Typical artificial forest soil bacterial community response to the soil environment of Grain for Green area in the Loess Plateau, China

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Research Article

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Posted Date: April 22nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1536487/v1

Typical artificial forest soil bacterial community response to the soil environment of Grain for Green area in the Loess Plateau, China

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Abstract

Purpose: This study aims to analyze the differences of soil bacterial community structure under different vegetation restoration types, and to determine feasible strategies for revegetation of grain for green area in the Loess Plateau.

Methods: High-throughput sequencing technology was used to analyze the alpha diversity of soil bacteria, community structure characteristics and the correlation between soil environmental factors and bacterial communities in different vegetation restoration types.

Results: The findings reveal that soil microbial C and N in different soil layers shows a decreasing trend with an increase in soil layers. Across all samples, the dominant groups are Proteobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes, Nitrospirae, Bacteroidetes, GAL15, and Firmicutes. Soil nutrient supply, such as TOC, TN, AN, AP, and AK, had crucial roles in shaping the composition and diversity of the bacterial communities.

Conclusions: Based on the results, our data provides a starting point for establishing soil bacterial databases in the Loess Plateau, as well as for the plants associated with the vegetation restoration.

Keywords: Revegetation; High-throughput sequencing; Soil bacterial community; Soil environment factor; The Loess Plateau

1 Introduction

Ecological restoration is a complex process that involves improvement of green vegetation coverage, prevention of soil erosion, and reduction of natural disasters. Currently, revegetation is a hot topic of research in ecology, encompassing soil physical and chemical properties and soil and water conservation (Zeng et al. 2014), ecological stoichiometric characteristics of vegetation and soil (Sun et al. 2020), soil nutrient element preservation (Ma et al. 2014), biodiversity of undergrowth vegetation (Chen and Cao 2014), and soil microbial communities (Liu et al. 2018). As

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the agents of decomposition in the ecosystem, soil microorganisms play a key role in forest succession and nutrient cycling. They are the link between the aboveground and underground components of the ecosystem and represent an important index for evaluation of ecosystem restoration. Plant community in forest ecosystems by changing the species composition, biomass, litter–earth system components, forest and soil environment factors, such as soil microbial community structure and composition (Okubo et al. 2016a; Spohn and Widdig 2017a), and other relevant studies have reported the species and soil physical and chemical properties of soil microbial community composition and diversity (Ding et al. 2017; Nacke et al. 2016; Pei et al. 2016; Zhalnina et al. 2015).

It is a long-term and arduous task to harness the ecological environment and restore the vegetation in the Loess Plateau. Revegetation is not only the first step in ecosystem reconstruction but also an important measure of ecological environment management and soil and water conservation on the Loess Plateau, and it can effectively reduce soil erosion and improve soil quality (You et al. 2020; Tan et al. 2019). Wuqi County in Shaanxi Province is located in an interlaced area of wind erosion and water erosion on the Loess Plateau. In that area, the soil erosion is serious, and the ecological environment is fragile. Since 1998, the government has adopted a development strategy based on “closing hills and returning farmland, afforestation to grass, indoor-sheep production, and planting trees to poverty eradication.” The change in vegetation development and species composition in Wuqi County following the closure reflects the trend of increasing stability of the degraded matrix and enhancement of the community environment (Shan et al. 2019).

In recent years, the revegetation project on the Loess Plateau has increased the area of vegetation considerably, and an increasing number of studies have examined the revegetation of the returned farmland in this area (Chen et al. 2015; Feng et al. 2016; Nie et al. 2015). In general, these studies focus on the impacts of revegetation on soil properties, changes of species diversity, the benefits of soil and water conservation, and the allocation mode of revegetation in the process of land restoration. However, there has been no systematic analysis of the interaction between revegetation, soils, and microorganisms in the process of land restoration.

With the development of soil microbial research methods, high-throughput sequencing technology, also known as either "next-generation sequencing technology" or deep sequencing, has gradually become a conventional method for analyzing the diversity of soil microorganisms.

Currently, this method is used widely to determine the effects of vegetation, crop cultivation and land management, organic pollutants, heavy metals, seasonal changes, climatic conditions, and other factors on soil microbial community structure and biomass (Ren et al. 2018; Zeng et al. 2016; Zhang et al. 2018).

Therefore, to explore the role of microorganisms in the process of vegetation restoration on the soil ecosystem of Grain for Green Project area in the Loess Plateau and to determine feasible strategies for revegetation in this area, the present study investigated the dynamics of soil properties and the structure of soil bacterial communities at sites on the Grain for Green are in the Loess Plateau using 16S rRNA high-throughput sequencing.

2 Materials and methods

2.1 Study site and sampling

The vegetation types in the area are characterized by the transition from forest shrub steppe vegetation to steppe forest shrub steppe vegetation. The vegetation composition is dominated by the flora of North China. Since the project of returning farmland to forestry was implemented, there vegetation coverage has increased significantly. Different artificial *H. rhamnoides* forests as the research objects were selected. Typical sample plots were were selected in stands and 20 m × 20 m arbor sample plots were set up to observe and record the position (coordinate information, altitude) and site condition (such as slope and canopy closure) of the sample site in August 2017. Table 1 shows the specific situation. At each sampling site, soil was collected from three locations ca. 20m apart from one another by pooling five soil cores from 1m×1m area at each location. The surface soil samples were collected from each quadrat at random and mixed immediately to form a single soil sample. Visible matter, such as loose gravel and plant debris, was removed and divided into two parts. One part was passed through a 2 mm sieve into a 15 mL centrifuge tube, placed into a liquid N canister, transferred to the laboratory, and stored in a freezer at −80 °C for microbial analyses. The other part was subjected to air-drying treatment and used to determine soil nutrients.

