

EFFECTS OF PLANTING *XANTHOCERAS SORBIFOLIUM* BUNGE ON HEAVY METAL ELEMENTS AND RHIZOSPHERE ARCHAEA DIVERSITY OF FLY ASH

BAI, X. L.¹ – ZHAO, J. G.^{1,2*} – HUO, J. X.^{1*} – SONG, J.¹ – CHEN, Z. W.^{2*}

¹*School of Chemistry and Chemical Engineering, Shanxi Datong University, Datong 037009, P.R. China*

²*Institute of Carbon Materials Science, Shanxi Datong University, Datong 037009, P.R. China*

**Corresponding authors*

*e-mail: jgzhaoshi@163.com (J.G. Zhao), hjx511@163.com (J.X. Huo),
chenzhiwen@sxdtu.edu.cn (Z.W. Chen)*

*phone: +86-1313-3329-150 (J.G. Zhao), +86-1350-3525-637 (J.X. Huo), +86-1381-0289-899
(Z.W. Chen)*

(Received 20th May 2022; accepted 14th Sep 2022)

Abstract. The utilization of fly ash is of great significance for the sustainable development of waste resource utilization and environmental protection. In this study, inductively coupled plasma mass spectrometry (ICP-MS) and high-throughput sequencing techniques were used to investigate physicochemical properties and archaea composition in rhizosphere fly ash, respectively. The results showed that heavy metal elements in rhizosphere fly ash could be transported and accumulated in the taproot, fibrous root, stem and leaf of *Xanthoceras sorbifolium* Bunge. Meanwhile, the contents of P, K, Mg, total nitrogen, available phosphorus, available potassium, and the organic matter in rhizosphere fly ash increased after planting *X. sorbifolia*, suggesting that planting *X. sorbifolia* can increase the fertility of fly ash. The planting of *X. sorbifolia* decreased the number of archaea (Operational Taxonomic Units) OTU and species abundance of fly ash. But the abundance of beneficial archaea such as *Firmicutes* and *Euryarchaeota* increased after planting *X. sorbifolia*. In the potential phenotypes predicted by BugBase, the number of aerobic, mobile element containing, facultatively anaerobic, biofilm forming, potentially pathogenic decreased. anaerobic, gram-negative, gram-positive, stress tolerant amount increased. These results indicated that planting *X. sorbifolia* has positive effect on the utilization of fly ash by phytoremediation.

Keywords: *Xanthoceras sorbifolium* Bunge, fly ash, physicochemical properties, archaea diversity, phytoremediation

Introduction

Thermal power generation dominates the world's total power supply, and coal accounts for the largest share of thermal power generation in China (Wang et al., 2015). Fly ash is a waste product from the combustion of pulverized coal in power plants, and its production is increasing, requiring large scale disposal. Worldwide, fly ash has been successfully used in the concrete industry for many years, but its application is still limited due to insufficient understanding of the properties of fly ash itself and the properties of concrete mixed with fly ash (Ghazali et al., 2019). Other combustion products are stored underground. If not handled properly, fly ash will occupy a large amount of storage land and pose a serious threat to the environment and public health (Wang et al., 2020). In windy weather, fly ash easily generates dust and cause serious air pollution in a large area (He et al., 2012). Fly ash consists of fine and powdery particles that can be a source of water pollution, but it also contains macronutrients (N,

P, K, Ca, Mg and S) and micronutrients (B, Co, Cu, Fe, Mn, Mo, Ni and Zn), which are essential nutrient elements for plant growth (Yu et al., 2019). However, fly ash also contains Al, Cr, Pb, Hg, Ni, As and other heavy metals that are toxic to plants and animals (Dzantor et al., 2015). Moreover, these heavy metal elements aggravate air and water pollution and ultimately affect human health (Ma et al., 2017). In view of this, we urgently seek a simple and reasonable method to remediate fly ash and make it have potential application value.

Xanthoceras sorbifolium Bunge, belonging to Sapindaceae, is an oil-bearing seed shrub unique to China. It has a long life span of up to 200 years (Zu et al., 2009). It has been identified as the main biodiesel tree species, and the Chinese government has provided special support to help its development (Zhang et al., 2010). *X. sorbifolia* is mainly distributed in northern China, with high resistances to the drought, cold and salt stresses (Zhang et al., 2010). The stems and fruits of the *X. sorbifolia* are used in folk medicine to treat rheumatoid arthritis, night enuresis, etc. (Li et al., 2010). It is also an excellent quality for eliminating wind, sand fixation and desertification control, as well as can be used as a green ornamental tree in parks (Liu et al., 2013).

