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Influence of highway construction of alpine grassland area in Gannan on soil properties and microbiota

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In order to explore microorganisms which may utilized in soil rehabilitation and vegetation restoration, the present study evaluated the impacts of highway construction on soil chemical properties and microbiota and their interaction based on highway construction area in Gannan on the Qinghai-Tibet Plateau. Soil samples were collected from highway construction area of Zhuoni-Hezuo highway including two representative locations at different distances and abandoned black and yellow soils in June and September. The properties of these collected soil samples, including the contents of nitrogen, phosphorus, potassium, microbial biomass carbon, pH value, enzyme activity and microbiota were analyzed. A total of 26 phyla, including 369 genera of bacteria, and 14 phyla, including 307 genera of fungi, were identified in all soil samples. Only sampling time had a great impact on the soil, as the values of most of the tested soil chemical indices and microbial α-diversity indices in September were significantly higher than in June ($P < 0.05$). However, the fungal ACE index in September was significantly lower than that in June ($P < 0.05$). There was no significant difference between the two distances within a single location or the two locations at the same distance, except for a few indices in September. The nutrient contents of the black soil were significantly higher than those of the yellow soil ($P < 0.05$), although both were significantly lower than those of the soil from plant-growing areas ($P < 0.05$). The soil chemical properties had a significant correlation with most of the bacterial α-diversity indices, which revealed the interaction between soil properties and microbiota. Specifically, *Sphingomonas* and *Acaromyces* et al. were recognized as biomarkers after LEfSe analysis. The soil total nitrogen content significantly affected plant fresh weight and the bacterial community, which was also the main factor affecting the biomarkers. These results were consistent with vegetation growth, suggesting that soil away from the red line of the highway construction had its own adaptation mechanisms; however, highway construction destroyed and affected the surrounding natural environment. Improved understanding of the impacts of highway construction on ecology will be critical in building decision support tools to help improve the ecosystem.

Keywords Highway construction, Microbial community, Soil chemistry properties, Ecological restoration, Growth seasons

With the rapid development of the global economy and society, major transport infrastructure projects in China have progressed rapidly, and there is an urgent need for ecological restoration and roadside greening, especially in ecologically fragile areas such as the Qinghai-Tibet Plateau. Ecological restoration is the process of restoring the structure, function, and biodiversity of degraded ecosystems through human intervention and management. Traditionally, restoration ecology has focused on aboveground features[1,2] with less attention given to belowground microbial aspects.

Soil microorganisms are one of the largest reservoirs of biodiversity on Earth and play a key role in soil remediation and vegetation restoration, this influences numerous ecosystem processes, including nutrient cycling, energy flow, developing stable soil systems and establishing sustainable plant communities[3–6]. It has long been proposed that soil microbial communities consider as reliable indicators of success in monitoring the spectrum of ecological restoration, including plantation forests, invasive species management and soil stabilization[7–9]. Consequently, an increasing number of scholars are directing their research towards the field of soil microbial ecology in diverse ecosystems and fragile areas. However, the implementation of soil microbiome

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in ecological restoration remains in the experimental stage due to the interference of extreme events and the complexity of governance measures. In some instances of restoration, microbial communities have recovered. For example, According to Li et al. (2022), soil microorganisms have a significant role in the process of plant succession and are intimately associated with the roots of vegetation[10]. Gao et al. (2016) found that application of microbial agents has effectively promoted the restoration and reconstruction of soil ecosystem functions in mining areas[11]. while in others, the establishment of microbial communities and nutrient cycling did not occur even after decades[12]. These indicated that the ecological restoration functions of microorganisms are comprehensively influenced by multiple factors, including their own characteristics, environmental conditions, research site and the degree of ecosystem damage et al. The restoration of soil functionality continues to present a significant challenge due to the inherently complex, non-linear, and unpredictable nature of microbial ecosystem dynamics[11–14]. However, some successful cases demonstrate that utilizing microorganisms for ecological restoration is indeed feasible. Moreover, microbial fertilizers are more environmentally friendly and efficiently in the ecological restoration[15,16]. Few studies have reported the function of ecological restoration of microorganisms in highway construction. Therefore, it is necessary to strengthen the exploration of important microorganisms in the local area and to promote the achievement of ecological restoration goals.

Vegetation plays a crucial role in protecting highway slopes by slowing erosion, intercepting rainfall, and stabilizing slope surfaces with well-developed root systems[17]. The recovery of vegetation on highway slopes can mitigate the adverse impacts, particularly during the initial phase of highway construction. Although the response of plant communities to nutrient enrichment has been extensively studied, it has been shown that vegetation restoration can increase the nutrient content and microbial activity of soils. However, the ecological and environmental conditions in China are highly complex, making the relationship between plant growth and nutrient cycling during land recovery largely uncertain[18]. To optimize soil nutrient utilization for vegetation restoration and roadside greening, it is necessary to aware the effects of highway construction on plant communities and soil nutrients. In addition to the uncertainty surrounding plant growth and nutrient cycling, the responses of the soil microbial community to these factors remain litter understood, despite their critical importance for the functioning of the ecosystem. Soil microorganisms play a vital role in maintaining ecosystem function and soil fertility. A number of studies have shown that there is a strong relationship between soil chemical properties and the microbial community[19–21]. A recent study showed that nitrogen fertilisation decreased both soil microbial diversity and the relative abundances of Actinobacteria and Nitrospirae, while the relative abundances of Ascomycota and Basidiomycota remained unchanged[22]. Another investigation has demonstrated that the microbial community affect soil chemistry, which was significantly positively correlated with soil pH, carbon/nitrogen ratio (C/N), and other environmental factors[23–25]. These studies suggest that soil microbial diversity and composition also respond differently to soil chemical properties, with relationships that can vary across different regions and time periods. Understanding the relationships between soil nutrients and microbial communities in this area is key to guiding effective local ecological restoration efforts. In fact, highway construction on the soil quality is various at different researches. On the one hand, the differences due to the local topography, soil types, and vegetation cover; on the other hand, it is because of environmental factors, such as temperature and humidity. Seasonal variations are one of the main natural causes of temperature and humidity variations[26,27]. Therefore, a comprehensive understanding of the impact of highway construction on soil quality at different growing seasons in this region, as well as the influence of seasonal variations on soil quality, is conducive to a thorough grasp of the current ecological status of the highway construction site and self - restoration capacity of the ecological environment in this area. Subsequently, by integrating microbial ecological restoration technology, ecological restoration can be carried out efficiently and precisely, thereby achieving the goal of ecological recovery.

Therefore, the aims of this study were to (1) evaluate the effects of highway construction and seasonal variations on microbial communities and soil chemical properties; (2) identify microbes that could serve as biomarkers; and (3) reveal the relationship between soil chemical properties and microbial communities, as well as their influence on vegetation.

Materials and methods

Study area and experimental design

The survey sites were located in the Zhuoni-Hezuo highway construction area in Gannan Tibetan Autonomous Prefecture, Gansu Province, China. Two representative locations were at linear distances of 17 km; representative area 1 (section 8) was located in Zhuoni County, belong to the transitional zone between the Qinghai-Tibet Plateau and the Loess Plateau and representative area 2 (section 11) was located in Hezuo City, belong to a typical alpine meadow area of the Qinghai - Tibet Plateau. Considering that the effect of highway construction on vegetation diminishes with increasing distance from the highway construction. This impact is most evident within the first 50 m, tending to stabilize at approximately 200 m[28,29]. Therefore, for each location, the two sample sites were 10 m (disturbed) and 500 m (undisturbed) away from the highway construction red line. The soil samples were: 8–10: 10 m away from the construction red line at section 8; 8-500: 500 m away from the construction red line at section 8; 11 – 10: 10 m away from the construction red line at section 11; 11–500: 500 m away from the construction red line at section 11. Except these soil samples, the black soil is on the surface layer of the soil and underneath is yellow soil. Black and yellow soils were excavated and piled up for further construction utilization, these soil samples at section 8 (8-H: abandoned yellow soil; and 8-B: abandoned black soil) and section 11 (11-H: abandoned yellow soil; and 11-B: abandoned black soil) were also included.

Each sample site had four blocks (1 × 1 m) at 100 m intervals. The survey was conducted from June 19 to June 21, 2021, and September 7 to September 9, 2021. In each block, five soil samples were collected at a depth of 10 cm using a five-point sampling method[30] the collected soil samples were the surface black soil. In addition,

black and yellow soils generated during highway construction piled up on roadsides were also collected by mixing them separately and then packed in Ziplock bags as replicates in these two representative areas.

A part of the soil samples was sieved through a 2-mm screen after removing the dead branches and stones, and stored in a refrigerator at -20 °C until further analysis. The other part of the soil was stored to measure the chemical indices.

Soil chemical properties

The soil nutrients, soil enzyme activities were determined according to the protocol of chen et al.[31]. More details regarding the assays are provided in the Supporting Information.