2.2 Soil sample determination

2.2.1 Sequencing of soil microbial genes

Extraction of DNA: Following the manufacturer's instructions, the genomic DNA was isolated from 0.5 g of each soil sample from each sample site using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, CA, USA). Extracts of three technical repeats were mixed into a single DNA sample. Extracted genomic DNA was detected by 1% agarose gel electrophoresis. DNA concentration and purity were measured with an ultramicro spectrophotometer (Nano Drop2000, US).

PCR amplification：Based on previous reports, the primers 338F-806R for the 16S rRNA of bacteria were used. Amplified products were detected by 2% agarose gel electrophoresis, recovered from the gel using the AxyPrep DNA gel extraction kit (Axygen Biosciences, Union City, CA, USA), washed with Tris-HCl, and verified by 2% agarose gel electrophoresis.

PCR products were quantified by the QuantiFluorTM-ST Fluorometer (Promega Biotech, Beijing, China) and the samples adjusted as required for sequencing. High-throughput sequencing was performed using Illumina Miseq PE300 platform (Shanghai Majorbio Bio-Pharm Technology Co., Ltd, China).

2.2.2 Soil microbial carbon and nitrogen determination

Soil microbial biomass C and N were determined using fumigation extraction (Vance et al., 1987). Briefly, 25 g of the oven-dry equivalent of field-moist soil was fumigated at 25.8 °C for 24 h with CHCl3. The soil was added to 100 ml of 0.5 M potassium sulfate by shaking at 200 rpm for 1 h and then filtered after fumigant removal. Another 25 g of non-fumigated soil was simultaneously extracted. The TOC and TN contents of the extracts were determined using a TOC analyzer (multi N/C 3100, TOC/TNb analyzer, Analytik-Jena AG, Germany)().

2.2.3 Determination of soil physical and chemical properties

The content of soil organic carbon (TOC) , total nitrogen (TN) , total phosphorus (TP), and total kalium(TK) were determined using the vario EL cube CHNOS Elemental Analyzer(Elementar Analysensysteme GmbH，Germany). Soil alkali-hydro N (AN) were measured using the alkali-hydrolyzed diffusion method, and available phosphorus (AP) was determined by UV-Visible Spectrophotometer (UV-2550, Shimadzu, Japan). And available kalium were determined by iCAP 6300 ICP-OES Spectrometer(Thermo Fisher, USA).

2.3 Data Processing and Analysis

2.3.1 Basic data processing

All data were analyzed using IBM SPSS statistics, R and the free online platform of Majorbio Cloud Platform (www.majorbio.com).

2.3.2 Operational Taxonomic Units (OTU)Analysis

To obtain the species classification information of each OTU, RDP classifier was used to analyze the representative sequences of OTU with 97% similarity level, and the species composition of each sample was counted in each taxonomic level: domain, Kingdom, phylum, class, order, family, genus, and species. The taxonomic identity of each phylotype was determined by the bacterial 16S rRNA Silva reference database (http://www.arb-silva.de).

2.3.3 Alpha diversity analysis and Rarefaction curve

Indices of community richness Chao 1 estimator, abundance-based coverage estimator (ACE) and indices of community diversity Shannon, Simpson estimator were calculated. And the coverage index reflects whether the sequencing results represent the real situation of microorganisms in the sample. A certain number of sequences were randomly selected from the samples, and the alpha diversity index of the corresponding samples was counted. Taking the extracted data as the abscissa and the alpha diversity index as the ordinate, the curve was drawn, and whether the sequencing data volume was enough was judged according to whether the curve was smooth or not. In this study, OTUs with 97% similarity were selected to calculate the alpha diversity index under different random sampling by MOTHUR (http://www.mothur.org/), and the curve was made by using R language tool.

2.3.4 Statistic analysis

Principal coordinates analysis (PCoA) was used to evaluate the overall differences in the structures of the plant and microbial communities based on Bray-Curtis distances. Redundancy analysis (RDA) using Monte Carlo permutation (999repetitions) was used to test the relationships among the soil properties. Correlations between the soil environment factor and composition of the soil bacterial communities were investigated using Pearson's correlation analysis. Heatmap adopts the Average level clustering method.

3 Results

3.1 Analysis of soil microbial carbon and nitrogen

3.1.1 Characteristics of soil microbial carbon and nitrogen

As shown in Table 2, what was revealed is that soil microbial C and N content in different soil layers showed a trend of small change with increasing soil layers. That is, soil microbial activities weaken gradually as the soil depth increased. There is a slight increase in soil microbial C content in the 60–80 cm soil layer of *H. rhamnoides* pure forest (shady slope), the 80–100cm soil layer of *H. rhamnoides* pure forest (sunny slope), the 80–100 cm soil layer of *H. rhamnoides* and Chinese arborvitae mixed forest and the 60–80 cm soil layer of *H. rhamnoides* and *Pinus tabulaeformis* mixed forest. There is no clear vertical variation of soil microbial N content, and there are no significant differences between different soil layers. Over the four plots, the soil microbial C content was highest in *H.rhamnoides and Pinus tabulaeformis* mixed forest, whereas there are few differences in the soil microbial C contents of the other three plots. Soil microbial N content is highest in *H. rhamnoides* pure forest (sunny slope), and the lowest value is in *H. rhamnoides* and *P. tabulaeformis* mixed forest.