Fly ash has been used as a soil ameliorant in agricultural fields for several decades (Nayak et al., 2015). Previous studies have shown that fly ash has a high water holding capacity, a good pH value and significant plant nutrients, making it a potential soil conditioner (Ram and Masto, 2014). The application of fly ash can often improve the absorption of soil nutrients and plant nutrients and increase the biomass and yield of crops (Ram et al., 2007; Thind et al., 2012; Schönegger et al., 2018). But there are other studies showing that the heavy metal elements in fly ash can be accumulated in plants (Liu et al., 2013). Therefore, we took advantage of this phenomenon to plant *X. sorbifolia* in fly ash, thereby reducing the heavy metal content in fly ash. We use the well-developed root system of *X. sorbifolia* to remediate the fly ash, reduce the heavy metal elements in the fly ash, and change some of the chemical properties of the fly ash. And the archaeal diversity of fly ash in the rhizosphere before and after planting was determined to further explore the mechanism of heavy metal reduction. These results implied that planting *X. sorbifolia* can be used for phytoremediation of fly ash.

Materials and Methods

Experimental design

This experiment was performed in the plant growth greenhouse of the Institute of Carbon Materials Science, Shanxi Datong University. Fly ash samples were taken from Datong Power Plant. The experiment was set up in three replicate groups. The fly ash was mixed evenly, and taken into sterile tubes, then stored in -80°C refrigerator for the amplicon sequencing. Fly ash samples were also dried naturally and passed through a 1mm sieve for the determination of chemical property indicators (the fly ash samples without planting *X. sorbifolia* were defined as CK group). The remaining fly ash were placed in three seedling pots, and 4 L 100% fly ash was added to each seedling pot. Thirty seeds of *X. sorbifolia* were selected with the same size and planted in the pots by performing three replications (the fly ash samples planting *X. sorbifolia* were named as S group). Both CK and S groups were watered once every two weeks with 3L distilled water in each pot. After growing for three months, three biological samples were randomly selected, and each biological sample was a mixture of five randomly collected *X. sorbifolia* rhizosphere fly ash. Then, the rhizosphere fly ash samples were put into

the sterile tubes and stored in a refrigerator at -80°C for the determination of chemical property indicators and the extraction of soil DNA for amplicon sequencing analysis. The *X. sorbifolia* seedlings were rinsed with distilled water, and then the leaves, stems, taproots and fibrous roots were immediately frozen with liquid nitrogen (three biological replicate samples were taken), and stored in -80°C refrigerator.

Analysis of the properties of different X. sorbifolium tissues

Microwave-assisted digestion was used to digest the root, stem and leaf samples of *X. sorbifolia*. The contents of heavy metal elements (Pb, Hg, Cr, Cd and As) of different tissues in *X. sorbifolia* were determined by using Inductively Coupled Plasma-Mass Spectrometry (ICP- MS) (ICAP6300). For each plant sample, 7 ml nitric acid (HNO₃ 63%) and 2 ml hydrogen peroxide (H₂O₂ 70-72%) were added for digestion. The samples were digested with a microwave digester (Milestone ETHOS ONE, *Leutkirch im Allgau*, Germany) for 2 h. After digestion, the sample was heated on a hot plate at 135 °C for 2h with about 1 mL evaporated. It was then cooled at room temperature using double distilled water to achieve a final volume of 50 ml. Finally, the samples were filtered by 0.22-μm membrane filter and analyzed by ICP-MS (ThermosXSERIES2) (Tadesse et al., 2019).

Fly ash chemical properties analysis

Analyzing the chemical properties of fly ash mainly involved in the determination of contents of Pb, Hg, Cr, Cd, Pb, P, K, Mg elements, total nitrogen (TN), available phosphorus (AP), available potassium (AK), organic matter (OM). The heavy metal elements in the ash were digested by microwave digestion (HJ 832-2017), followed determination by ICP-MS. P element was determined with alkali-soluble-molybdenum-antimony anti-spectrophotometry (LY/T 1232-2015). Determination of K element adopts alkali solution-flame photometry (GB-7854-87). Mg element was measured by Hydrogen krypton acid-perchloric acid digestion method (NY/T 296-1995). Kjeldahl nitrogen determination method was used to analyze the total nitrogen content of fly ash (Nozawa et al., 2005). The contents of available phosphorus, available potassium and organic matter in fly ash were determined using a soil nutrient rapid tester (TPY-8A) and corresponding reagents (purchased from Zhejiang Top Yunnong Technology Co., Ltd.) according to the instructions.