Analysis of soil microbial diversity

DNA extraction and sequencing

Soil DNA was extracted using a DNA extraction kit (TGuide S96, DP812) according to the manufacturer's instructions. The bacterial 16 S rRNA gene fragments were amplified with the universal primers 27F_(16 S-F) (5'- AGRGTTTGATYNTGGCTCAG-3') and 1492R_(16 S-R) (5'-TASGGHTACCTTGTTASGACTT-3') fused with a unique barcode. The identification region for fungal diversity was the ITS1 and ITS4 region (primers: 5'-CTTGGTCATTTAGAGGAAGTAA-3'; 5'-TCCTCCGCTTATTGATATGC-3'). Each bacterial PCR mixture contained 1.5 µl genomic DNA, 15 µl KOD OneTM PCR Master Mix, 1.5 µl forward primer, 1.5 µl reverse primer, and 10.5 µl ddH_2O. PCR was performed under the following conditions: one cycle at 95 °C for 2 min, followed by 25 cycles at 95 °C for 10 s, 55 °C for 30 s, 72 °C for 90 s, and a final extension at 72 °C for 2 min. Each fungal PCR mixture contained 1.5 µl genomic DNA, 15 µl KOD OneTM PCR Master Mix, 0.9 µl forward primer, 0.9 µl reverse primer, and 11.7 µl ddH_2O. PCR was performed according to the following conditions: one cycle at 95 °C for 5 min, followed by 8 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, followed by 24 cycles at 95 °C for 30 s, 60 °C for 30 s, 72 °C for 45 s, and a final extension at 72 °C for 5 min. Purified PCR products were sequenced on an Illumina NovaSeq 6000 platform. Using the NEXTFLEX™ Rapid DNA-Seq Kit (Bioo, USA), we constructed metagenomic paired-end libraries, which were subsequently pooled and loaded onto NovaSeq Reagent Kits (Illumina, USA) for Bridge PCR. The sequencing of metagenomic libraries was performed by Baimaike Inc. (Beijing, China) on the NovaSeq 6000 platform (Illumina, USA). Lima v1.7.0, Cutadapt 1.9.1, and UCHIME v4.2 software were used to preprocess the original data. When the sequence similarity was not less than 97%, the sequence was classified as an operational taxonomic unit (OTU) using Usearch software (version 10.0). Representative bacterial and fungal OTU sequences were taxonomically classified using a BLAST alignment against the SSU rRNA database of SILVA138 (http://www.arb-silva.de/) and the Unit (v8.2) fungal ITS database (http://unite.ut.ee/), respectively[32,33]. The OTUs were annotated and subjected to bioinformatic analysis using QIIME2 2020.6, KRONA v2.6, and mothur1.34.4 classifier software.

Sequencing data analysis

QIIME2 software (version 2020.6) was employed to compute the amplicon sequence variants (ASVs) and the Shannon, Simpson, and ACE indices, while also generating a rarefaction curve. QIIME2 software was used to calculate the UniFrac distance, and non-metric multidimensional scaling (NMDS) dimension reduction graphs were plotted using the ade4 and ggplot2 packages in R software (version 4.3.2). Subsequently, the ANOSIM function in the QIIME2 software was used to analyze the significant differences between the community compositions of the groups. The BMKCloud platform (www.biocloud.net) was used to draw LEfSe chart used in this study.

Statistical analysis

One-way ANOVA was used to test the differences in soil chemical properties, microbial diversity indices, and relative abundances of the most abundant microorganisms at different sampling sites and an independent samples T-test of variance was used to analyze the differences between different growing seasons. Pearson's correlation coefficients were calculated to analyze the relationships between microbial diversity indices, most abundant microorganisms, and soil chemical properties. The soil chemical properties mentioned above were recorded using Excel (version 2019) and analyzed with SPSS 23.0 (SPSS Inc., Chicago, IL, USA) and Sigmaplot 12.5, Origin 2021 software package and statistical significance was considered when $P < 0.05$.

Random forest analysis was performed, including different soil characteristics as predictors of the different microorganisms and availability as response variables, these analyses were performed using the random forest package for R statistical software (version 4.3.2). Mantel test was used to analyze the interaction between the content of soil chemical properties and microbial community distribution and plant growth using Lianchuan online tools (https://www.omicstudio.cn), the screening threshold was defined as a correlation coefficient greater than 0.5, with a significance of $P < 0.05$. Plant growth data were obtained from Li et al. (2023)[34].

Results

Soil nutrient contents at different sampling sites and different growing seasons

The total nitrogen content of soil from highway construction sites was significantly higher than that of abandoned black and yellow soils ($P < 0.05$). The total nitrogen content of the black soil was significantly higher than that of the yellow soil at section 8 in June, and the total nitrogen content of the black soil was significantly higher than that of the yellow soil at both sections in September ($P < 0.05$). A significant increase in total nitrogen content was observed 500 m away from the construction red line at Sects. 8 and 11 compared to 10 m away from the construction red line in September ($P < 0.05$). Total nitrogen content of soil from highway construction sites in September was significantly higher than in June ($P < 0.05$) (Fig. 1A). The total phosphorus content of soil from highway construction sites was significantly higher than that of the black and yellow soils in September, except

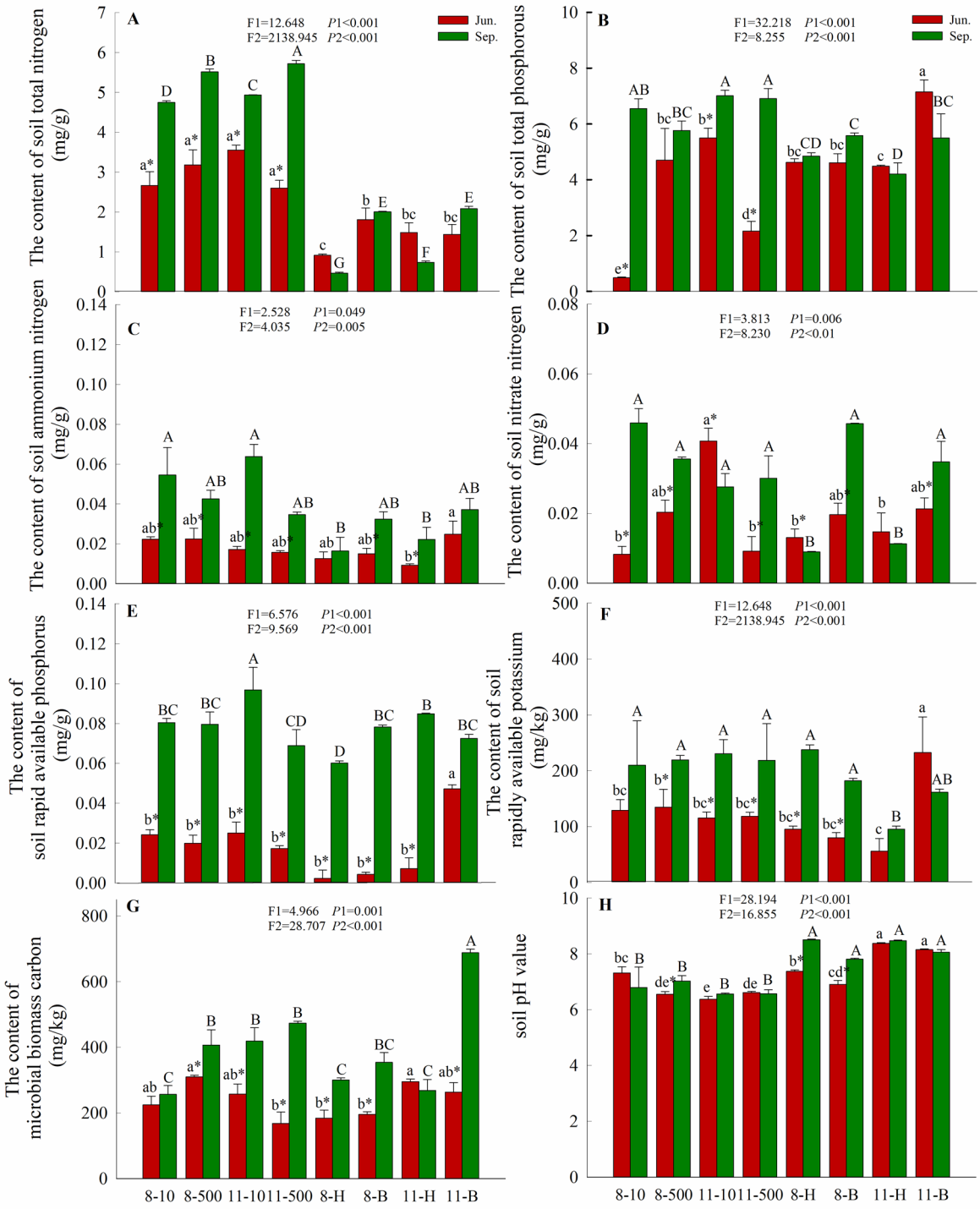

Fig. 1. The contents of soil total nitrogen (A), soil total phosphorous (B), soil ammonium nitrogen (C), soil nitrate nitrogen (D), soil rapidly available phosphorus (E), soil rapidly available potassium (F), soil microbial biomass carbon (G) and soil pH value (H) at different sampling sites and different growing seasons. 8–10, 8-500, 11 – 10, 11–500 showed that 10 m and 500 m away from the construction red line on the highway of 8 section and 11 section, respectively; 8-H, 8-B, 11-H, 11-B showed that the yellow soil in the deep abandoned soil and the black soil in the shallow abandoned soil of the highway of 8 section and 11 section, respectively. Different lowercase (a, b, c) letters indicated significant differences between different treatments in June ($P < 0.05$, $n = 4$). Different uppercase (A, B, C) letters indicated significant differences between different treatments in September ($P < 0.05$, $n = 3$). "*" stand for significant differences between June and September at the same plot. The same below.