3.1.2 Regression analysis of soil microbial carbon and nitrogen contents and soil carbon and nitrogen contents

Regression analysis, as a method for statistical analysis of the inter-dependent quantitative relationship between two or more variables, can be used to analyze the relationship between one variable and the change of another variable. The soil microbial C and N content and the soil C and N content in different *H. rhamnoides* forests were analyzed via exponential and linear regression analysis and established a regression model. Table 3 shows the results. Apart from the *H. rhamnoides* and *P. tabulaeformis* mixed forest, the regression equations of the other three samples are well fitted. Of the three well-fitting plots, the R^2 of the index model of soil microbial biomass N and alkali-hydrolysable N of *H. rhamnoides* pure forest (sunny slope) is 0.73, whereas the remainder are all above 0.8, with some even exceeding 0.9. Therefore, the model can well explain the relationship between soil microbial C content and soil organic C content, soil microbial N content and soil total N content, and soil microbial N content and alkali-hydrolyzed N content. Only the soil microbial N content and soil alkali-hydrolyzed N content regression model shows a good fit in the case of *H. rhamnoides* and *P. tabulaeformis* mixed forest.

3.2 High throughput sequencing results

By sequencing the V4-V5 region of bacterial genes, all samples were subsampled according to

the minimum number of sample sequences. And the OTU with sequence number ≥ 5 in at least one sample is reserved. A total of 299,625 effective sequences were obtained from soil bacterial community, with an average length of 438.21 bp. RDP classifier was used to cluster OTU representative sequences with 97% similarity level. And the results showed that the bacterial communities of the samples consisted of 24 phyla, 55 classes, 110 orders, 206 families, 348 genera, 680 species and 1989 OTUs. Combining OTUs of the same species and statistically analyzing the changes of species composition among different samples enables the diversity of microflora among different samples to be understood.

Table 4 shows the sequence number of test samples and alpha diversity index of microbial community. The coverage of each sample is greater than 98%, which indicates that the probability of gene sequence detection in soil samples is very high, and the sampling is basically reasonable, which can truly reflect the microbial community characteristics of soil samples. According to the rarefaction curves of each diversity index (Fig. 1), with the increase of random sequencing data, the dilution curve basically tends to be flat, which indicates that the confidence degree of microbial community in real environment is high, and the sequencing depth and data volume are enough. What's more, the richness indices and diversity indices of bacterial community in arbor shrub mixed forest are higher than that in shrub pure forest, and the indices of shrub forest on sunny slope are higher than those on shady slope (Table 4).

3.3 Soil bacterial community structure

The dominant phylum in bacterial community is Actinobacteria (37.27% on average), followed by Proteobacteria (23.91%), Acidobacteria (12.75%), Chloroflexi (12.27%), Gemmatimonadetes (5.85%), Nitrospirae (1.66%), Bacteroidetes (1.26%), GAL15 (1.14%), and Firmicutes (1.1%) (Fig. 2). There are differences in relative abundance of dominant phylum between the pure and mixed artificial forests, but the differences are not significant. Among the Proteobacteria, Alpha-, Beta-, Gamma-, and Deltaproteobacteria are found in all samples (Fig.3). The members of Alphaproteobacteria dominating this phylum, occups 14.67% of all populations，highest at GL compared to the others. Betaproteobacteria, Deltaproteobacteria, and Gammaproteobacteria comprises 4.30%, 2.75%, and 2.19% of the total populations, respectively.

The plot of PCoA (Fig. 4) clearly identifies variations in bacterial community composition among the sites, with the abundance of each group varying with different afforestation modes. At

the OTU level, the interpretation variance of PC1 and PC2 is 24.46% and 19.37%, respectively, and the accumulated interpretation ability is 43.83%. The profiles at HrN and HrBo sites tend to group together and are clearly separated from those at GL, HrS, and HrPt sites, which are clearly separated from each other.

3.4 Relationships among soil environment factor and bacterial communities

The Pearson correlation coefficient were used to analyze the correlation between bacterial communities and soil environmental factors. The results of the analysis are illustrated by a heatmap in Fig. 5. TOC, AN, AP, TN, and AK contents are correlated positively with the abundance of Alphaproteobacteria and Acidobacteria and negatively with the abundance of Gemmatimonadetes and Bateproteobacteria. AP and AK are correlated negatively with the relative abundance of Nitrospira.

The RDA confirmed the results of the correlation analysis and further identified the effect of the soil on the bacterial communities at the class level(Fig. 6). The first two axes explain 87.77% of the total variance, indicating that TOC, TN, AN, AP, and AK were the most influential factors driving the changes in the composition and diversity of the bacterial communities.

4 Discussion

Restoration mainly affects the quantity and community of soil microorganisms by affecting the litter, root morphology and secretion, and the material and energy transformation process in the whole ecosystem(Okubo et al. 2016; Spohn and Widdig 2017). *Hippophae rhamnoides*, which is a pioneer and associated species of vegetation structure in the Loess Plateau, has clear ecological and economic benefits. Its root system can co-exist with nitrogen (N)-fixing actinomycetes to form N-fixing nodules and fix free N. Furthermore, this root system can decompose insoluble organic matter and minerals, thereby helping to improve the soil (Hu et al. 2014; Hu 2012).

Based on analysis of microbial biomass C and N in *H. rhamnoides* pure forest (shady slope), *H. rhamnoides* pure forest (sunny slope), *H. rhamnoides* and Chinese arborvitae mixed forest, and *H. rhamnoides* and *Pinus tabulaeformis* mixed forest, the driving capacity of the soil carbon (C) cycle and the mineralization and fixation of soil microorganisms on nitrogen (N) were examined. The soil microbial C and N analysis showed that soil microbial biomass N and N in different forest layers exhibited a trend of a small change with increasing soil layers; that is, as the soil depth

increased, the soil microbial activity weakened gradually. Yao et al.(2016) studied the variation rules of soil microbial biomass carbon in different vegetation restoration types in the Loess Plateau, and the results showed that soil microbial biomass carbon content in mixed forest of trees was the largest. Hu et al. (2010) studied the influence of vegetation patterns on soil microbial biomass and functional diversity in a hilly area of the Loess Plateau. They also showed that, in terms of increasing soil microbial biomass carbon and nitrogen content, the mixed mode of forest and grass was more effective than the single vegetation and could effectively promote soil remediation. Therefore, it is suggested that the mixed forest should be the main way to implement the Grain for Green Project in the Loess Plateau.