Total DNA extraction of fly ash and Illumina HiSeq 2500 sequencing

Total DNA from fly ash was extracted by the Biomarker Soil Genomic DNA Kit (Beijing, China). For each fly ash sample, total DNA extractions were performed with three replications. Then, archaeal V4–V5 region of the 16S rRNA gene was amplified with forward primer 524F: 5'-TGYCAGCCGC-CGCGGTAA-3' and reverse primer Arch958R: 5'-YCCGGCGTTGAVTCCAATT-3'. The PCR thermocycling program was as follows: 95 °C for 3 min, followed by 35 cycles of 30 s at 95 °C, 30 s at 55 °C, and 45 s at 72 °C, with a final extension at 72 °C for 10 min and a hold temperature of 16 °C. The PCR products from each sample were examined by electrophoresis on a 1% agarose gel. The PCR products were recovered from the agarose using the AxyPrep DNA Gel Recovery Kit (AXYGEN, USA). Finally, high-throughput sequencing was performed on Illumina HiSeq 2500 sequencing platform at Beijing Biomarker Technologies Co, LTD (Beijing, China).

Quality screening of sequencing data

The original data were assembled by using FLASH software, version 1.2.11 (Magoc and Salzberg, 2011), and the high quality sequences were filtered by Trimmomatic software, version 0.33 (Bolger et al., 2014). We then used UCHIME, version 8.1 (Edgar et al., 2011), to remove any potential undesirable artifacts in the data such as adapters or low quality or “N” bases and reads shorter than 50 bp, to get the final high-quality tags sequence. The sequencing raw tags data has been submitted to the Genome Sequence Archive (<http://gsa.big.ac.cn/>) under access number CRA005791.

Analysis of archaea diversity in fly ash

USEARCH software (Edgar, 2013) was used to cluster the clean tags into OTU (Operational Taxonomic Units) with a cutoff of 97% similarity, and taxonomic annotations of OTU sequences were finished based on SILVA (bacteria) (<http://www.arb-silva.de>) and UNITE (fungi) (<https://unite.ut.ee>) database, respectively. The Alpha diversity index of the samples was evaluated using Mothur (version v.1.30) software (Chappidi et al., 2019). The QIIME2 software was used to analyze Beta diversity (Hall and Beiko, 2018). The biochemical properties data of *X. sorbifolia* tissues and fly ash were analyzed by GraphPad Prism 6.1 (Mitteer et al., 2020). The redundancy and correlation clustering labeled heat map analyses were finished using R version 3.6.1 (<https://www.r-project.org/>). The GraphPad Prism version 6.1 and Data Processing System (DPS) version 7.05 softwares were used for statistical analysis by using the analysis of variance (ANOVA) and LSD multiple range test to calculate the significant difference among treatments ($p < 0.05$) (Mitteer et al., 2020).

Results

Planting Xanthoceras sorbifolium Bunge reduced the contents of heavy metal elements but improved the fertility of rhizosphere fly ash

After three months growth of *X. sorbifolia* plants, the contents of five heavy metals (Pb, Hg, Cr, Cd and As) in rhizosphere fly ash decreased compared with CK group (Table 1). Among them, Pb and Cd elements were detected in the taproots, fibrous roots, stems and leaf of the *X. sorbifolia*, while Cr and As elements were mainly detected in the taproots, fibrous roots, stems. In addition, the content of Hg decreased significantly in S group, but it was not detected in roots, stems, or leaves of *X. sorbifolia* plants. These results indicated that *X. sorbifolia* can absorb these heavy metals through its root system and migrate them to other tissues, such as stem and leaf.

The contents of P, K, and Mg of CK group were 0.40 g/kg, 2.34 g/kg, 0.62 g/kg, respectively, while the contents of P, K, and Mg in S group were higher than that of CK, which were 0.46 g/kg, 2.66 g/kg, 0.71 g/kg, respectively. These results suggested that planting *X. sorbifolia* on fly ash can increase the contents of P, K, and Mg elements (Fig. 1). Also as shown in Table 2, the total nitrogen, available P, available K, and organic matter of S group increased compared with CK. Taken together, the soil fertility of fly ash was improved after planting *X. sorbifolia*.

Table 1. Heavy metal contents of different *X. sorbifolia* tissues and two groups of rhizosphere fly ash samples

Heavy metal content	Pb (mg/kg)	Hg (mg/kg)	Cr (mg/kg)	Cd (mg/kg)	As (mg/kg)
CK	45.54±0.48a	0.20±0.04a	74.50±1.81a	0.22±0.02a	4.91±0.06a
S	45.26±0.57a	0.09±0.01b	69.92±1.1b	0.17±0.02a	4.23±0.14c
Taproot	0.52±0.11	Not detected	4.25±0.12	0.13±0.03	0.05±0.01
Fibrous root	3.24±0.21	Not detected	14.71±0.06	0.39±0.03	0.45±0.11
Stem	0.28±0.05	Not detected	9.69±0.05	0.10±0.01	Not detected
Leaf	0.10±0.01	Not detected	Not detected	0.10±0.02	Not detected