for the soil 500 m away from the construction red line at section 8 ($P<0.05$). The total phosphorus content of the black soil was significantly higher than that of the yellow soil at section 11 ($P<0.05$). A significant increase in total phosphorus content was observed 500 m away from the construction red line at section 8 compared to 10 m away from the construction red line in June ($P<0.05$). Total phosphorus content of soil from highway construction sites in September was significantly higher than in June, except for the soil 500 m away from the construction red line at section 8 ($P<0.05$) (Fig. 1B). Ammonium nitrogen and nitrate nitrogen contents of soil in September were significantly higher than in June at most sampling sites ($P<0.05$) (Fig. 1C, D). Soil rapidly available phosphorus content at 10 m away from the red construction line at section 11 was significantly higher than that of the black and yellow soils ($P<0.05$). The rapidly available phosphorus content of the black soil was significantly higher than that of the yellow soil at section 8 in September, and the rapidly available phosphorus content of the black soil was significantly higher than that of the yellow soil at section 11 in June ($P<0.05$). Soil rapidly available phosphorus content in September was significantly higher than in June, except for the yellow soil at section 11 ($P<0.05$) (Fig. 1E). Rapidly available potassium content of soil from highway construction sites was significantly higher than that of the yellow soil at section 11 in September ($P<0.05$). The rapidly available potassium content of the black soil was significantly higher than that of the yellow soil at section 11 in June ($P<0.05$). Soil rapidly available potassium content in September was significantly higher than in June at most sampling sites ($P<0.05$) (Fig. 1F). Microbial biomass carbon content of soil from highway construction sites was significantly higher than that of the yellow soil at both sections in September, except for the soil 10 m away from the red construction line at section 8 ($P<0.05$). Microbial biomass carbon content of the black soil was significantly higher than that of the yellow soil at section 11 in September ($P<0.05$). A significant increase in soil microbial biomass carbon content was observed 500 m away from the construction red line at section 8 compared to 10 m away from the construction red line in September ($P<0.05$). Soil microbial biomass carbon content in September was significantly higher than in June at most sampling sites ($P<0.05$) (Fig. 1G). The soil pH value from the highway construction site was significantly lower than that of the black and yellow soils in September ($P<0.05$). In addition, the soil pH value in September was significantly higher than in June at 500 m away from the construction red line, as well as in the black and yellow soils in section 8 (Fig. 1H).

Soil enzyme activity at different sampling sites and different growing seasons

As shown in Fig. 2, Urease activity of soil from highway construction sites was significantly higher than that of the black and yellow soils in September ($P<0.05$). The soil urease activity of black soil was significantly higher than that of the yellow soil at section 8 in June ($P<0.05$) (Fig. 2A). Sucrase activity of soil from highway construction sites was significantly higher than that of the black and yellow soils at Sects. 8 and 11 in the two growing seasons ($P<0.05$) (Fig. 2B). Soil catalase activity of black soil was significantly higher than that of yellow soil at Sects. 8 and 11 in September ($P<0.05$). Soil catalase activity was significantly higher in September than in June ($P<0.05$) (Fig. 2C).

Soil microbial diversity and composition at different sampling sites and different growing seasons

Microbial diversity analysis

The rarefaction curves (Fig. S1) and coverage estimates (Table S1) suggest that the current sequencing depth is sufficient to accurately represent the microbial composition of the samples and to uncover the diversity of the microbial communities.

Based on the OTUs analysis (Fig. S2), We further conducted microbial diversity analysis (Fig. 3). Bacterial Shannon and Simpson indices of soil from highway construction sites were significantly higher than those of the yellow soil at section 8 in September ($P<0.05$). The bacterial Shannon index of the black soil was significantly higher than that of the yellow soil at section 11 in June, and the bacterial Shannon index of the black soil was significantly higher than that of the yellow soil at both sections in September ($P<0.05$). The bacterial Simpson index of the black soil was significantly higher than that of the yellow soil at section 11 in September. Bacterial Shannon and Simpson indices of yellow soil at section 11 in September were significantly higher than in June ($P<0.05$) (Fig. 3A, B). Bacterial ACE index of soil from highway construction sites was significantly higher than that of yellow soil at both sections in June ($P<0.05$). Bacterial ACE index of black soil at section 11 in

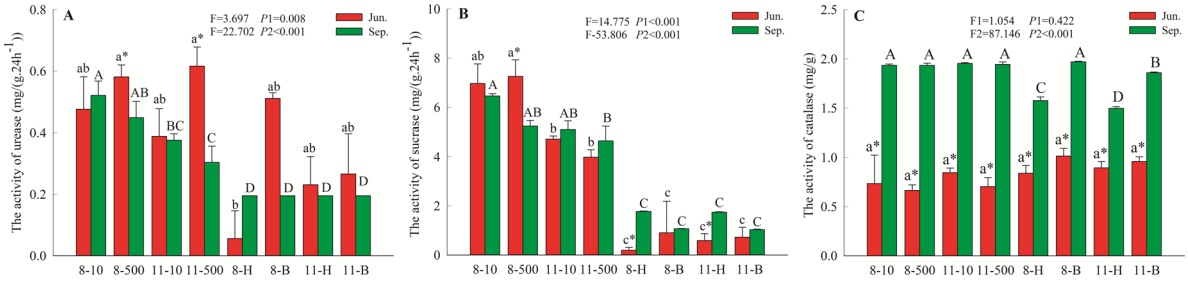

Fig. 2. The activities of soil urease (A), sucrase (B) and catalase (C) at different sampling sites and different growing seasons.

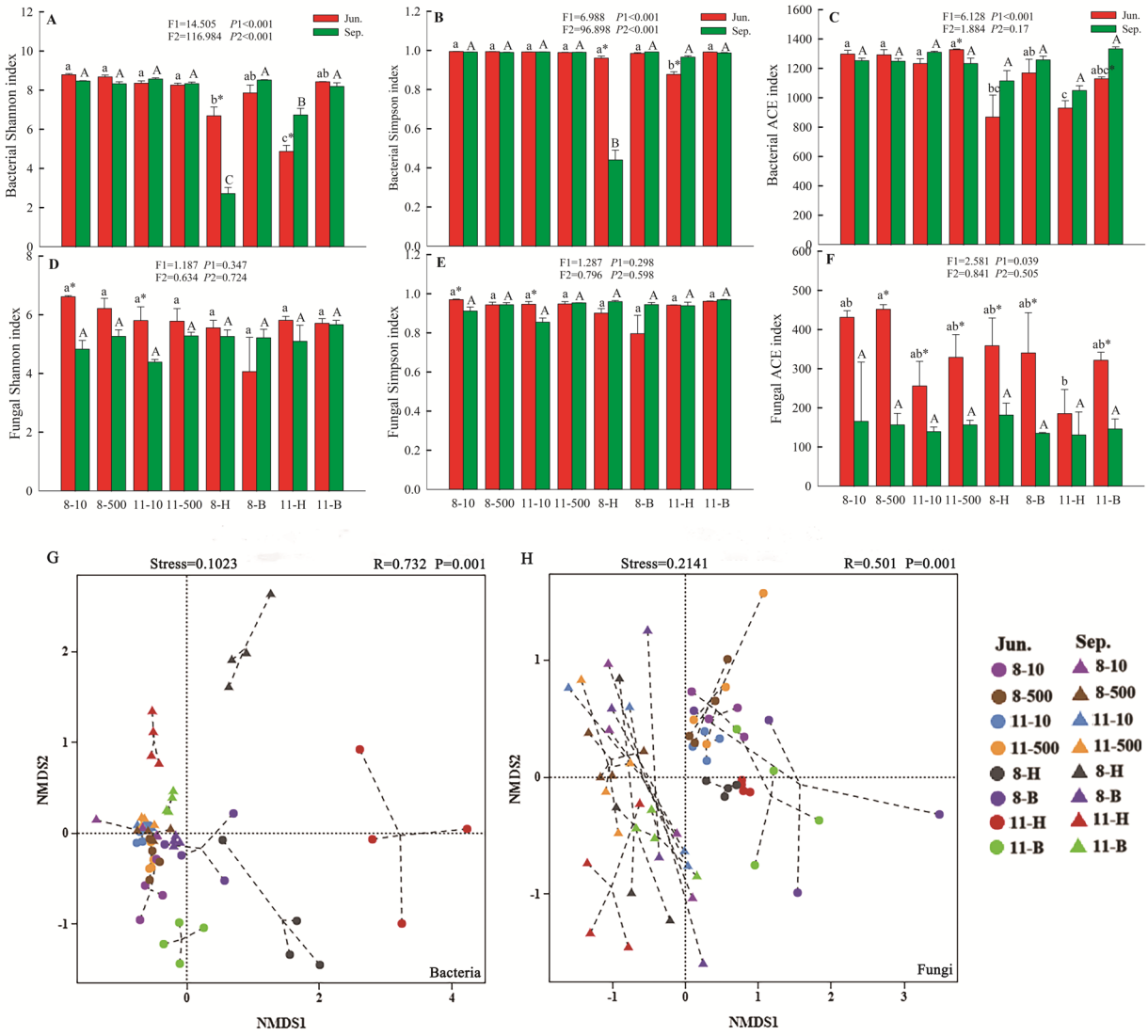

Fig. 3. Soil bacterial (alpha, A, B, C; beta, G) and fungal (alpha, D, E, F; beta, H) diversity at different sampling sites and different growing seasons. ACE, Abundance-based coverage estimator; NMDS, Non-metric multidimensional scaling. The Shannon, Simpson and ACE, index is used to estimate the microbial diversity in the sample. The larger the Shannon and Simpson value, the higher the community diversity. Different colors represent varying sampling sites and different shapes represent different times. R-value is the ANOSIM statistic R, and P-value is the significance from permutation.