Studies have shown that Actinobacteria is the main functional bacterium that degrades lignin and cellulose (Pankratov et al. 2011). Alpha-, Beta-, Gamma-, and Deltaproteobacteria are the most important microorganisms in saline alkali soil (Lin et al. 2019). In this study, we found that the relative abundance of Actinobacteria and Proteobacteria was 37.23% and 23.91%, respectively, which was the most dominant bacteria in the community, which was consistent with the research results of Liu et al. (2016) on the characteristics of soil bacterial community in different arbor forests in the Loess Plateau, and the vegetation type was an important factor affecting the diversity of soil actinobacteria (Zhang et al. 2015). TOC, TN, AN, AP, and AK were the most influential factors driving the changes in the composition and diversity of the bacterial communities. Xu et al. (2019) reported the responses of soil *nosZ*-type denitrifying microbial communities to the various land-use types of the Loess Plateau, and the results showed that AN was the key environmental factor affecting microbial composition. An et al. (2017) reported that the loss of microbial diversity affects the carbon mineralization rate and the pattern of carbon source utilization, leading to functional changes in terrestrial ecosystems. Zhao et al.(2018) found that the change of soil carbon composition after afforestation had a significant impact on the diversity and structure of soil microbial community.

This study only addresses the soil microbial characteristics of different stands in growing season during revegetation in Wuqi City, Shaanxi Province. To better analyze the mechanism of influence of vegetation on microorganisms and the influence of site conditions and environmental factors on soil microbial community diversity, the litter and root exudates under various artificial forests can be studied further. To better analyze the microbial characteristics under the effect of

revegetation in the Loess Plateau, similar studies can be conducted in other areas of the Loess Plateau. The results of these experiments can be analyzed to better explore the soil under *H. rhamnoides* forest in the process of vegetation diversity of soil microbial community and is expected to provide some data supporting the study of revegetation in the Loess Plateau.

5. Conclusions

In this study, the soil bacteria were analyzed as a function of different vegetation restoration types on the Loess Plateau. Soil microbial biomass C and N in different forest layers exhibited a trend of a small change with increasing soil layers; that is, as the soil depth increases, the soil microbial activity weakens gradually. Across all samples, the dominant groups were Proteobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes, Nitrospirae, Bacteroidetes, GAL15, and Firmicutes. Soil nutrient supply, such as TOC, TN, AN, AP, and AK, have crucial roles in shaping the composition and diversity of the bacterial communities. Furthermore, the data also provide a starting point for establishing soil bacterial databases in the Loess Plateau, as well as for the plants associated with the vegetation restoration.

Acknowledgements

We would like to thank Shanghai Majorbio Bio-pharm Technology Co.,Ltd and the State Key Laboratory of vegetation and environmental change, Institute of Botany, Chinese Academy of Sciences for their sample testing support.

Authors' contributions

XL and TW conceived and designed the experiments. XL carried out the field work, analyzed the data and drafted the manuscript. TW revised the manuscript and provided editorial advice. All authors have read and agreed to the published version of the manuscript.

Funding

The work was supported by the 13th Five-year National Key Research and Development Project (No.2016YFC0501705) funded by the Ministry of Science and Technology (MOST), P.R. China.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All the authors have approved the manuscript that is enclosed.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Figure Captions

Fig. 1. Rarefaction curves at cutoff level of 3%.

Fig. 2. Relative abundance (a) and Circos (b) of bacterial community at the phylum level. Others: Relative abundance <1% in all samples.

Fig. 3. Distribution of Proteobacteria populations at the class levels.

Fig. 4. Principal coordinates analysis (PCoA) of bacterial composition based on Bray-Curtis distances.

Fig. 5. Heatmap of bacterial and soil environmental factors. R and P values are obtained by calculation. The R value is shown in different colors in the graph. The color card on the right-hand side of the heatmap is a color partition with different R values. $0.01 < p \leq 0.05$ * $0.001 < p \leq 0.01$ ** $p \leq 0.001$ ***. BC: microbial biomass C, BN: microbial biomass N, TOC: total organic C, TN: total N, AN: alkali-hydro N, TP: total P, AP: available K, TK: total K, AK: available K, the same as followings.

Fig. 6. Ordination plots of the results from the redundancy analysis to identify the relationships among the bacterial populations (blue arrows) and soil environmental factors (red arrows).