Note: CK represents the fly ash without planting *X. sorbifolia*, S represents the fly ash after planting *X. Sorbifolia*. Statistically significant differences (“a” is different from “b” or “c”, $\alpha = 0.05$ level) of values are indicated with different letters with analysis of variance

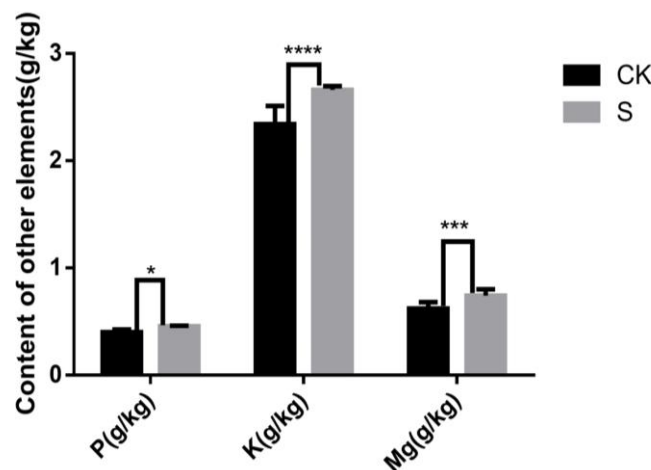


Figure 1. The contents of P, K and Mg in CK and S group samples. Error bar indicates standard deviation, *, ***, **** were significantly different at $P < 0.05$, 0.001 and 0.0001 levels, respectively (Student's t-test, $n = 3$)

Table 2. The total nitrogen (TN), available P (AP), available K (AK), and organic matter (OM) contents in CK and S group samples

Chemical properties	TN (mg/kg)	AP (mg/kg)	AK (mg/kg)	OM (mg/kg)
CK	880.00±25.50a	41.80±0.92a	150.01±1.49a	9640.00±237.91a
S	894.66±10.81a	51.90±0.81c	153.54±1.73a	10,370.00±161.71b

Note: CK represents the fly ash without planting *X. sorbifolia*. S represents the fly ash after planting *X. Sorbifolia*, TN, total nitrogen; AP, available phosphorus; AK, available potassium; OM, the organic matter. Statistically significant differences (“a” is different from “b” or “c”, $\alpha = 0.05$ level) of values are indicated with different letters with analysis of variance

Archaea composition and structure analysis of fly ash

A total of 481,184 archaeal 16S rRNA raw sequences were generated. After removing the short sequences, adapters and low-quality sequences, 314,803 16S rRNA high-quality sequences were obtained for the subsequent analysis with at least 48,690

clean tags produced in each sample. Based on these clean tags, we identified 405 Operational Taxonomic Units (OTU) in CK1, 383 in CK2, 180 in CK3 (Fig. S1A), while 202 for S1, 211 for S2 and 220 for S3 (Fig. S1B). Finally, a total of 991 OTU were identified in these two groups (Fig. 2), among which 526 OTU were unique to CK group, 263 OTU unique to S group and 202 OTU were shared by both groups. Compared to the CK group, the number of OTU in fly ash decreased by 50% due to the planting of *X. sorbifolia*.

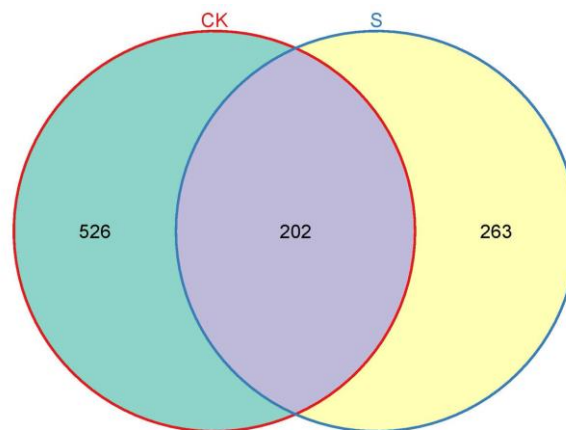


Figure 2. Venn diagram of operational taxonomic units (OTU) in Archaea composition between CK and S groups

To get the corresponding species classification information of each OTU, the OTU representative sequences were aligned to microbial reference database, then the community composition of each sample was analyzed at the taxonomic level of phylum, class, order, family, genus, species. At the phyla level (Fig. 3A), most of the archaea from two groups were assigned to *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Verrucomicrobia* and *Euryarchaeota*, among which *Firmicutes* are the most abundant. Compared to the CK group, the relative abundance of *Firmicutes*, *Euryarchaeota* and *Patescibacteria* increased by 12%, 20% and 117.3%, respectively. *Chloroflexi*, *Acidobacteria* and *Planctomycetes* decreased by 40.02%, 27.08% and 51.64%, respectively. In addition, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Verrucomicrobia* decreased slightly (Fig. 3A).