September was significantly higher than in June ($P<0.05$) (Fig. 3C). The fungal Shannon index of soil from highway construction sites in September was significantly lower than in June, except for the soil 500 m away from the red construction line at both sections ($P<0.05$) (Fig. 3D). The fungal Shannon index of soil at the 10 m away from the red construction line at both sections in September was significantly lower than in June (Fig. 3E). Soil fungal ACE index in September was significantly higher than in June, except for the yellow soil at the section 11 (Fig. 3F) ($P<0.05$).

The NMDS had very low stress, indicating that the two axes of our NMDS were a good representation of the entire soil microbial community in our survey. The differences in soil microbial community composition between the different sampling sites and growing seasons were significant (Fig. 3G) ($P<0.05$), especially in the fungal communities, the points were significantly separated in the different growing seasons (Fig. 3H).

Microbial community structure analysis

As shown in Fig. 4, the microbial community structure and relative abundances of bacteria and fungi were significantly different at different sampling sites and during different growing seasons. In total, 602 bacterial species belonging to 26 phyla and 424 fungal species belonging to 14 phyla were identified. Of these, bacterial species increased the most from June to September, with a 24.9% increase. (Fig. 4A, B).

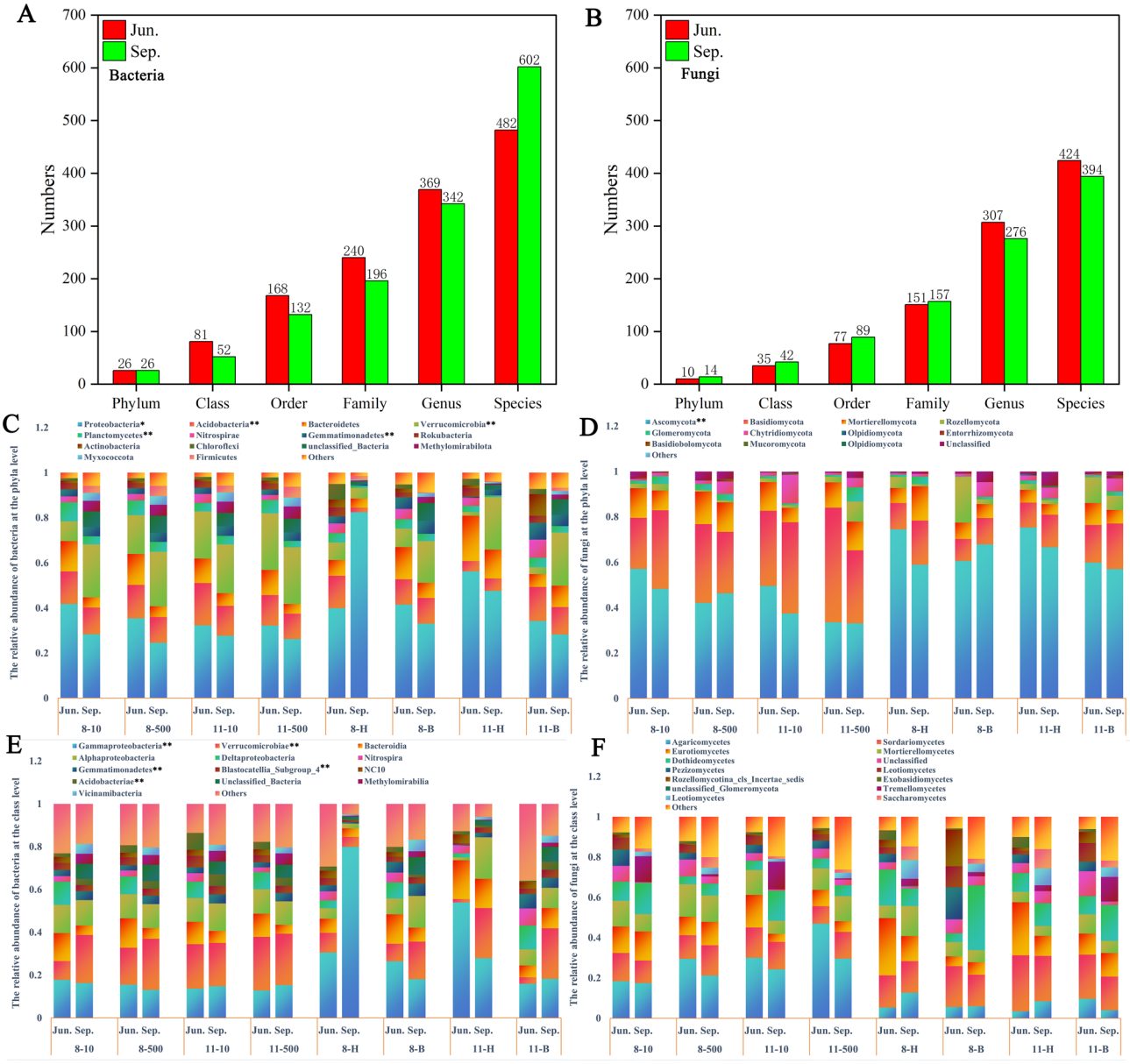

Fig. 4. Bacterial community structure (A), fungal community structure (B) and the relative abundance of the top 10 phyla of bacteria (C) and fungi (D) and the relative abundance of the top 10 class of bacteria (E) and fungi (F). "*", "**" indicate significant differences at the confidence levels of 0.05 and 0.01, respectively. "ns" means no significant difference.

At the phylum level, the relative abundances of Proteobacteria, Acidobacteria, Gemmatimonadetes, and Verrucomicrobia in bacterial communities showed significant variation across various sampling sites and growing seasons (Fig. 4C). Only the relative abundance of Nitrospirae was significantly different at different sampling sites in June, and the relative abundances of Myxococcota and Methylomirabilota were significantly different at different sampling sites in September (Fig. 4C). For fungal communities, only the relative abundance of Ascomycota was significantly different at different sampling sites and growing seasons at the phylum level (Fig. 4D); the relative abundances of Olpidiomycota, Chytridiomycota, and Basidiomycota were all significantly different at different sampling sites in June. Only the relative abundance of Rozellomycota was significantly different at different sampling sites in September. At the class level, for bacterial communities, the relative abundances of Gammaproteobacteria, Gemmatimonadetes, Acidobacteria, Verrucomicrobia, and Blastocatellia_Subgroup_4 were all significantly different at different sampling sites and in different growing seasons (Fig. 4E); the relative abundances of *Nitrospira*, Deltaproteobacteria, and Alphaproteobacteria were all significantly different in June; and the relative abundances of Vicinamibacteria, Methylomirabilia, Bacteroidia, and Alphaproteobacteria were all significantly different in September. For the fungal communities, there were no microorganisms that were significantly different during the different growing seasons at the class level (Fig. 4F).

However, the relative abundances of Exobasidiomycetes, Dothideomycetes, Eurotiomycetes, Sordariomycetes, and Agaricomycetes differed significantly in June (Fig. 4F).

LEfSe analysis

The linear discriminant analysis (LDA) effect size (LEfSe) method was used to detect microbial groups causing significant differences based on sampling points and sampling times (Fig. 5, Fig. S4). There are 105 bacterial biomarkers exhibited statistically significant differences with an LDA threshold of 4.0 at different sampling points, including 25 bacterial genus (Fig. 5A). Specifically, *Limnobacter* was enriched in yellow soil at section 8 in September; *Uncultured-Vicinamibacterales* was enriched in black soil at section 8 in September; *Uncultured-SC-I-84* was enriched in 10 m away from the construction red line at section 8 in September; *Candidatus-Udaeobacter*, *Sphingomonas*, *Flavisolibacter*, *Caenimonas*, *Lysobacter* and *Noviherbaspirillum* were enriched in yellow soil at section 11 in September; *P3OB-42* was enriched in 500 m away from the construction red line at section 11 in September; *Glaciimonas*, *Terrimonas*, Uncultured-*Chloroflexi-bacterium* were enriched in yellow soil at section 8 in June; *Massilia* was enriched in black soil at section 8 in June; unclassified-*Chitinophagales* was enriched in 500 m away from the construction red line at section 8 in June; *Polaromonas*, *Pseudomonas*, *Flavobacterium* and *Unclassified-TRA3-20* were enriched in yellow soil at section 11 in June; *Bifidobacterium*, *unclassified-Gemmatimonadaceae*, *Nitrospira* and *NMD1* were enriched in black soil at section 11 in June; *RB41* and unclassified-*Acidobacteriales* were enriched in 10 m away from the construction red line at section 11 in June.

