Effects of shade stress on morphophysiology and rhizosphere soil bacterial communities of two contrasting shade-tolerant turfgasses

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Abstract
Background: Perturbations in the abiotic stress directly or indirectly affect plants and root-associated microbial communities. Shade stress presents one of the major abiotic limitations for turfgrass growth, as light availability is severely reduced under a leaf canopy. Studies have shown that shade stress influences plant growth and alters plant metabolism, yet little is known about how it affects the structure of rhizosphere soil bacterial communities. In this study, a glasshouse experiment was conducted to examine the impact of shade stress on the physiology of two contrasting shade-tolerant turfgrasses and their rhizosphere soil microbes. Shade-tolerant dwarf lilyturf (Ophiopogon japonicus, OJ) and shade-intolerant perennial turf-type ryegrasss (Lolium perenne, LP) were used. Bacterial community composition was assayed using high-throughput sequencing. Results: Our physiochemical data showed that under shade stress, OJ maintained higher photosynthetic capacity and root growth, thus OJ was found to be more shade-tolerant than LP. Illumina sequencing data revealed that shade stress had little impact on the diversity of the OJ and LP’s bacterial communities, but instead impacted the composition of bacterial communities. The bacterial communities were mostly composed of Proteobacteria and Acidobacteria in OJ soil. Further pairwise fitting analysis showed that a positive correlation of shade-tolerance in two turfgrasses and their bacterial community compositions. Several soil properties (NO3--N, NH4+-N, AK) showed a tight coupling with several major bacterial communities under shade stress, indicating that they are important drivers determining bacterial community structures. Moreover, OJ shared core bacterial taxa known to promote plant growth and confer tolerance to shade stress, which suggests common principles underpinning OJ-microbe interactions. Conclusion: OJ was more shade-tolerant than LP. Shifts in rhizosphere soil bacterial community structure play a vital role in shade-tolerance of OJ plants.

Background

Urban greening currently employs a combination of trees, shrubs and grass, but beneath the trees and shrubs is the inevitable shady lawn. As a result, shade stress presents a major challenge to turf
grass growth. It has been estimated that approximately 20–25% of all grassed areas in the USA [1], and 50% of turfgrass in China, is subjected to varying degrees of shade [2]. Thus, insight into the mechanism of turf grass resistance or adaptation to shade stress is vital for turf management and selection of shade-tolerant turf grass varieties.

The negative effects of shade on plant morphology and physiology have long been established. The morphology of shaded leaves is characterized by an increase in specific leaf area and a decrease in thickness [3, 4]. Particularly, a lack of light adversely impacts chlorophyll content, chloroplast ultrastructure, as well as photosynthetic physiological processes [5, 6].

Root-associated bacterial communities play a crucial role in maintaining the health of the plant host [7]. These communities possess complex relationships where the composition and abundance of microbial communities depends on factors such as: soil chemical properties, plant genotype and phenotype, and perturbations in the surrounding abiotic or biotic stresses [8, 9]. The root distribution pattern in soil reflects plant ecological adaptation and may increase plant survival under stress [10]. However, there is little understanding how shade influences root morphology and root-associated bacterial communities. Clarifying exactly how shade stress affects soil bacterial communities is an essential step in developing strategies to combat shade in turfgrass management.

Dwarf lilyturf (Ophiopogon japonicus (Linn. f.) Ker-Gawl.) is an important green plant in the Liliaceae family with strong inherited shade tolerance. We used a high-throughput Illumina sequencing platform to characterize the responses of soil bacterial communities in O. japonicus (OJ) to shade stress, with shade-intolerant perennial turf-type ryegrasss (Lolium perenne,LP) as a control. The following hypotheses were tested: i) Shade-tolerant OJ had better photosynthetic capacity and root distribution than shade-intolerant LP; ii) the different shade-tolerance in the two plants correlate with an altered soil bacterial community composition; iii) shade stress affects root bacterial communities by altering soil chemistry and root morphology.