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Figures

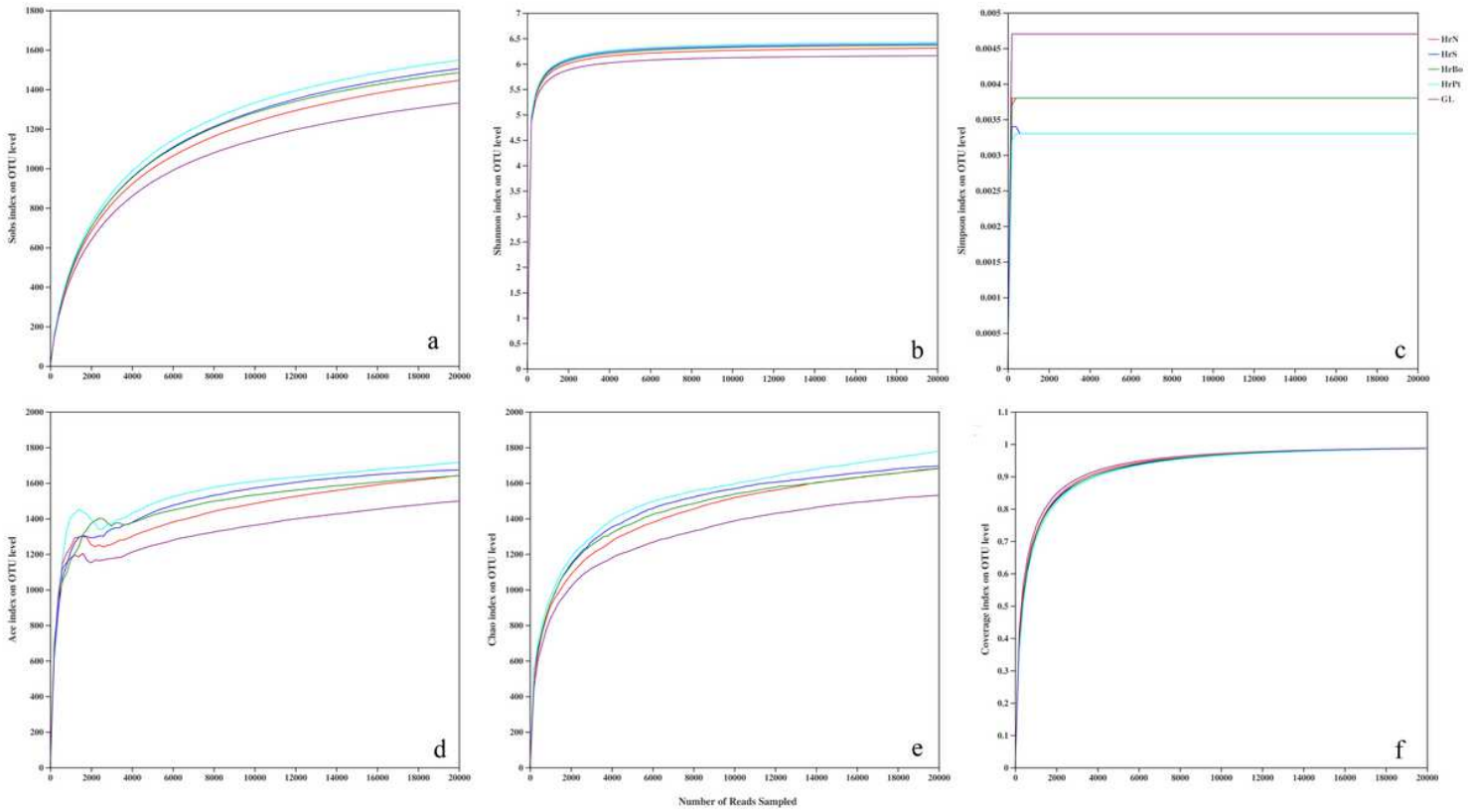

Figure 1

Rarefaction curves at cutoff level of 3%.

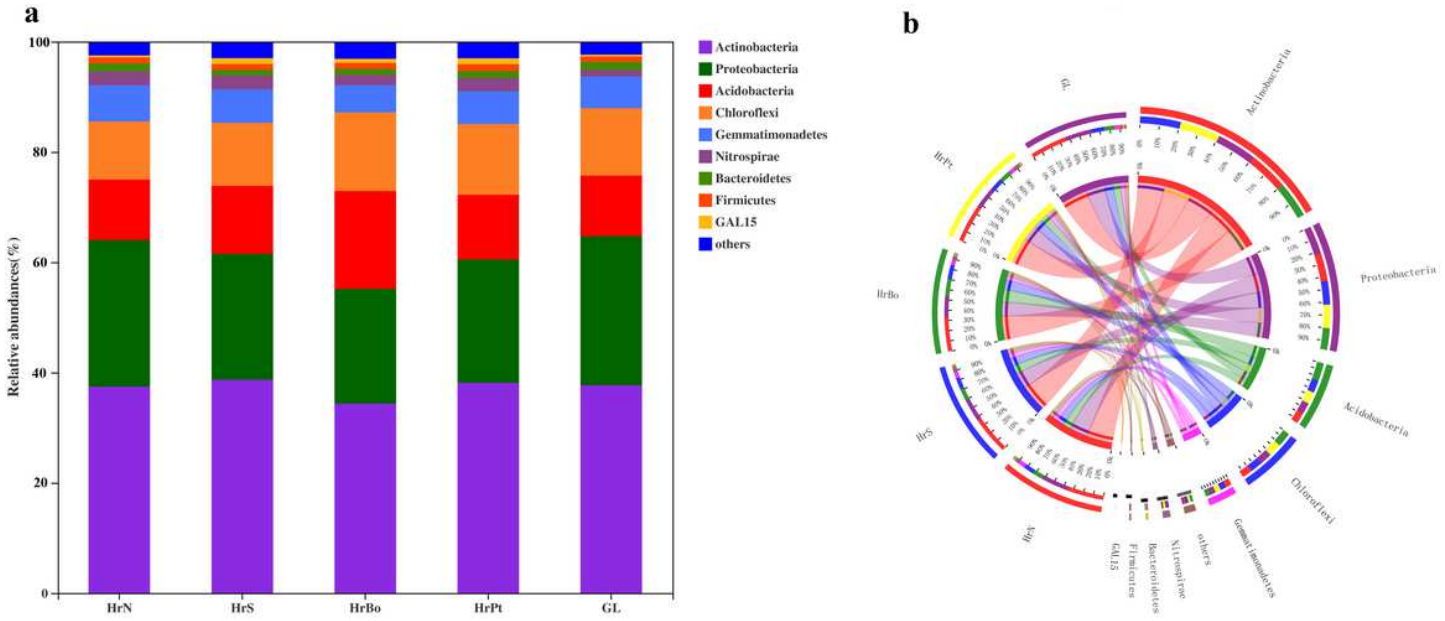

Figure 2

Relative abundance (a) and Circos (b) of bacterial community at the phylum level. Others: Relative abundance <1% in all samples.