There are 86 fungal biomarkers exhibited statistically significant differences with an LDA threshold of 4.0 at different sampling points, including 28 fungal genus (Fig. 5B). Specifically, *Geotrichum* was enriched in yellow soil at section 8 in September; *Cladosporium* was enriched in black soil at section 8 in September; *Coprinopsis* was enriched in 500 m away from the construction red line at section 8 in September; *Fusicolla* and *Ascobolus* were enriched in yellow soil at section 11 in September; *Monascus* and *Solicoccozyma* were enriched in black soil at section 11 in September; *Unclassified-Agaricales* and *Diversispora* were enriched in 500 m away from the construction red line at section 11 in September; *Achaetomium* was enriched in 10 m away from the construction red line at section 11 in September; *Aspergillus* and *Setophoma* were enriched in yellow soil at section 8 in June; *Trichoderma* and *Unclassified-Leotiomycetes* were enriched in black soil at section 8 in June; *Hygrocybe* was enriched in 500 m away from the construction red line at section 8 in June; *Archaeorhizomyces* was enriched in 10 m away from the construction red line at section 8 in June; *Fusarium*, *Acaromyces*, *Zygosaccharomyces* and *Tricharina* were enriched in yellow soil at section 11 in June; *Mycena*, *Arachnopeziza*, *Chaetosphaeria* and *Ochroconis* were enriched in black soil at section 11 in June; *Inocybe*, *Clavulinopsis*, *Clavaria* and *Sebacina* were enriched in 500 m away from the construction red line at section 11 in June.

In addition, there was 6 and 7 bacterial biomarkers for June and September, respectively, including 3 bacterial genus; Specifically, *Candidatus-Udaeobacter* and *Sphingomonas* were enriched in September (Fig. S5A, C). There were 6 and 6 fungal biomarkers for June and September, respectively, including 4 fungal genus; Specifically, *Acaromyces* and *Aspergillus* were enriched in June, *Achaetomium* and *Monascus* were enriched in September (Fig. S5B, D).

Random forest analysis

Based on the above LEfSe analysis. Some microbial biomarkers are simultaneously present in different sampling points and sampling times. Furthermore, random forest analysis was performed on these microorganisms. Random forest analysis indicated that soil sucrase activity, soil rapidly available phosphorus content, and soil total phosphorus were the strongest predictors of the abundance of *Nitrospira* (Fig. 6A), while soil sucrase activity and pH value were the strongest predictors of the abundance of *Terrimonas* (Fig. 6A). The abundances of *Candidatus-Udaeobacter* and *Sphingomonas* were significantly predicted by soil total nitrogen and soil nitrate-nitrogen contents (Fig. 6B). Soil ammonium nitrogen content was the strongest predictor of the abundance of *Acaromyces* and *Aspergillus* (Fig. 6C). The abundance of *Achaetomium* was significantly predicted by the soil sucrase activity (Fig. 6D). These indicated that, especially, soil nitrogen content and soil sucrase activity make a relatively large contribution to biomarkers.

The relationship among soil chemical properties, microbial community and plant growth

Spearman correlation analysis and Mantel test to study the relationships among soil chemical properties, microbial community and plant growth (Tables 1 and 2; Fig. 7). The results should that Shannon, Simpson, and ACE indices of bacterial community were significantly negatively correlated with soil pH ($P < 0.05$). Except for soil pH, The Shannon and Simpson indices of the bacterial community were significantly positively correlated with 6 of the 11 tested indices in June and 8 of the 11 tested indices in September shown in Figs. 1 and 2 ($P < 0.05$), which suggested the interaction between soil properties and bacterial α-diversity. However, fungal α-diversity indices significantly correlated with only a few tested soil properties, which suggested that soil properties had less impact on fungal α-diversity (Table 1).

The relative abundances of the most abundant phyla varied significantly between the sampling sites and growing seasons (Fig. S3). As shown in Table 2. The most abundant bacteria had a significant correlation with only a few tested soil chemical properties in June. However, the relative abundance of Acidobacteria was significantly positively correlated with 8 of the 11 tested indices, and the relative abundance of Proteobacteria was significantly negatively correlated with 8 of the 11 tested indices in September shown in Figs. 1 and 2, which suggested the interaction between soil properties and bacterial community compositions in September ($P < 0.05$). The relative abundant of Ascomycota was significantly negatively correlated with the 5 of 11 tested indices in June and 6 of 11 tested indices in September shown in Figs. 1 and 2 ($P < 0.05$). The relative abundant of Basidiomycota was significant correction with the 6 of 11 tested indices in June and 5 of 11 tested indices in September shown in

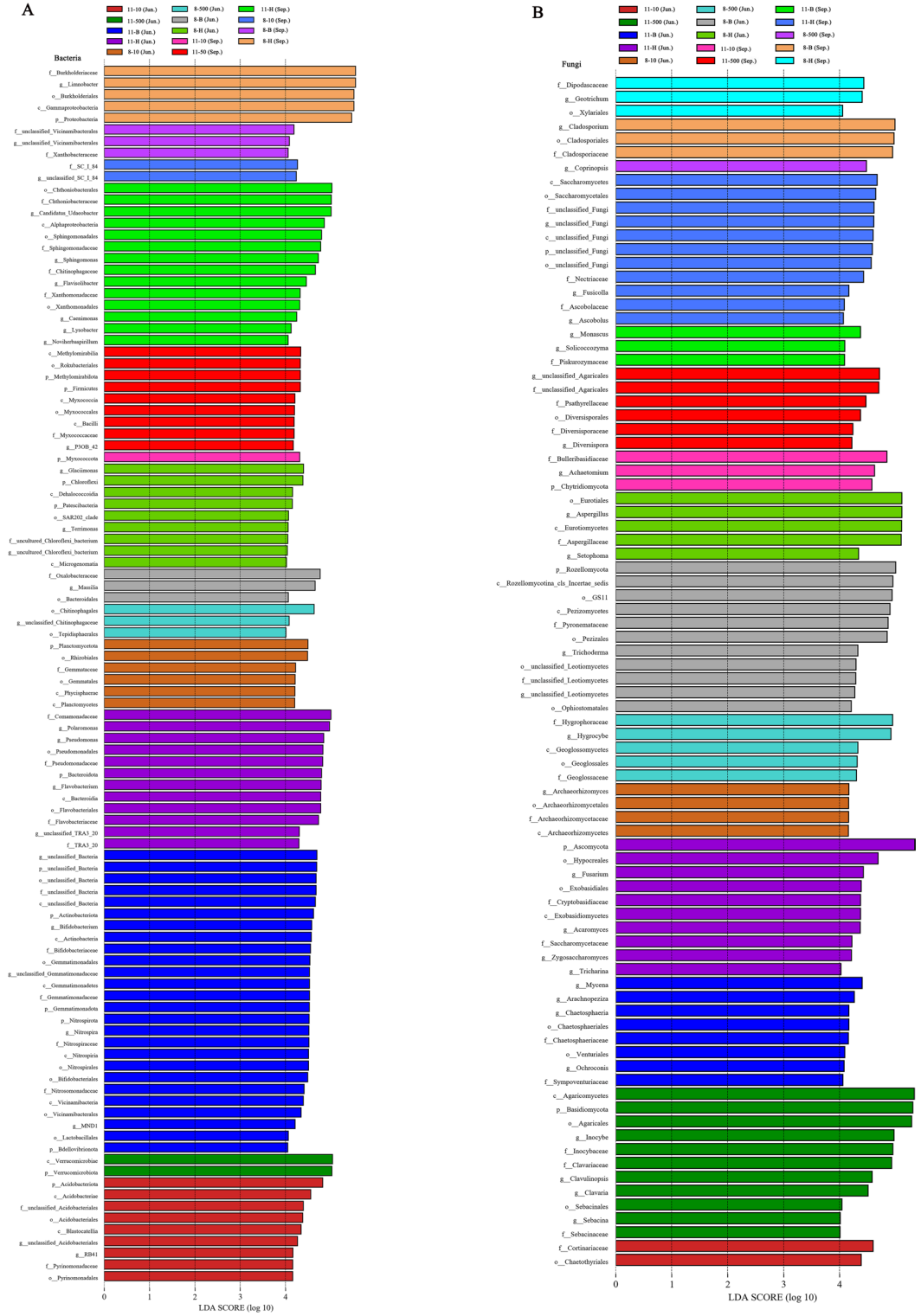

Fig. 5. Taxa identified by linear discriminant effect size (LEfSe) analysis that explains the differences of bacterial (A) and fungal (B) at different sampling points. The threshold on the logarithmic LDA score for discriminative features was set to 4 ($P < 0.05$).