Results
Plant growth characteristics in response to shade stress

OJ and LP seedlings were exposed to shade stress and examined to determine their growth response to this stress (Fig. 1). Shade treatment resulted in different growth suppression in the two plants. Shade did not significantly influence leaf area in OJ but resulted in a 14.9% decrease ($P < 0.05$) in LP leaf area when leaves were exposed to 14 d of shade stress compared to non-shade (Fig. 1A, Additional file 1: Table S1). Shade treatment significantly decreased ($P < 0.01$) total root length, root surface area, and root volume in LP, while OJ exhibited superior acclimation to shade stress (Fig. 1B-D). In addition, OJ and LP had different changes in chlorophyll content in response to shade stress. Shade stress increased chlorophyll content in OJ, while shade stress reduced chlorophyll content in LP (Fig. 1E). Fluorescence parameters ($F_v/F_m$) for chlorophyll $a$ were reduced significantly in LP compared with OJ, indicating that OJ maintained higher photosynthetic capacity under shade stress (Fig. 1F). These results demonstrate that OJ is more shade-tolerant than LP.

Soil chemical characteristics

Shade stress significantly influenced most of the physicochemical properties analyzed (Table 1). Both OJ and LP soil significantly increased the NO$_3^-$-N content with shade treatment ($P < 0.001$). Conversely, shade treatment decreased TP, TK and AK in both soil types compared to non-shade treatment, however there was a greater effect with LP soil. Shade treatment of OJ resulted in a significant decrease in rhizosphere NH$_4^+$-N and a significant increase in the rhizosphere AP content. The opposite trend was observed with LP. Shade treatment had a small effect on the soil TN, SOC, C:N ratio, and rhizosphere pH level.

Bacterial diversity and community composition response to shade stress

Amplicon products of the V4 region of the 16S rRNA gene were obtained from each of the 60 samples and sequenced using the Illumina HiSeq 2500 platform. A total of 5371314 bacterial clean reads were
obtained. These sequences were grouped into 11485 OTUs according to a 97% similarity threshold. According to the rarefaction curves (Additional file 2: Figure S1), the sequencing depth in these samples was sufficient to cover the full diversity.

The bacterial communities did not have similar alpha diversity features between OJ and LP rhizosphere soil, as measured by the OTU richness, Shannon's diversity index (H) and Simpson's Evenness (E) (Fig. 2). The richness and diversity of OTUs did not show significant differences between the two rhizosphere soils. However, the evenness increased ($P < 0.05$) in OJ soil under shade stress but decreased in LP soil. This suggests that a few numerically dominant OTUs inhabit the LP rhizosphere. The patterns of bacterial community composition between treatments in OJ and LP soils were analyzed using PCoA based on Bray-Curtis dissimilarity. The PCoA analysis explained 64.06% of variation (two axes) in bacterial community composition. Shade treatments led to a distinct bacterial community structure (PERMANOVA, $P < 0.05$), and the bacterial community structures between the OJ and LP rhizosphere soils were also obviously different (Fig. 3). Further evidence showed that the bacterial communities collected at OJ rhizosphere on the one hand, and JP rhizosphere on the other, overlapped partially in the PCA plot (Additional file 3: Figure S2), indicating that OJ and LP soils had different bacterial community structures.

In both OJ and LP rhizospheric soil, the edaphic bacterial communities harbored principally 11 different phyla (accounting for more than 93% in each sample). The most numerically dominant phyla were Proteobacteria followed by Acidobacteria and Thaumarchaeota (Fig. 4A). Proteobacteria, Actinobacteria, and Chloroflexi decreased in LP soil in response to shade stress, but an increase or a lower degree of change was observed in OJ soil. In contrast, shade led to higher abundances of Verrucomicrobia and Acidobacteria in LP soil, compared to OJ soil (Kruskal-Wallis, $P < 0.01$). There were 12 genera (> 0.5%) within the classes Alpha and Gamma Proteobacteria, Flavobacteria, Planctomycetia, Spartobacteria, Nitrospira, and Thaumarchaeota. In all the samples, the taxonomic structure of the bacterial community was characterized by a clear numeric dominancy of the genus Candidatus_Nitrososphaera (Fig. 4B). The most evident differences between OJ and LP rhizosphere soil bacterial communities were the opposing trends in the abundance of Nitrospira, Steroidobacter,
Kaistobacter and Pirellula. These genera increased or no significant change was observed with increasing shade treatment in OJ soil, but they tended to decrease in LP soil. In contrast, the relative abundance of Rhodoplanes, Planctomyces, and Pseudomonas was larger (Kruskal-Wallis, $P < 0.01$ or $P < 0.001$) in OJ soil under shade treatment, compared to LP soil. In LP soil Gemmata had a larger relative abundance than in OJ soil (Kruskal-Wallis, $P < 0.001$), although shade stress decreased the relative abundance of this genus in both soils.