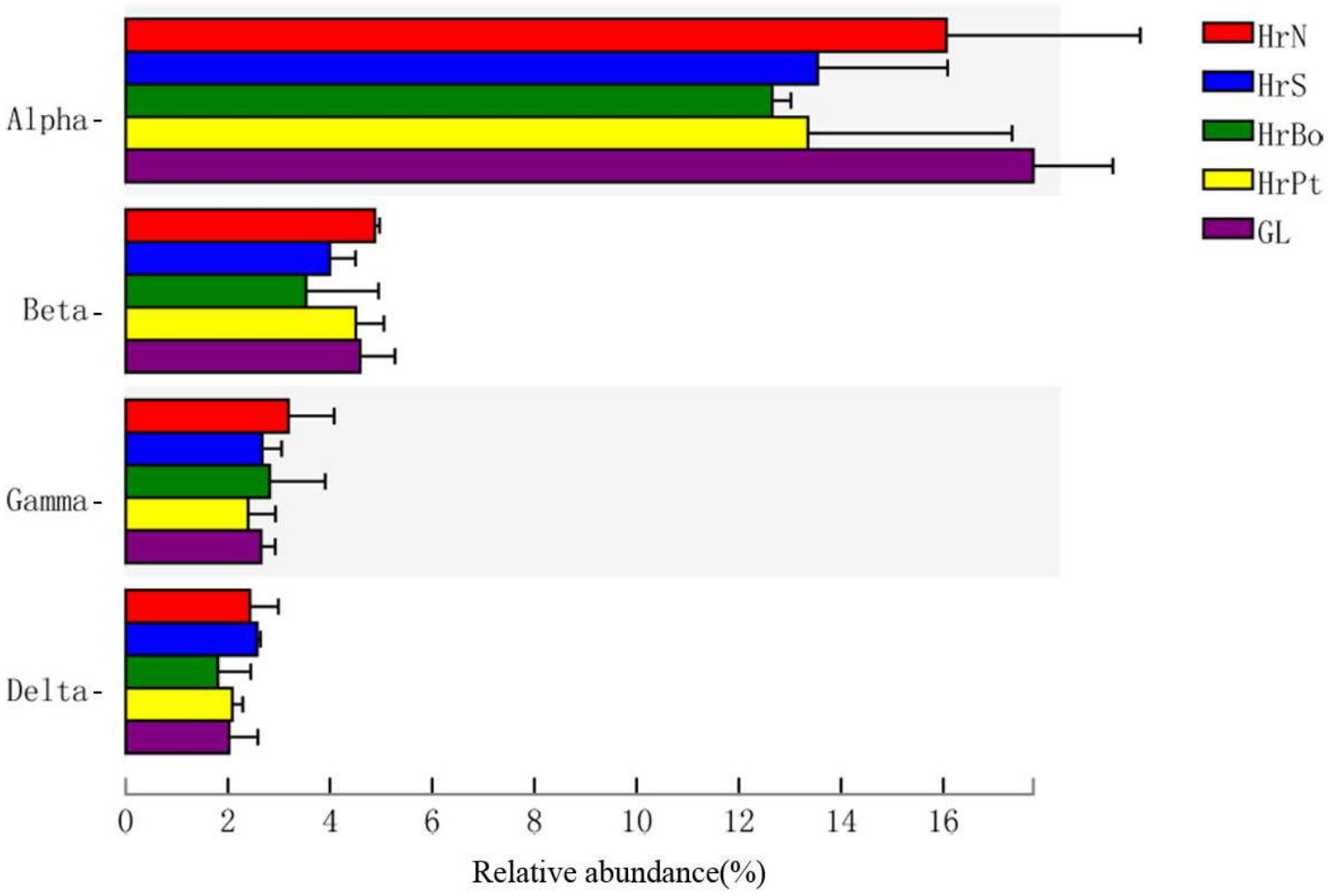

Figure 3

Distribution of Proteobacteria populations at the class levels.