Figs. 1 and 2 ($P < 0.05$). However, the relative abundant of Mortierellomycota had significantly correlated with only very few tested soil chemical properties. These results indicated that there are strong correlations between the microbial diversity indices, the relative abundant of most abundant microorganism and soil chemical properties.

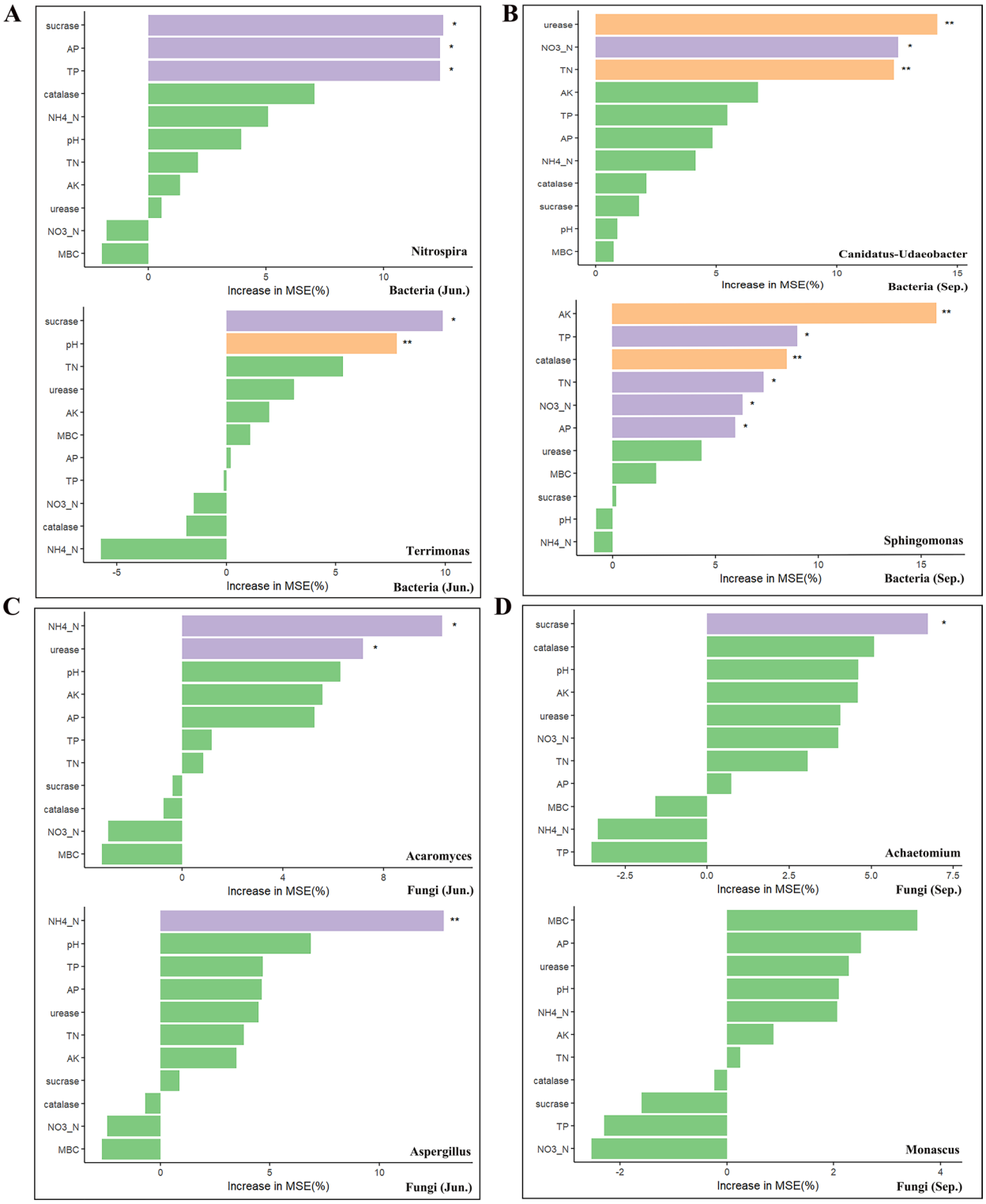

Fig. 6. Random forest analysis indicating the effects of soil chemical properties on the relative abundances of *Nitrospira* and *Terrimonas* (A), *Candidatus-Udaeobacter* and *Sphingomonas* (B), *Acaromyces* and *Aspergillus* (C) and *Achaetomium* and *Monascus* (D). MBC, soil microbial biomass carbon; TN, soil total nitrogen; TP, soil total phosphorus; NH4+-N, soil ammonium nitrogen; NO3–N, soil nitrate nitrogen; AP, soil rapidly available phosphorus; AK, soil rapidly available potassium. Different bar colors denote the direction of effects and asterisks above the bars indicate the significance levels (*, $P<0.05$; **, $P<0.01$).

Soil chemical properties	Bacterial α-diversity indices						Fungal α-diversity indices					
	Shannon index		Simpson index		ACE index		Shannon index		Simpson index		ACE index	
	June	September	June	September	June	September	June	September	June	September	June	September
Microbial biomass carbon	-0.018	**0.394***	-0.068	0.238	-0.091	**0.476****	0.112	0.015	0.054	0.028	-0.266	-0.161
Total nitrogen	**0.537****	**0.537****	**0.538****	**0.521****	**0.657****	0.273	0.147	-0.12	0.130	-0.217	0.005	-0.028
Total phosphorus	-0.032	**0.499****	-0.043	**0.580****	-0.28	0.239	-0.205	-0.234	-0.151	-0.253	-0.181	0.072
Ammonium nitrogen	**0.489****	**0.694****	**0.479****	**0.602****	0.243	**0.507****	0.054	0.001	0.196	-0.052	0.226	0.017
Nitrate nitrogen	0.139	**0.446***	0.098	**0.448***	0.032	0.413*	0.011	-0.115	-0.058	-0.205	0.123	-0.072
Rapidly available phosphorus	**0.360***	**0.488****	**0.373***	**0.442***	0.174	0.017	0.222	-0.112	**0.416***	-0.206	0.060	-0.079
Rapidly available potassium	**0.565****	0.257	**0.538****	0.162	0.372*	0.193	0.224	-0.234	**0.352***	-0.273	0.322	**0.412***
pH value	**-0.378***	**-0.659****	**-0.384***	**-0.581****	**-0.563****	**-0.428***	-0.113	0.274	-0.07	**0.375***	-0.281	0.111
Urease activity	**0.372***	**0.452****	0.308	**0.491****	**0.526****	0.141	0.168	-0.055	0.069	-0.165	0.269	0.186
Sucrase activity	**0.645****	0.173	**0.624****	0.198	**0.730****	-0.068	**0.387***	-0.221	0.346	-0.309	0.268	0.214
Catalase activity	-0.321	**0.683****	-0.331	**0.648****	**-0.368***	**0.431***	**-0.376***	-0.211	-0.3	-0.296	-0.336	-0.148

Table 1. Spearman correlations analysis between the contents of soil chemical properties and the microbial α-diversity indices. The value stand for correlation coefficient, "*", "**" indicate significant differences at the confidence levels of 0.05 and 0.01, respectively. The same below.

Soil chemical properties	Bacterial community compositions						Fungal community compositions					
	Proteobacteria		Acidobacteria		Bacteroidetes		Ascomycota		Basidiomycota		Mortierellomycota	
	June	September	June	September	June	September	June	September	June	September	June	September
Microbial biomass carbon	-0.038	**-0.489****	0.015	**0.424***	-0.111	0.049	0.009	-0.276	-0.049	0.15	0.115	-0.027
Total nitrogen	-0.346	**-0.770****	0.332	**0.583****	0.312	-0.314	**-0.585****	**-0.658****	**0.657****	**0.428***	**0.385***	0.267
Total phosphorus	-0.146	**-0.525****	0.345	**0.569****	**-0.396***	**-0.409***	0.084	**-0.615****	-0.283	**0.399***	-0.03	0.101
Ammonium nitrogen	-0.292	**-0.468****	**0356***	**0.408***	0.008	-0.235	**-0.399***	-0.251	0.215	0.220	0.298	0.287
Nitrate nitrogen	-0.339	**-0.483****	0.266	**0.354***	0.014	-0.051	-0.008	-0.124	0.031	0.094	0.213	-0.001
Rapidly available phosphorus	-0.213	-0.145	0.318	**0.354***	-0.11	**0.373***	-0.301	-0.061	**0.417***	0.048	0.188	-0.080
Rapidly available potassium	-0.24	-0.293	**0.383***	0.139	-0.016	**-0.440***	**-0.387***	**-0.421***	**0.450****	0.268	0.231	**0.434***
pH value	**0.423***	**0.741****	**-0.423***	**-0.057****	-0.335	0.208	**0.504****	**0.554****	**-0.565****	**-0.396***	**-0.364***	-0.284
Urease activity	-0.151	**-0.710****	-0.103	**0.517****	**0.400***	-0.253	**-0.486****	**-0.558****	0.33	**0.467****	0.275	0.178
Sucrase activity;	-0.234	**-0.474****	0.255	0.169	**0.360***	**-0.458****	**-0.511****	**-0.488****	**0.566****	**0.507****	**0.405***	0.183
Catalase activity	-0.042	**-0.502****	0.044	**0.593****	-0.053	-0.286	0.232	**-0.356***	**-0.457****	0.114	-0.073	0.118

Table 2. Spearman correlations analysis between the contents of soil chemical properties and the relative abundant of the most abundant bacterial and fungal phyla.