At the genus level (Fig. 3B), *Lactobacillus*, *Streptococcus*, and *Gardnerella* are the first three dominant archaea genera of two groups, and the other archaea genera include *Natronomonas*, *Thermoactinomyces*, *Aerococcus*, *Prevotella*, *Ruminococcaceae_UCG-014*, *Uncultured_bacterium_c_kD4-96*, *Weissella*. Compared to the CK group, *Streptococcus*, *Gardnerella*, *Ruminococcaceae_UCG-014* and *Weissella* increased by 39.51%, 15.84%, 110.47% and 28.76%, respectively. *Thermoactinomyces* and *Aerococcus* increased slightly, while all the other genera decreased slightly (Fig. 3B).

The heat map and cluster analysis results of 100 genera in all samples were shown in Fig. 4, clearly showing the differences in the archaea composition of rhizosphere fly ash between CK and S groups. The results show that the abundance of *Blautia* and *Weissella* increased after planting *X. sorbifolia*, but the abundance of *Bacillus*, *Mycoplasma*, *Bacteroides*, *Sneathia*, *Bradyrhizobium*, *Aeromonas*, *Anaerolinea* decreased.

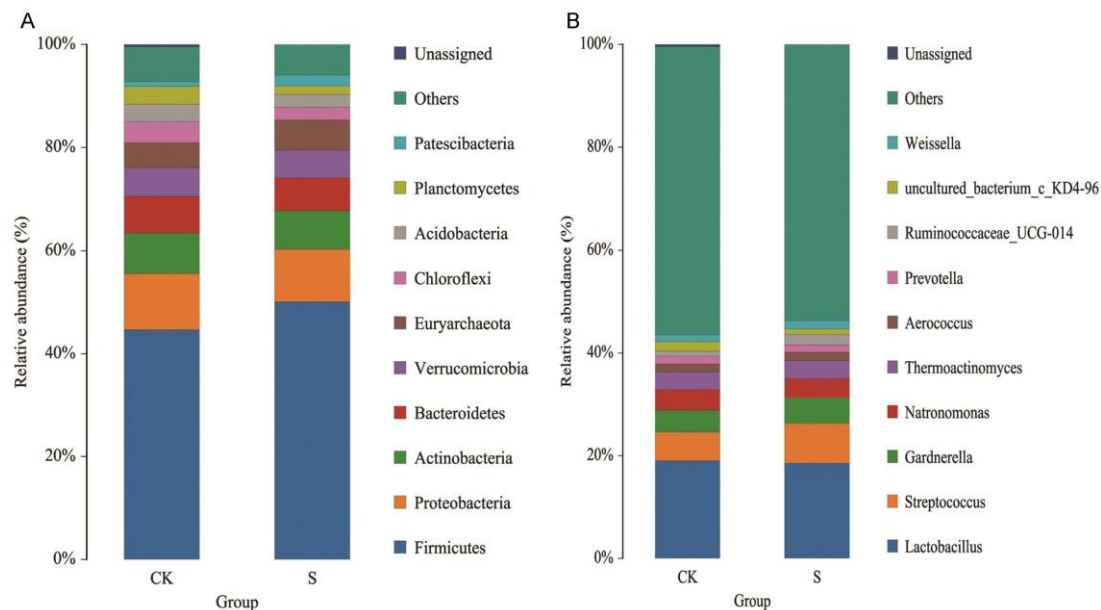


Figure 3. Species distribution map of operational taxonomic units (OTU) in Archaea composition between CK and S groups. (A) the distribution of species at phylum level, (B) the distribution of species at genus level

Alpha index analysis

The OTU coverage of CK and S groups were 99.96% and 99.97%, respectively, indicating that the sample size was large enough and the sequencing data could represent the true situation of the microbes in the fly ash samples. Alpha diversity reflects the species abundance and diversity, and it has a variety of metrics: Chao1, Ace, Shannon, and Simpson (Willis, 2019). Chao1 and Ace index measure species abundance, that is, the number of species (Chernov et al., 2015). Compared to the CK, Ace and Chao1 values both decreased after planting *X. sorbifolia* (S), indicating that the number of archaea in the rhizosphere fly ash decreased after planted *X. sorbifolia* (S) (Table 3). Shannon and Simpson indices were used to measure species diversity and influenced by species abundance and community evenness in sample communities (Kim et al., 2017). Under the condition of the same species abundance, the greater the evenness of each species in the community, the greater the diversity of the community (Grice et al., 2009). The larger the Shannon index value is, the smaller the Simpson index value is, indicating the higher the species diversity of the sample (Wang et al., 2012). Compared with non-planting *X. sorbifolia* (CK), Shannon index decreased after planted *X. sorbifolia* (S), while Simpson index was on the contrary, indicating that the diversity of rhizosphere fly ash decreased after planted *X. sorbifolia*. Rarefaction curve is used to reflect the species diversity and indirectly reflect the species richness in the sample (Dubois et al., 2010; Izsák and Pavoine, 2012). The rarefaction curve (Fig. 5A) indicated that the OTU number decreased after planting *X. sorbifolia*. The rank abundance curve (Fig. 5B) also showed that the species abundance of rhizosphere fly ash decreased after planting *X. sorbifolia*.