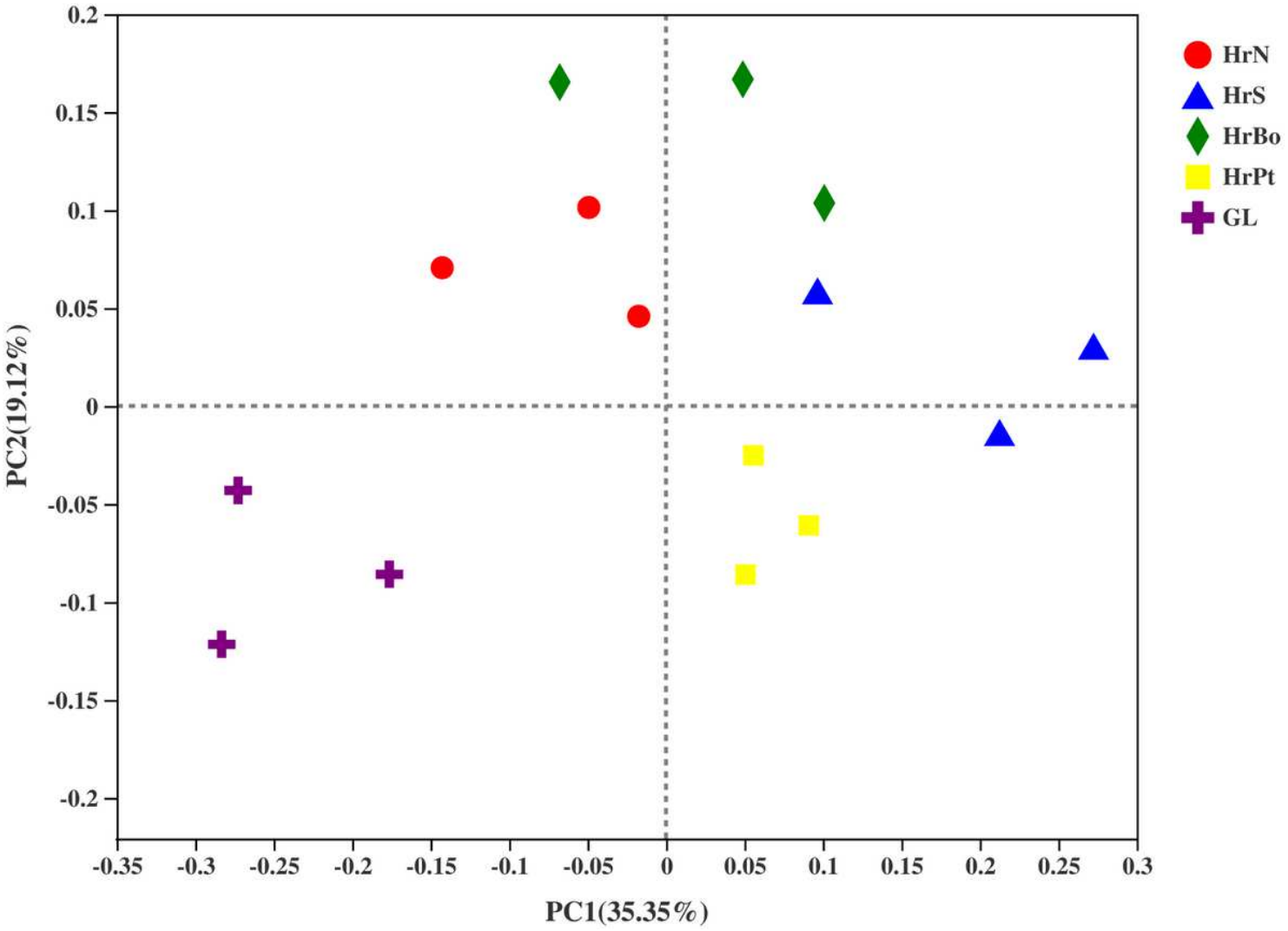

Figure 4

rincipal coordinates analysis (PCoA) of bacterial composition based on Bray-Curtis distances.