Soil total nitrogen content may be related to plant total fresh biomass, soil ammonium nitrogen content was significantly related to the plant Shannon index, pH value was significantly related to the plant evenness index in June (Fig. 7A), and the above factors were significantly correlated with the bacterial community in June (Fig. 7C). Soil total nitrogen content was significantly related to plant total fresh biomass, soil microbial biomass carbon was significantly related to the plant Shannon index in September (Fig. 7B). The above factors were also significantly correlated with bacterial and fungal communities in September (Fig. 7D). In addition, the soil total phosphorus, soil nitrate nitrogen, soil rapidly available phosphorus, soil pH, and soil catalase activity were most strongly correlated with the bacterial community and the soil ammonium nitrogen, soil nitrate nitrogen, soil rapidly available phosphorus, soil pH, and soil sucrase activity were most strongly correlated with the fungal community in September (Fig. 7D). These results indicated that there is a close relationship among soil chemical properties and bacterial communities, as well as plant growth.

Discussion

To ensure that highway construction is compatible with natural resources and the environment, it is necessary to evaluate the impact of highway construction on the ecological environment. Our results found that the contents of total nitrogen and microbial biomass carbon decreased significantly at 10 m away from the construction red line compared to 500 m away from the construction red line in September. This indicated that highway construction has significantly reduced total nitrogen content in September. This is consistent with previous research[35]. Previous study found microbial biomass carbon play a significant role in enhancing soil aggregation, and promoting C and N turnover and thus nutrient cycling[36]. Therefore, highway construction may directly lead to the utilization and absorption of plant nutrients. The distance from the highway construction had no significant effect on the other chemical properties in the two growing seasons, except for the two indices mentioned above. The

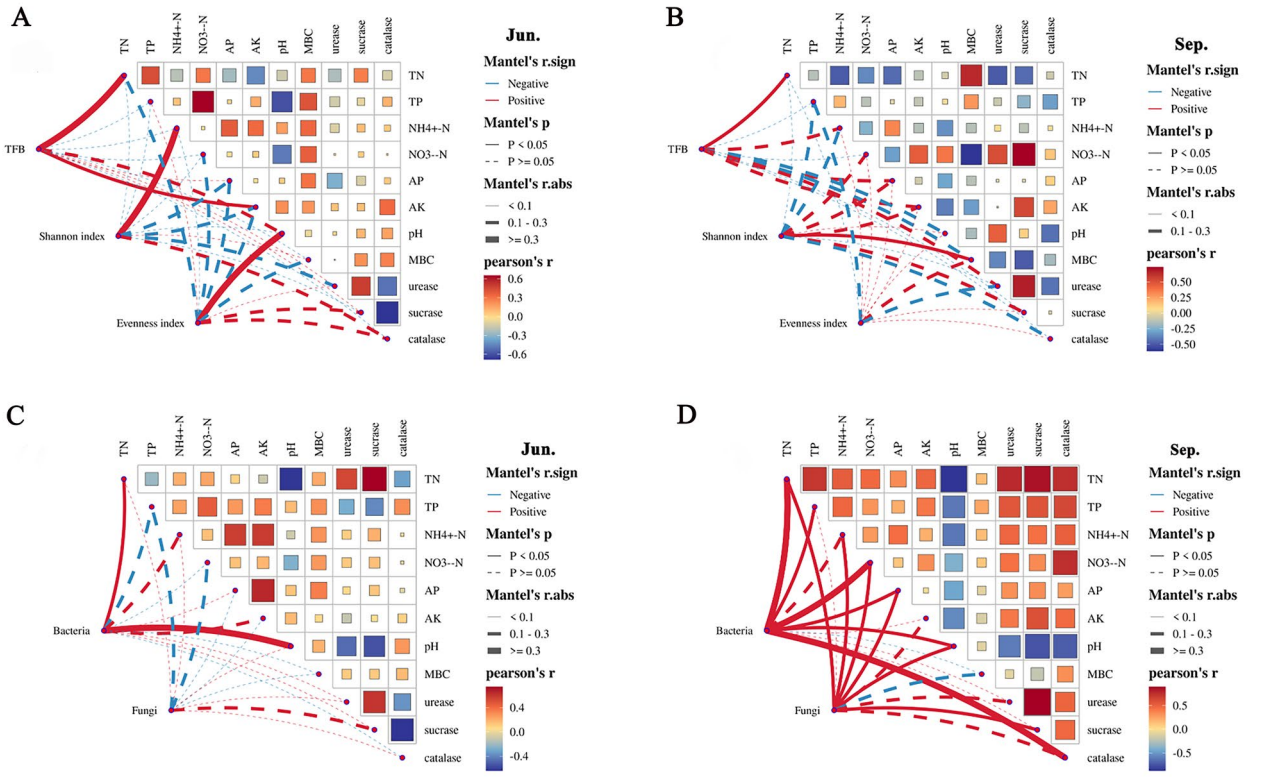

Fig. 7. The interaction between plant growth, microbial community and soil chemistry properties at different growing seasons. (A) plant growth and soil chemistry properties in June; (B) plant growth and soil chemistry properties in September; (C) microbial community and soil chemistry properties in June; (D) microbial community and soil chemistry properties in September. TFB, total fresh biomass; MBC, soil microbial biomass carbon; TN, soil total nitrogen; TP, soil total phosphorus; NH_4^+-N, soil ammonium nitrogen; NO_3^--N, soil nitrate nitrogen; AP, soil rapidly available phosphorus; AK, soil rapidly available potassium.

reason may be that nutrient cycling is a long-term process of accumulation, and short-term disturbances cannot significantly alter soil nutrient conditions, this is also consistent with the study of Maynard (2014), they through meta-analysis comparing soil nutrient concentrations between harvested and unharvested (control) areas in the boreal forest of Canada (one study from Alaska) found that the overall changes to soil nutrient concentrations and content were minimal in the short- to medium-term (< 20 years)[37]. On the other hand, ecosystems possess self-repair capabilities, maintaining nutrient balance through intrinsic recovery mechanisms within a certain range of damage[38]. It is worth noting that the values of most soil chemical parameters were significantly higher in September than that in June in this study. This indicated that the seasonal changes were the main factors effected the soil nutrients. This also indicates that the plants in this region possess a certain capacity for self-repair. This is consistent with the previous study[39]. There are significant differences in temperature and precipitation in different seasons, particularly in the Qinghai-Tibet Plateau region[27]. This may also be the reason for the seasonal variations in changes of soil chemical properties. The above discussion suggests that the impact of highway construction distance is relatively small compared to seasonal variations. However, highway construction also significantly affected total nitrogen content and microbial biomass content in September. Our team through field surveys discovered that plants grow vigorously in the Gannan in September. Therefore, it is recommended to minimize construction during the peak growing season of plants.

Seasonal variations affect soil biological properties[40]. Soil microbial communities are crucial in grassland ecosystems as they regulate the processes of organic matter decomposition and nutrient availability to plants. However, there is no consistent pattern of seasonal dynamics in the soil chemical properties or soil microbial diversity and composition[31]. In this study, the two sampling periods were plant reproductive and vegetative growth. We originally speculated that soil chemical properties would vary with the change in seasons because climate and plant inputs varied greatly with the seasons in previous study[31]. It is not surprising that our results showed significant variations in the soil chemical properties between the two growing seasons. Given the large seasonal variations in soil chemical properties, we further inferred that soil microbial communities varied significantly with the seasons. The change of soil microbial communities are consistent with our speculation. The soil microbial communities were significantly different during the different growing seasons, especially the soil fungal communities, according to NMDS analysis. These results are broadly in line with those of previous studies conducted in different grassland ecosystems[41–43]. This study also demonstrated that there

was a significant increase in some bacterial diversity from June to September. On the one hand, this may be because plant roots gradually expand as the plant grows; therefore, communication with the soil environment becomes stronger. During plant growth, a large number of root metabolites are produced, and root metabolite exudation is an important mediator of plant interactions with soil microbes[44]. On the other hand, the field survey found plant biomass is most luxuriant in Gannan during September, which is the reproductive growing season for the plants. Most previous study found that microbial community abundance and diversity increased with greater plant diversity and biomass[45,46]. In addition, good soil quality is more conducive to the structure and activity of microorganisms[47]. Most of the soil nutrient contents are significantly higher in September than that in June in this study, this indicated that the soil nutrients were better in September compared with June. This may also be a reason for the increase in some bacterial diversity indices in September. Temperature is an important factor affecting species diversity. Sharp et al. (2014) found that species richness and diversity indices were strongly correlated to temperature, with peak diversity at 24 °C and the microbial richness decreased with decreasing temperature[48]. Surprisingly. Gannan, located in the Qinghai-Tibetan Plateau, the temperature is relatively low about 0–10℃ in September[27]. Therefore, we inferred that the significant decrease in the fungal ACE index in September is primarily due to the decreasing temperature. The NMDS analysis also found that the fungal community was affected by the seasonal changes in this study, this further confirms our hypothesis that temperature may be the main factor responsible for the significantly decreased in the fungal ACE index.