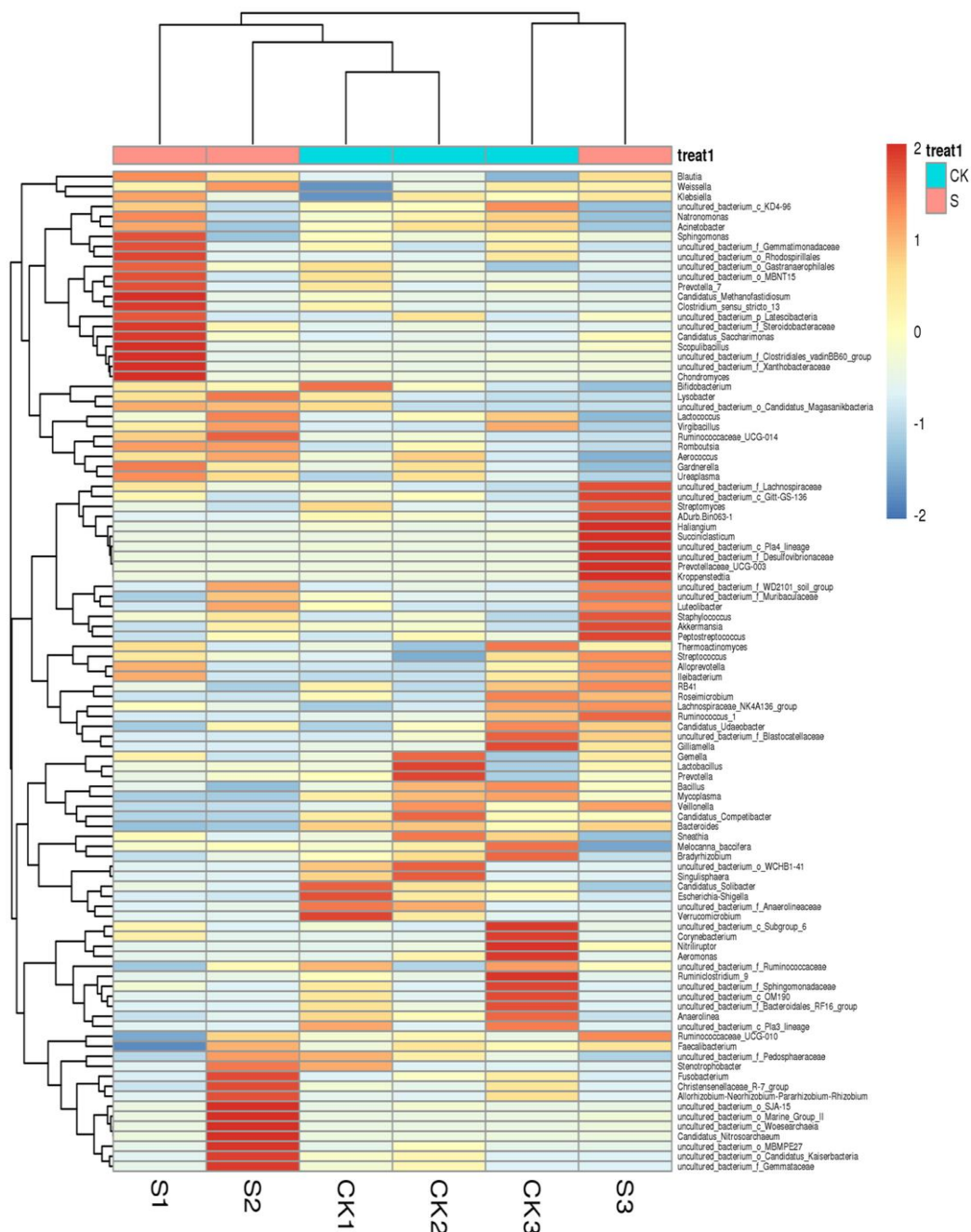


Figure 4. The clustering heat map of Archaea species abundance at the genus level between CK and S groups