Core microbial players associated with rhizosphere soil in OJ and LP

The core bacteriome of OJ and LP rhizosphere soils was determined to examine shifts in the bacterial communities observed with the different host types. This analysis suggested that a specific taxonomy may exist which is particularly well adapted and prominent under different growth conditions. We found that OJ rhizosphere soil was dominated by OTUs identified as: Nitrosovibrio (19.1% of total core bacterial OTU), Aquicella (12.5%), Planctomyces (11.8%), Pseudomonas (11.2%), Nitrospira (10.3%), Steroidobacter (10.3%), Flavobacterium (8.8%), Kaistobacter (5.2%), Bacillus (6.8%), and Rhodoplanes (5.1%), which mostly belongs to Proteobacteria. In contrast, the rhizosphere soil of LP was dominated by Acinetobacter (21.0%), Flavisolibacte (19.3%), and Skermanella (17.1%) (belonging to Proteobacteria and Bacteroidetes, respectively). Notably, Nitrosovibrio_tenuis and Candidatus_Nitrososphaera_SCA1145 (both 5.9%) were identified in the OJ rhizosphere soil core (Additional file 4: Table S2).

Relationships between shade-tolerant parameters and bacterial communities

There was a significant positive relationship between plant shade tolerance in OJ and LP plants and soil bacterial community composition (Fig. 5). Among all the shade-tolerant indicators measured, leaf area, $F_v/F_m$, chlorophyll content, and root morphology were significantly correlated with soil bacterial community composition ($P < 0.001$ for all).

Relationships between bacterial
community and environmental variables

The OJ and LP soil bacterial community structures displayed clear, individual correlations ($P < 0.001$ or $P < 0.05$) to soil physicochemical variables including $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and TK as shown by the Mantel test (Additional file 5: Table S3). CCA analysis revealed that the OJ and LP rhizosphere soil bacterial communities were affected differently by edaphic chemical parameters under the different shade treatments examined. The proportion of total variability of OJ and LP soil bacterial communities attributed to the explanatory variables was 73.21% and 82.57%, respectively. This partition of variability was significant (general permutation test, $P < 0.01$ or 0.05; 999 replicates; Fig. 6; Additional file 6: Table S4). AK and total N were the major factors affecting the bacterial assemblages in OJ soil as judged by the length of the vectors shown in our CCA plots. In OJ soil, AK and total N were positively correlated ($P < 0.05$) with *Gemmatimonadetes*, *Chloroflexi*, *Acidobacteria*, *Nitrospirae*, and *WS3*. For OJ soils, CCA was consistent with the trends revealed by PCA showing a clear separation between control and shade treatment (Additional file 3: Figure S2). The TN, $\text{NO}_3^-\text{-N}$, and $\text{NH}_4^+\text{-N}$ concentration, three directly interlinked parameters, had a strong effect on bacterial assemblages in the LP soil. TN and $\text{NH}_4^+\text{-N}$ were positively correlated ($P < 0.05$) with *Actinobacteria*, *Bacteroidetes*, and *Thaumarchaeota*, respectively. Taxa, such as *Verrucomicrobia*, *Chloroflexi*, *Acidobacteria*, *Planctomycetes*, *Gemmatimonadetes*, and *WS3* were positively correlated ($P < 0.01$) with $\text{NO}_3^-\text{-N}$.

Additionally, shade treatments of different durations separately clustered in LP soil.

Discussion

The current knowledge of the plant shade stress response has arisen from studies of physiology and morphology and has neglected the function of root-associated soil microorganisms. Because plant health is closely tied to the activity of these associated microbes, our work using two turfgrass genotypes with contrasting shade tolerance profiles provides a description of the physiological and rhizosphere soil bacterial response induced by shade stress.
Physiological responses of OJ and LP plant to shade stress

We uncovered several differences between OJ and LP through analysis of the physiological response and growth suppression that accompanied shade stress. Our physiochemical data demonstrated that shade stress resulted in more severe growth suppression in LP than in OJ. This was indicated by a larger decline in leaf area, total root length, root volume, and surface area in LP versus OJ. Similar results have been observed in several tree species, showing that shade-tolerant red oak had greater leaf area and dry mass than shade-intolerant species [11]. Plant photosystem II is sensitive to various environmental stresses, including shade stresses [12]. Chlorophyll a fluorescence ($F_v$/F$_m$) is a valuable indicator of stress tolerance [5, 13]. Our results show that under shade stress OJ maintained higher $F_v$/F$_m$ and chlorophyll ($a+b$) content, suggesting it has a better photosynthetic capacity under shade stress. The present data agrees with our first hypothesis, listed above. These findings suggest that OJ is more shade-tolerant than LP.