To gain a deeper understanding of the role of specific microorganisms, Lefse analysis screened the biomarkers at different sampling points and different growing seasons. *Candidatus-Udaeobacter*, *Sphingomonas*, *Achaetomium*, and *Monascus* et al. were found to be the biomarkers. The genus *Sphingomonas* is not only an important regulator of *Arabidopsis thaliana* leaf microbiota[49] but also the most characteristic microorganism in the rice seed disease resistance phenotype[50]. Members from this genus play the role of "extending the immune system" in the "disease triangle" and can be passed from generation to generation in the microbiome of healthy plant seeds. A previous study revealed that *Achaetomium* produces a secondary metabolite with a high content of phenolics, including flavonoids and tannins, which contribute to its significant biological potential, exhibiting remarkable antibacterial, antioxidant, and hepatoprotective potential, and can serve as a substantially sustainable resource for novel secondary metabolites[51]. The genus *Monascus* can be characterized as aerobic, saprophytic, prototrophic, mesophilic (temperature optimum 30–35 °C), weakly xerophilous (growth up to 0.85 water activity), with respiro-fermentative metabolism. It can produce lytic enzymes that enable its growth on a spectrum of substrates, including monosaccharides, disaccharides, starch, pectin, and in the case of *M. ruber*, cellulose and ethanol[52]. These microorganisms may ultimately influence plant growth and development by regulating metabolic pathways. However, their role in plant growth-promotion is unknown. As a result, it is crucial to undertake further studies to uncover the specific mechanisms by which rhizosphere microbiomes modify plant growth and to experimentally assess the positive impacts of these genera on plant development. This can be achieved by taking multiple samples in a way that does not damage the plant. Genetic characteristics and transcriptomic information of plants can also be studied in depth by combining DNA and RNA biomarkers. In addition, modern techniques, such as high-throughput sequencing technologies and stable isotope analysis, allow us to study the physiological characteristics of plants and their ecological adaptations more comprehensively[53–55]. This will ultimately contribute to the advancement of plant science. This study further evaluated the contribution of soil chemical properties to these important microorganisms using random forests and found that the total nitrogen content contributed the most to the effects of these important microorganisms. We also found that the total soil nitrogen content, whether in September or June, had a significant positive correlation with fresh plant biomass. The above research further indicated that nitrogen also plays an important role in plant growth. This is consistent with Chen et al. (2000)[35].

Based on studies on soil chemistry and microbial communities, it is clear that soil chemical properties and microbial communities vary greatly with the growing seasons in this study. Therefore, we hypothesized that there is a close relationship between soil chemical properties and microbial communities. In the present study, we found that the total nitrogen content and pH value were significantly positively correlated with the soil bacterial community in June. This was generally consistent with the results of Bossio (1998) and Lauber (2008)[56,57]. That might because nitrogen is often a key limiting factor for soil organisms and addition of N can change microbial biomass, activity and species composition[58]. In addition, the present study showed that total nitrogen content was significantly positively correlated with the plant fresh biomass and the pH value was also significantly positively correlated with the evenness index, suggesting that total nitrogen content and pH value may promote plant growth by influencing bacterial communities. What is more, despite the demonstrated influences of soil chemical properties on soil microorganisms and further effect the plant growth, the mechanism of the effect are still requires further study. Microbial communities are important indices of soil quality. A well-functioning microbial community is a prerequisite for resilience to external factors and soil fertility[59]. In addition, seasonal variations have impact on soil microbial community according to the NMDS results. Most of soil nutrient contents are significantly higher in September than that in June. This may be that the accumulation of plant photosynthetic products, which increases soil nutrient content and improves soil quality. In the present study, Mental test should that the contents of soil total nitrogen, soil total phosphorus, soil nitrate nitrogen, soil pH value, and soil catalase activity were significantly positively correlated with the soil bacterial communities in September. The result should that the relationship between soil chemical properties and soil microbial communities is more closely in September compared to June. which is consistent with previous research[60,61]. the relationships between plant and soil, as well as between soil and soil, will change as the plant grows and develops. Most plants depend on soil properties; similarly, plants and their associated microorganisms play a crucial role in the formation or modification of soil[62]. The interactions between microorganisms and soil chemistry ultimately promote plant growth[63]. The Mantel test showed that soil total nitrogen content was significantly positively correlated with the total fresh biomass of plants. There was also a significant correlation between total

nitrogen content and soil bacterial communities, indicating that bacterial communities may modulate N-cycling processes to promote plant growth. This result is similar to that obtained by Xu (2014)[64] and Philippot (2013)[65]. Therefore, based on the above research, we suggest that nitrogen supplementation should be considered in the process of phytoremediation and nitrogen fixation can also be improved by planting more locally adapted legumes to increase the soil nitrogen content.

The yellow soil was sandy soil, which generally contains low organic matter content and is unable to retain moisture and nutrients; therefore, it generally has low fertility[66]. Some sandy soils contain high contents of base cations and micronutrients with less inorganic phosphorus (P) and nitrogen (N), which leaches out with heavy rain or irrigation[67]. In this study, the nutrients in the yellow soil were significantly lower than those in the black soil and is not conducive to nutrient retention because of its large soil porosity; therefore, the yellow soil can be used as a road fill. Our previous systematic study on yellow soil in greenhouses also found that yellow soil did not promote plant growth[68]. Plant ash incorporation into the soil can lead to substantial alterations in the structure of the microbial community and enhance soil fertility[69] which was confirmed in our previous research. Our previous studies found that adding plant ash to black soil increased the germination number and plant biomass[70] suggesting that the addition of plant ash can increase the soil nutrient content, thus further promoting plant growth. Additionally, the price of plant ash is relatively low and the addition of plant ash to restore vegetation growth is feasible. Since the 1990s, with the implementation of various highway slope protection technologies, engineering protection of slopes has gradually been replaced by vegetation protection, and green slopes can reduce driver fatigue[68] on the other hand, green slopes can prevent flooding and waterlogging, effectively strengthening the slope, which is the most important purpose[71]. Through a field investigation of the Gannan S10 Zhuohe highway, we found that all bags with seeds for slope greening were purchased, as well as the soil inside, which increased the cost of highway construction. The nutrient survey showed that although the nutrient content of the black soil was higher than that of the yellow soil, the nutrient content of the black soil was also relatively lower than that of the plant growth site, such as total nitrogen, which is a key element influencing plant growth, bacterial community and keystone taxa. Therefore, the nutrient content of the black soil should be increased if it is to be utilized. Additionally, several microbial agents that have emerged in recent years can be used to further improve soil quality and contribute to slope greening[72,73]. Microbial agents are active microbial preparations produced from the fermentation broth of target microorganisms (such as active bacteria or fungi) after industrial production and expansion. These agents typically use porous substances as adsorbents, such as peat and vermiculite, to enhance the survival and activity of the microorganisms[74]. Based on the above analysis of the taxa, *Candidatus-Udaeobacter*, *Sphingomonas*, *Achaetomium*, and *Monascus* were considered target microorganisms for preparing microbial agents. Additionally, this study revealed that the total nitrogen content is crucial for plant growth, bacterial community and key taxa. Therefore, we can produce a nitrogen-retentive and decomposition-promoting microbial agent based on the nitrogen-retentive microbial agent from previous studies to restore green slopes[75]. Therefore, black soil can be reused for slope greening and remediation by the addition of plant ash and microbial agents to improve its quality. This measure can ultimately improve a city's appearance and reduce highway construction costs.

Conclusion

Soil chemical properties and microbial communities are primarily influenced by seasonal variations. However, highway construction can also significantly affect some indicators, such as soil total nitrogen content and microbial biomass carbon content in September. There is a close relationship between the soil bacterial community and soil chemical properties and soil bacterial communities can promote vegetation growth by regulating soil nitrogen cycling. Furthermore, our results indicate that these effects are driven by specific microbial taxa that may play a crucial role in soil functioning due to their keystone role. Yellow soils can be reused as road fill because of their low nutrient content but black soils can be reused for slope revegetation and remediation by adding plant ash, microbial agents, etc. This study showed that the costs of highway construction and ecological restoration can be reduced through the reuse of black soil.

Data availability

The datasets generated during the current study are available in the NCBI repository, [ACCESSION NUMBER TO DATASETS: BioProject ID PRJNA1205081].

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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