique mutualism may grant *F. tikou*a Bur. resistance against invasive plants.

Conclusions

The photosynthetic parameters of *F. tikoua* Bur. were found to be lower than those of *A. philoxeroides*. However, *F. tikoua* Bur. exhibited stronger resistance to light inhibition. The two species were shown to dissipate residual light energy through different pathways. DEGs involved in carbon metabolism, protein processing in the endoplasmic reticulum, and plant-pathogen interaction may contribute to the interactions observed between *F. tikoua* Bur. and *A. philoxeroides*. By maintaining rhizosphere soil microbial diversity, *F. tikoua* Bur. can recruit more beneficial bacteria such as *Actinomarina*, *Nitrosotenuis*, and *Laccaria* to improve soil DMSP and carbon cycle pathways, thereby reducing pathogen damage and resisting invasion by *A. philoxeroides*. This preliminary observation suggested *F. tikoua* Bur. could potentially be used to control the invasion of *A. philoxeroides*, though further study is needed to fully evaluate the protective effects of establishing *F. tikoua* Bur. plantings against encroachment by this invasive species.

Supplementary Materials: S table 1 Summary of RNA-Seq data. STable 2 Adonis test for differences in community composition of bacteria, archaeal, viruses and fungi. SFig.1 The comparison of leaf area. SFig.2 PCA of RNA-seq. SFig.3 RNA-seq correlation analysis. SFig.4 Wenn diagram of DEGs base on RNA-seq. SFig.5 Taxonomy summary. SFig.6 NMDS. SFig.7 Venn diagram on soil bacterial taxa. SFig.8 Virulence factor test. SFig.9 The results of CARD and SARG analysis. A: Drug class, B: resistance, C: type, D: mechanism. SFig.10 KEGG pathway on functional contribution in As vs Fs. SFig.11 Differences in abundant genera of bacteria in As vs Fs.

Author Contributions: For research articles with six authors, Jiyue Wang, Nian Chen, Xiaojian Tian, Jiajun Xu, Denghong Shi and Wenjia Yang. methodology, Xiaojian Tian; validation, formal analysis, Nian Chen, Denghong Shi; investigation, Nian Chen, Xiaojian Tian, and Jiajun Xu; data curation, Jiyue Wang and Nian Chen; writing-original draft preparation, Jiyue Wang; project administration, Denghong Shi;funding acquisition,Wenjia Yang. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: None.

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