Plant shade tolerance is related to bacterial community composition

Plants and their root-associated microbial communities are strongly interlinked, as a result, perturbations in the abiotic stress directly or indirectly influence plants, their associated microbial communities as well as the interaction between these organisms[8]. In both OJ and LP, we found that shade stress had little impact on bacterial richness and soil community diversity, which is consistent with other studies showing that community diversity is not significantly impacted by drought [14, 15]. Similar results have been reported in salt stress demonstrating that increasing salinity has no effect on total bacterial community richness [16]. The observed shifts in the soil microbiome when OJ and LP were shade stressed involved changes in relative bacterial abundance, rather than outright abolition of shade susceptible taxa and concomitant appearance of tolerant ones. This helps explain the lack of change in alpha-diversity.

Bacterial community composition was significantly different between OJ and LP rhizosphere soils with
various shade treatments. The relative abundance of *Proteobacteria* and *Actinobacteria* have been shown to accumulate in OJ soil in response to shade stress, while they have been shown to decrease in LP soil. Accumulating evidence shows that *Proteobacteria* and *Actinobacteria* display different trends in response to various environmental stresses, such as drought, salt and heavy metal stresses [17-20]. *Actinobacteria* are implicated in promoting plant growth under stress [21]. Many of them are known to form spores, which are resistant to adversity and can survive under stress conditions [22, 23].

Alpha and gamma *Proteobacteria* play a vital role in OJ soil in response to shade stress, as indicated by greater increase in genera of *Kaistobacter*, *Steroidobacter*, *Pseudomonas* observed in OJ soil. Inoculants of plant growth promoting rhizobacteria (PGPRs) genera such as *Pseudomonas*, *Flavobacterium*, and *Arthrobacter* have been shown to be effective candidates for stress amelioration in plants by mediating a variety of physiological and biochemical changes [24-27]. The present study has shown that species such as *Nitrosovibrio tenuis*, *Reyranella massiliensis*, *Arthrobacter psychrolactophilus*, and *Flavobacterium succinicans*, which belong to phyla of *Proteobacteria*, *Actinobacteria* and *Bacteroidetes*, respectively, are more abundant in OJ soil. This suggests that OJ preferred these genera and they may be markers of better shade-tolerance in OJ.

To further clarify this assumption and study the correlation of shade-tolerance parameters and bacterial community composition, pairwise fitting analyses were performed to compare above and below-ground morphology, photosynthetic capacity and bacterial community composition. We found that the leaf area, root volume, surface area, $F_v/F_m$, and chlorophyll $(a+b)$ content were positively and significantly related to soil bacterial community composition. This observation is in line with our second hypothesis, and with the last part of our third hypothesis. Similar observations have also been shown in maize with differing aluminum tolerances. It was observed that maize cultivars that depended on Al tolerance altered their root morphology and rhizosphere diazotrophic community composition [28].

**Changes in soil physicochemical**
properties play an essential role in shaping bacterial communities under shade stress

Soil acts as a strong ecological filter affecting the bacterial community structure and diversity. Numerous studies of microbial communities under abiotic stress have shown that soil factors govern microbial community structure [29-31]. Bottomley et al. [32] observed that soil NH$_4^+$-N was a dominant environmental factor that influenced bacterial community structures. This is due to the fact that it is the main nitrogen source for bacteria as seen by $^{15}$N isotope tracing [33]. Similarly, Nguyen et al. [34] also reported that bacterial diversity and composition were related to soil NH$_4^+$-N and total N content exposed to post-waterlogging or post-prolonged drought. Consistent with our third hypothesis, the major drivers in OJ rhizosphere soil were AK and total N which was positively associated with Gemmatimonadetes, Chloroflexi, Acidobacteria, Nitrospirae, and WS3. Thus we confirmed the importance of these two soil variables in regulating the OJ rhizosphere. In contrast, total N, NO$_3^-$-N, and NH$_4^+$-N concentration exhibit a strong effect on bacterial assemblages in LP soil. A strong relationship between soil physicochemical properties and bacterial communities was also observed in water-limited soils. In these soils, the abundance of Acidobacteria correlated positively with soil NH$_4^+$-N and total P and negatively with total N and Mg$^{2+}$, whereas Chloroflexi displayed the opposite trend [35].