Table 3. Alpha diversity index in CK and S groups

Sample ID	OTU	ACE	Chao1	Simpson	Shannon	Coverage
CK	323.0±101.3	339.2±95.6	342.6±103.6	0.0312±0.01	4.6±0.3	1.0±0.0
S	211.0±7.4	244.8±21.3	240.1±10.6	0.0345±0.00	4.2±0.1	1.0±0.0

Note: Fly ash without planting *X. sorbifolia* (CK) and fly ash after planting *X. sorbifolia* (S)

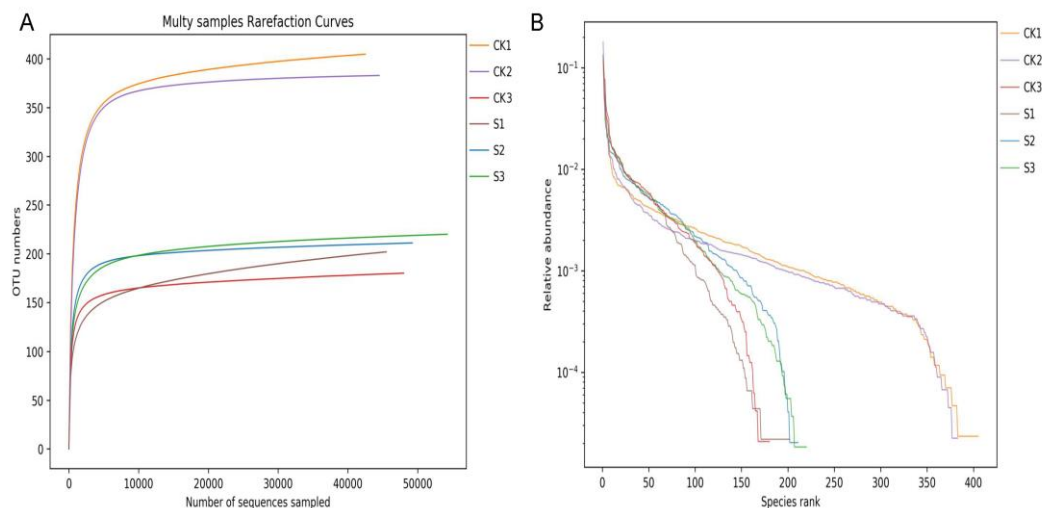


Figure 5. Alpha diversity index of Archaea species abundance between CK and S groups. (A) Sample rarefaction curve; (B) Sample rank abundance curve

Beta diversity analysis

Beta diversity analysis is used to analyze species composition changes on time and space scales (Socolar et al., 2016). Principal component analysis (PCA) was shown in Fig. 6A, the contribution value of the first principal component (PC1) to the sample variance is 32.32%, and the second principal component (PC2) is 22.51%. Principal coordinates analysis (PCoA) was shown in Fig. 6B, and the PCoA1 and PCoA2 are 23.53% and 21.40%, respectively. The results of Non-Metric Multi-Dimensional Scaling (NMDS) analysis showed that the stress value is equal to 0.003, suggesting that these points in each group are very close to each other (Fig. 6C). The Unweighted Pair-group Method with Arithmetic Mean (UPGMA) results (Fig. 6D) also supported that there existed significant differences between S and CK groups. In addition, sample clustering heat map (Fig. S2) still indicated the distance between CK and S samples is far. These results implied that the high correlations of the replicated samples were reliable for further analysis.

BugBase function prediction

BugBase is a tool for measuring high-level phenotypes in complex microbiome, such as oxygen tolerance, gram staining and pathogenic potential (Zhang et al., 2018). Fig. 7 showed nine phenotypes at the phylum level, respectively, including aerobic (Fig. 7A), anaerobic (Fig. 7B), mobile element containing (Fig. 7C), facultative anaerobic (Fig. 7D), biofilm forming (Fig. 7E), gram-negative (Fig. 7F), gram-positive (Fig. 7G), potentially pathogenic (Fig. 7H), stress tolerant (Fig. 7I). Among them, the relative abundance of aerobic (Fig. 7A), mobile element containing (Fig. 7C), facultative anaerobic (Fig. 7D), biofilm forming (Fig. 7E), gram-negative (Fig. 7F), potentially pathogenic (Fig. 7H) species decreased compared with CK group. However, the relative abundance of anaerobic (Fig. 7B), gram-positive (Fig. 7G) and stress tolerant (Fig. 7I) species increased.