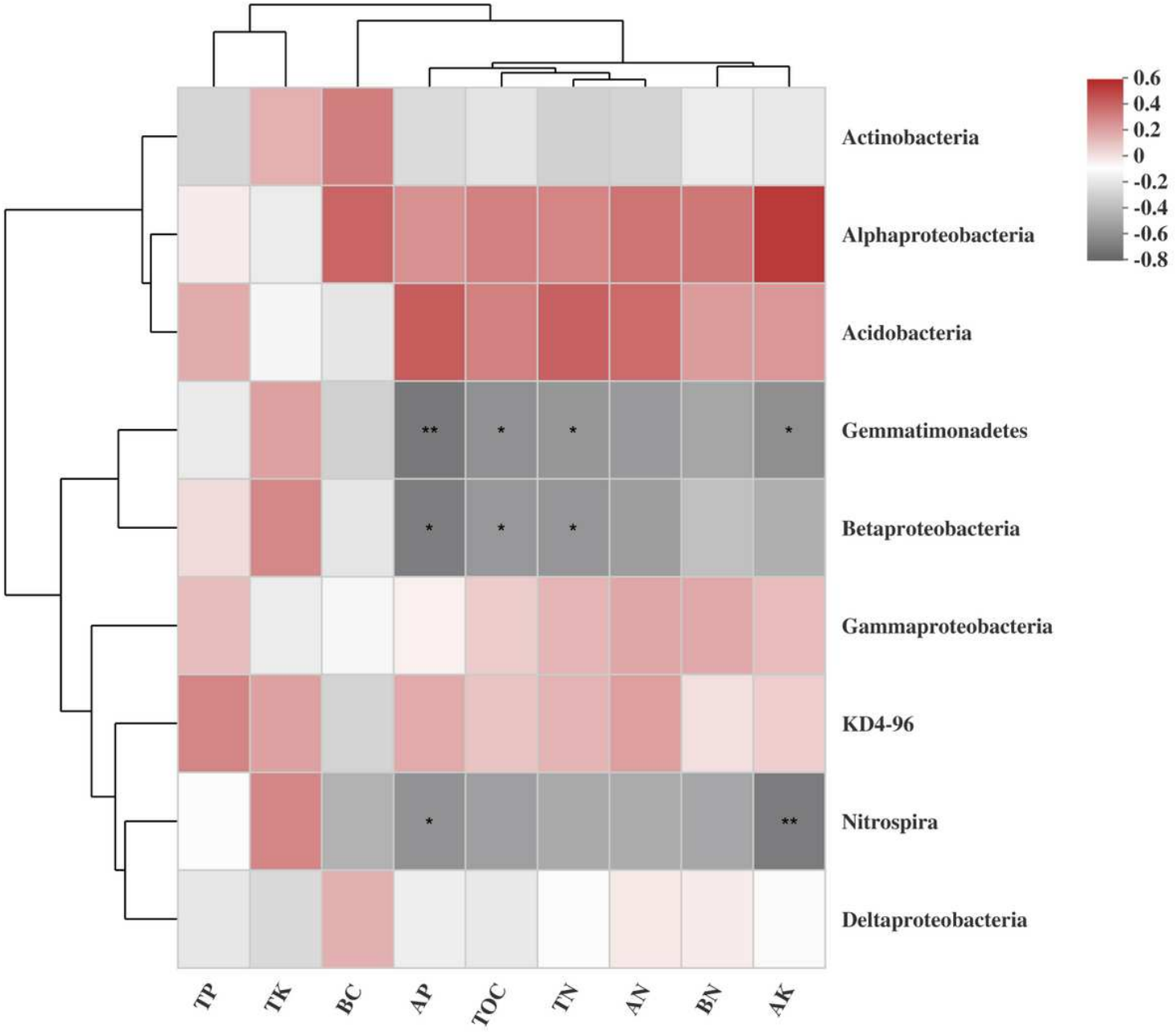

Figure 5

Heatmap of bacterial and soil environmental factors. R and P values are obtained by calculation. The R value is shown in different colors in the graph. The color card on the right-hand side of the heatmap is a color partition with different R values. 0.01 < p ≤ 0.05 * 0.001 < p ≤ 0.01 ** p ≤ 0.001 ***. BC: microbial biomass C, BN: microbial biomass N, TOC: total organic C, TN: total N, AN: alkali-hydro N, TP: total P, AP: available K, TK: total K, AK: available K, the same as followings.

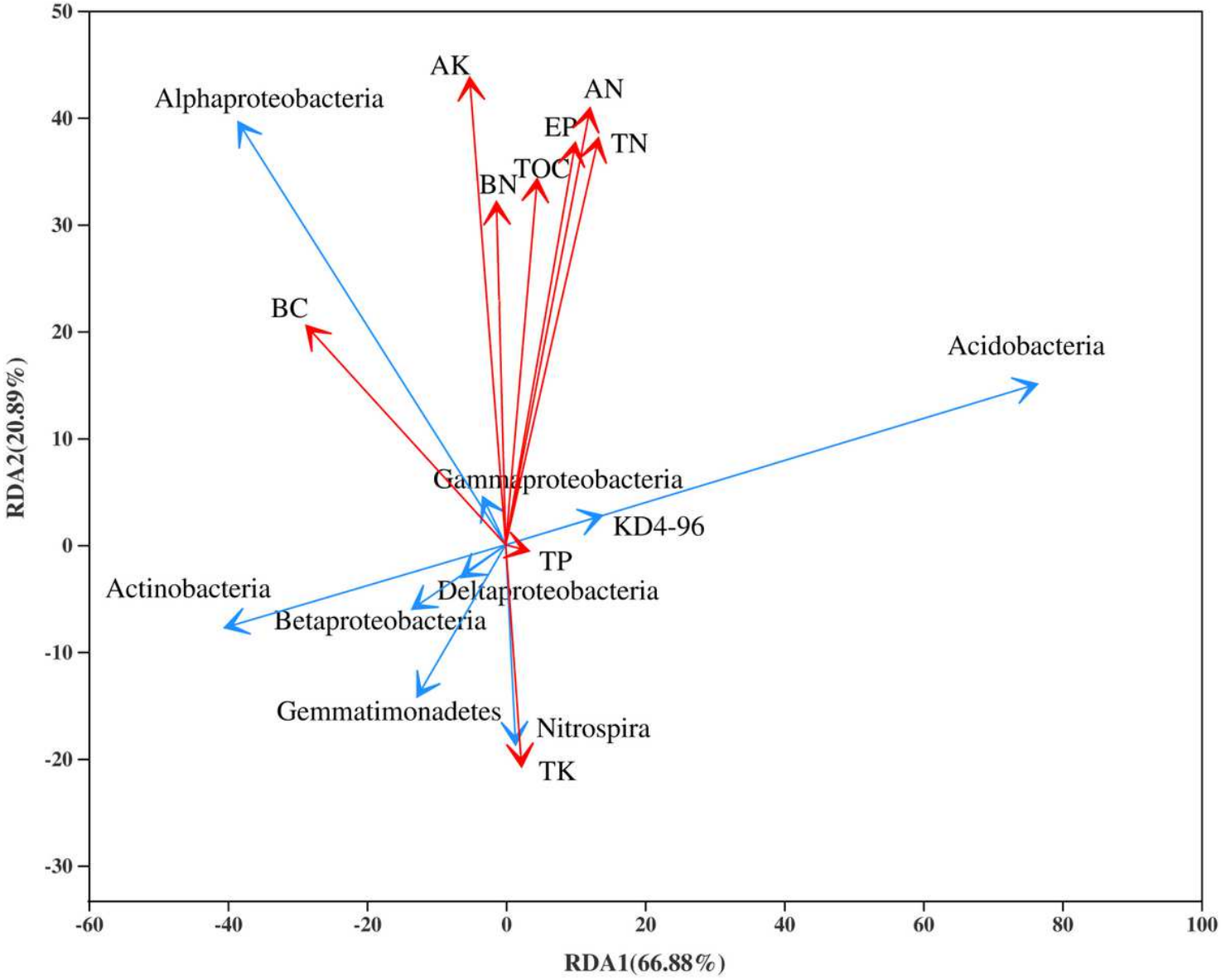

Figure 6

Ordination plots of the results from the redundancy analysis to identify the relationships among the bacterial populations (blue arrows) and soil environmental factors (red arrows).