In both control and stressed soil, host species were confirmed to exert a significant influence on bacterial community structures [36]. Our PCoA data shows that bacterial communities separated between OJ and LP soil. Al-tolerant maize cultivation significantly influenced the diazotroph populations [28], a result that aligns with our results with turfgrass. This may be mainly attributed to plant root exudates, which are key determinants of microbial community composition in plant-microorganism interactions [37].

Conclusions
This study describes the physiological plant shade stress response as well as the rhizosphere soil bacterial community shade stress response in two turfgrass genotypes with different shade tolerances. Maintenance of higher photosynthetic capacity and root growth during shade stress in OJ could make this more shade-tolerant than LP. Moreover, shifts in rhizosphere soil bacterial community structure play a vital role in shade-tolerance of OJ and LP plants. The study also shows that, under shade stress, some soil properties showed a tight coupling with several major bacterial communities, indicating that they are important drivers determining bacterial community structures.

Methods

Glasshouse experimental setup and soil sampling

A glasshouse experiment was conducted at Northwest Agriculture & Forestry University, China, using soils collected from two different turfgrasses: dwarf lilyturf (*O. japonicus*) and perennial ryegrass (*L. perenne* cv. Ph.D.). The seeds were obtained in June 2017, from Bcyseed Co., Ltd., located in Liwan District (23.072127N, 113.207089E), Guangzhou, China. The specimen was purchased by Bcyseed Co., Ltd., a professional seed production and sales company, who undertook the formal identification of the seeds used in this study. Based on the unlikeliness of an erroneous identification, we have not deposited a voucher specimen. In this study, dwarf lilyturf (OJ) was planted around buildings and trees and perennial ryegrass (LP) served as greening square in campus with good light conditions. The site has a warm temperate continental monsoon climate, with a mean annual air temperature of 12°C and 500 mm of mean annual precipitation. Before the experiment, the sites were managed as turfgrass for over 10 years.

To investigate impacts of shade stress on the rhizosphere bacterial communities, OJ was compared to LP as a control. The two plants were cultured in a plastic pot (13.5×17.5×11.0 cm) filled with soil collected from their respective areas of growth, as described above. Seeds were superficially disinfected with 0.1% sodium hypochlorite and washed three times with purified water. Three seeds were sown per pot directly into the soil. The plants were maintained in a greenhouse with an average
temperature of 23/18 °C (day/night), 700 µmol m⁻² s⁻¹ photosynthetic active radiation from natural sunlight, and 65 % relative humidity until the plants grew above 15 cm. Shade treatments (approximately 230 µmol m⁻² s⁻¹) were performed under the canopy using two layers of black nylon net using the same conditions described above. Pot treatments were randomized within a glasshouse compartment. We carried out five treatments including: (1) Control (0 d); (2) shade for 7 d; (3) shade for 14 d; (4) light (sunny) for 7 d; (5) light (sunny) for 14 d. There were six replicates per treatment, giving a total of 60 pots.

After shade treatment, total leaf area, root morphology, chlorophyll content and the maximum quantum yield of PSII ($F_{v}/F_{m}$) were measured. The roots of each plant were separated from the soil and shaken manually to remove the loosely attached soil. Rhizosphere soil was collected, as the soil adhering to the roots [28](Wang et al., 2018). A rhizosphere soil sample was obtained by pooling soil obtained from three plants growing in the same pot. As a result, each treatment had six replicate rhizosphere samples due to the six replicate pots. Bulk soil samples were also collected from the same pot at a depth of 0-10 cm. Soil samples were mixed thoroughly, divided into two parts, stored in sterile 50 mL Falcon tubes, and transported to the laboratory. One part was kept at 4°C for analysis of soil NH₄⁺-N and NO₃⁻-N, and to extract soil DNA within 3 days. The other part was air-dried for measurements of soil pH, total N (TN), total P (TP), total K (TK), soil organic C (SOC), available P (AP), available K (AK).

### Determination of plant growth characters

The total leaf area for each seedling was measured in the laboratory using a LI-3000A leaf area scanner (LI-COR Inc., USA). Root morphology including total root length, root surface area, and root volume was analyzed using a WinRhizo-V700 root scanner (Regent Instruments Inc., Quebec, Canada). The chlorophyll content was determined spectrophotometrically using 80% acetone as a solvent [38](Lichtenthaler, 1987). On the same leaf, a portable pulse-modulated fluorometer (PAM2100, Walz, Effeltrich, Germany) with the PamWin software was used to measure chlorophyll fluorescence ($F_{v}/F_{m}$).
Soil physicochemical analyses

Soil pH was measured using a pH meter (Mettler Toledo FE20, Switzerland) in a soil solution with a 1:2.5 soil: water ratio. The NH$_4^+$-N and NO$_3^-$-N were extracted with 2.0 M KCl and measured by a continuous flow analyzer (Flowsys, Systea Inc., Italy). Soil was processed for C content by first removing inorganic C through treatment with 1 M HCl. Following removal of inorganic C, soil organic C was analyzed using an auto-analyzer (Shimadzu, Kyoto, Japan). The total N in the soils were measured on an elemental analyzer (ECS 4024, Costech Inc., Italy). Total P was determined by digesting samples first with HClO$_4$-H$_2$SO$_4$, followed by the molybdenum blue method using an ultraviolet-visible spectrophotometer (UV-1000, AOE Instruments, Shanghai, China). Available soil P (AP) was extracted with 0.03 M ammonium fluoride-hydrochloric acid and measured colorimetrically as described above. Total K was determined using NaOH fusion method, and the available K (AK) was extracted with 1.0 M ammonium acetate and measured by flame photometry (Model 410, Sherwood, England).

Soil bacterial community analyses
DNA extraction, PCR amplification, and high-throughput sequencing

Total soil DNA was extracted 0.30 g soil collected from each soil sample using the Power Soil DNA extraction kit (MoBio Laboratories, Carlsbad, CA) as directed by the manufacturer’s instructions. The following PCR primers were used for amplification targeting the V4 region of the bacterial 16S rRNA gene: 338F (5’-XXXXXXXXGTACTCCTACGGGAGGCAGCAG-3’) and 533R (5’-TTACCAGGCTGCTGGCAC-3’). Paired-end sequencing was performed at Beijing Genomics Institute (BGI)-Shenzhen, Shenzhen, China, using a paired 250-bp Illumina HiSeq 2500 sequencing platform according to the manufacturer’s instructions.

Sequence processing

Illumina sequencing data were pair assembled using FLASH software (v1.2.11) [39] using a minimal overlapping length of 15 bp and mismatching ratio of overlapped region ≤ 0.1. Sequences were then
clustered into operational taxonomic units (OTUs) at a 97% identity threshold using USEARCH (v7.0.1090) [40]. UCHIME (v4.2.40) against the SILVA database was used to filter out chimeric sequences. USEARCH GLOBAL was used to align representative sequences from individual OTUs [41]. These were taxonomically classified using the Ribosomal Database Project (RDP) Classifier v.2.2 based on the SILVA database, using 0.6 confidence values as cutoff.

**Statistical analyses**

Analysis of variance (ANOVA) according to the general linear model procedure of SPSS17.0 (SPSS Inc., Chicago, IL USA) was used to determine the effects of shade treatment, turfgrass species, and the interactions between these factors on plant physiological indicators and the influence of shade treatment on soil properties. Differences between treatment means were separated by Fisher's protected least significance difference (LSD) test at \( P = 0.05 \). For these analyses, OTUs defined at 97% sequence similarity were used. Boxplots and heatmaps were obtained with the R package ggplot2 (v2.2.1). Rarefaction curves of observed OTUs were generated by software R (v3.1.1). The differences in OTU composition between different samples were displayed using principal component analysis (PCA). Alpha diversity [Richness, Shannon diversity index (H’) and Simpson's Evenness (E)] were analyzed based on randomly rarefied OTU abundance matrices using mothur (v1.31.2). Bray-Curtis distances of bacterial communities using QIIME (v1.80) was used to analyze beta diversity.

Principal coordinates analyses (PCoA), based on Bray-Curtis dissimilarity, were used to display differences in the composition of bacterial communities between OJ and LP rhizosphere soil treatments. Permutational multivariate analysis of variance (PERMANOVA) was conducted to test the significance of the Bray-Curtis dissimilarity. Kruskal-Wallis tests were performed using the R software (kruskal.test function) to assess the impact of shade stress on soil bacterial community structure in both species. A value of \( P < 0.05 \) was considered to be statistically significant.

To analyze the correlations between soil physicochemical parameters and bacterial community compositions, a Mantel test (9,999 permutations) with Spearman correlations of the R vegan package was used. Canonical correspondence analyses (CCA) were performed with the R package vegan (v2.4.2) to visualize the relationship between soil physicochemical properties and bacterial
communities. For the CCA analyses, the correlation of the canonical axes with the explanatory matrix was determined with the general permutation test and the “envfit” function was used to analyze the significance of soil physicochemical factors on the composition of bacterial communities. To analyze the correlations of above-and below ground phenotypes and the composition of bacterial communities, pairwise fitting analysis was carried out using the “lm” function in the R vegan package. Indicator species analysis was performed using the multipatt function implemented in the indicspecies package in R with 1000 permutations. The bioindicators of LP and OJ soil were designated as the OTUs that are part of the core microbiome of only LP or OJ soil under different treatments while also having abundances higher in OJ according to the permutation test ($P < 0.05$).

Abbreviations

AK: Available K; AP: Available P; CCA: Canonical correspondence analyses; OJ: Ophiopogon japonicus;

OTUs: Operational taxonomic units; LP: Lolium perenne; RDP: Ribosomal Database Project; SOC: Soil organic C; TK: Total K; TN: Total N; TP: Total P; PCoA: Principal coordinates analyses; PERMANOVA: Permutational multivariate analysis of variance

Declarations

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Availability of data and materials

All sequence files described in this paper have been submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database (accession number: SRP154594).

Authors’ contributions

JF and TH conceived and designed the study. PS, YL, JG and DZ performed the experiments. JF and PY analyzed the data and wrote the manuscript. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no potential conflict of interest.

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Table

Table 1 Responses of soil physicochemical variables to shade treatment. Values are the means(SD) of six pot replicates. Different lowercase or capital letters indicate significant differences (P < 0.05, LSD test) in OJ (Ophiopogon japonicus, shade tolerant) or LP (Lolium perenne, shade-intolerant) soil. ***P < 0.001, **P < 0.01, *P < 0.05

| Soil sample | Total N (g kg⁻¹) | Organic C (g kg⁻¹) | Soil C:N | Total P (g kg⁻¹) | Total K (g kg⁻¹) | NH₄⁺-N (mg kg⁻¹) |
|-------------|-----------------|-------------------|----------|-----------------|-----------------|-----------------|
| OJ.0d       | 1.07(0.23)      | 24.38(0.27)       | 22.79(1.79) | 0.52(0.045)b | 17.19(0.19)b | 6.99(0.026)a |
| OJ.7d       | 1.18(0.27)      | 27.43(3.16)       | 23.25(2.53) | 0.62(0.13)a  | 18.17(0.18)a | 2.93(0.27)b  |
| OJ.14d      | 1.19(0.19)      | 24.21(1.97)       | 20.34(1.70) | 0.58(0.035)a | 18.14(0.12)a | 2.91(0.61)b  |
| OJ.57d      | 1.08(0.14)      | 21.97(4.04)       | 20.34(1.38) | 0.51(0.05)b  | 17.83(0.36)a | 2.15(0.89)b  |
| OJ.14d      | 1.09(0.16)      | 22.13(3.62)       | 20.30(1.21) | 0.57(0.040)a | 16.98(0.20)b | 2.63(0.26)b  |
| OJ.7d       | 1.24(0.09)      | 25.36(0.87)       | 20.45(0.66) | 0.54(0.032)b | 16.88(0.13)bc | 4.78(0.96)b  |
| LP.0d       | 1.21(0.02)      | 21.88(0.74)       | 18.08(0.73) | 0.68(0.11)a  | 19.18(0.96)a | 4.67(0.47)b  |
| LP.7d       | 1.17(0.15)      | 24.99(1.27)       | 21.36(1.76) | 0.60(0.021)ab | 17.36(0.41)b | 5.94(1.44)a  |
| LP.14d      | 1.12(0.10)      | 22.97(3.19)       | 20.51(1.09) | 0.59(0.015)ab | 17.56(0.38)b | 5.08(0.62)ab |
| LP.57d      | 1.06(0.05)      | 21.95(2.79)       | 20.51(2.50) | 0.56(0.015)b  | 15.72(0.04)c  | 6.23(0.43)a  |

F value for ANOVA

| Treatments (T) | 0.504 | 0.919 | 0.738 | 3.279* | 12.394*** | 4.302* |
|----------------|-------|-------|-------|--------|-----------|--------|

| Species (S)   | 0.328 | 0.105 | 1.885 | 2.430 | 0.310 | 22.301*** |
|----------------|-------|-------|-------|--------|------|-----------|

| T x S          | 0.346 | 0.936 | 2.158 | 0.393 | 8.531*** | 7.487** |

Additional File Legend
Additional file 1: Table S1. Two-way ANOVA for the effect of shade stress on morphology and photosynthesis in shade-tolerant OJ (*Ophiopogon japonicus*) and shade-intolerant LP (*Lolium perenne*) plant. LA, Total leaf area; RL, Total root length; RV, Root volume; RSA, Root surface area; Chl, Chlorophyll. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Additional file 2: Figure S1. Rarefaction curve of bacterial 16S rRNA gene sequences obtained from amplicon sequencing.

Additional file 3: Figure S2. Principal Component Analysis (PCA) in rhizosphere soil microbial communities of shade-tolerant OJ (*Ophiopogon japonicus*) and shade-intolerant LP (*Lolium perenne*) under shade stress. OTUs delimited at 97% similarity.

Additional file 4: Table S2. List of the OTUs which comprise the core bacteriome of rhizosphere soil in OJ and LP. Those OTUs which are members of each core bacteriome are indicated in grey. Indicator species analysis was performed using the multipatt function implemented in the indicspecies package in R with 1000 permutations. Significance of each indicator value is represented: *$P < 0.05$; **$P < 0.01$ and ***$P < 0.001$.

Additional file 5: Table S3. Spearman's rank correlation coefficient of soil physicochemical variables and bacterial community composition revealed by Mantel tests ($r$ and $p$ values).

Additional file 6: Table S4. Relationship of single soil variable (OJ/LP) and microbial taxa according to CCA analysis ($r^2$ and $p$ values). ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Figures
Plant total leaf area (A), total root length (B), root surface area (C), and root volume (D), chlorophyll content (E), and chlorophyll fluorescence (Fv/Fm) (F) of shade-tolerant OJ (Ophiopogon japonicus) and shade-intolerant LP (Lolium perenne) under shade stress. Values are the means ± SD of six pot replicates. Lowercase or capital letters indicate significant differences (P < 0.05, LSD test) in OJ or LP.
Figure 2
Boxplots of Richness (A), Shannon's diversity index (B) and Simpson's Evenness (C) of bacterial communities based on OTUs defined at 97% sequence similarity. Black dots represent soil samples outliers.

Figure 3
Principal coordinates analysis (PCoA) based on Bray-Curtis distances between rhizosphere soil bacterial communities in shade-tolerant OJ and shade-intolerant LP under shade stress.
Figure 4

Changes in the bacterial community composition at the phylum level (relative abundance > 1%, A), and genus level (relative abundance > 0.5%, B) under shade stress. Asterisks indicate statistically significant differences according to Kruskal-Wallis tests (*P < 0.05; **P < 0.01 and ***P < 0.001)
Figure 5
Relationships between total leaf area (A), root volume (B), root surface area (C), Fv/Fm (D), and chlorophyll content (E) in bacterial community composition under shade stress. The relationship between total root length and bacterial community composition was not significant (data not shown). The plot shows the 95% confidence interval of the fit.
Figure 6

Canonical correspondence analysis (CCA) of bacterial communities based on Bray Curtis distances in rhizosphere soil bacterial communities of OJ (A) and LP (B) under shade stress.

Arrows indicate the direction and magnitude of bacterial taxa associated with soil physicochemical characteristics. Permutation tests confirmed the effect of the soil factors as drivers of the bacterial community

Supplementary Files
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