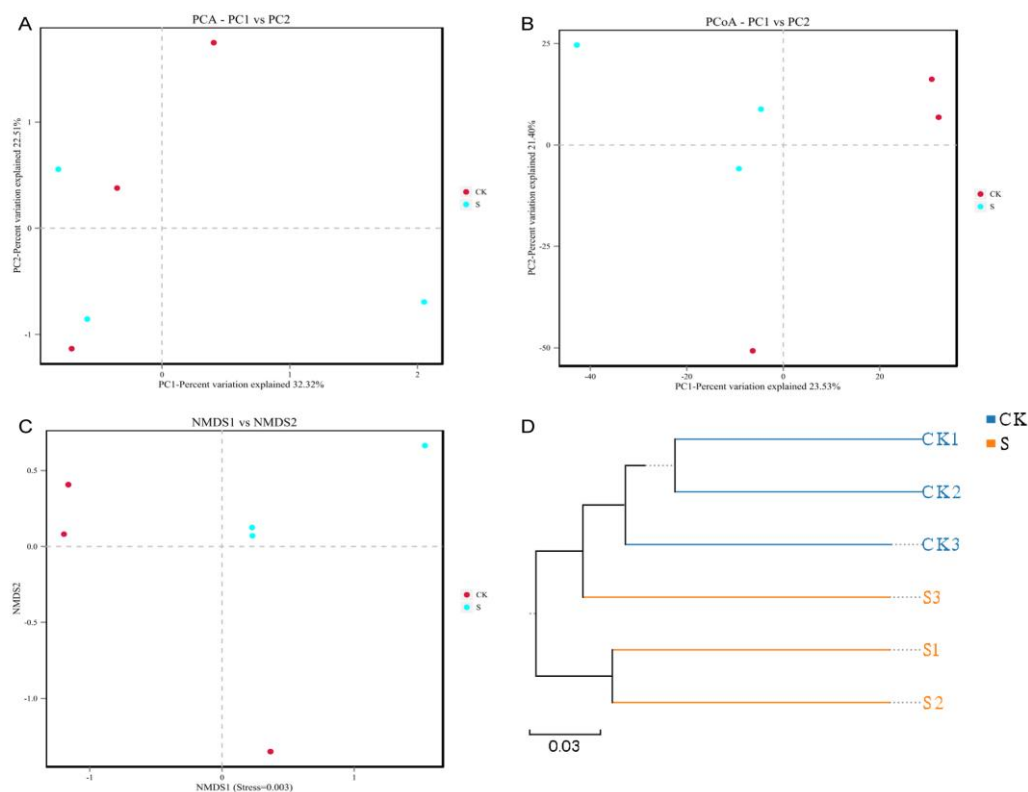


Figure 6. β -diversity analysis of rhizosphere fly ash archaea composition in CK and S groups. (A) PCA analysis; (B) PCoA analysis; (C) NMDS analysis

Relationship between archaea and physicochemical properties of rhizosphere fly ash

The redundancy analysis (RDA) results of the top ten archaea (*Planctomycetes*, *Acidobacteria*, *Chloroflexi*, *Verrucomicrobia*, *Bacteroidetes*, *Firmicutes*, *Euryarchaeota*, *Proteobacteria*, *Patescibacteria* and *Actinobacteria*) and environmental factors (heavy metals, P, K, Mg, TN, AK, OM and AP) of rhizo-fly ash were shown in Fig. 8. The impact of heavy metals on the community structure of top ten archaea was in the order of As > Hg > Cr > Pb > Cd (Red arrows in Fig. 8A). RDA1 and RDA2 explain 47.22% and 25.91% of the total variance, respectively (Fig. 8A). In detail, *Chloroflexi*, *Planctomycetes*, *Acidobacteria*, *Verrucomicrobia*, and *Bacteroidetes* were positively correlated with Hg, As, Cr and Cd, but the other five were negatively correlated (Fig. 8A). *Firmicutes*, *Euryarchaeota*, *Proteobacteria*, and *Patescibacteria* were positively correlated with Pb, while the others were positively correlated (Fig. 8A).

For the relationship of top ten archaea and P, K, Mg, the impact of these factors on the community structure of top ten archaea was in the order of K > Mg > P (Red arrows in Fig. 8B). *Firmicutes*, *Patescibacteria*, *Euryarchaeota*, *Proteobacteria*, and *Actinobacteria* were positively correlated with K, P, and *Firmicutes*, *Verrucomicrobia*, and *Bacteroidetes* were positively correlated with Mg (Fig. 8B). In addition, the impact of TN, AK, OM and AP on the community structure of top ten archaea was in the order of OM > AP > TN > AK (Red arrows in Fig. 8C). It showed that *Firmicutes*, *Euryarchaeota*, and *Actinobacteria* were positively correlated with AK, OM, AP. *Patescibacteria*, *Verrucomicrobia*, *Planctomycetes*, *Bacteroidetes*, and *Acidobacteria* was positively correlated with TN (Fig. 8C).

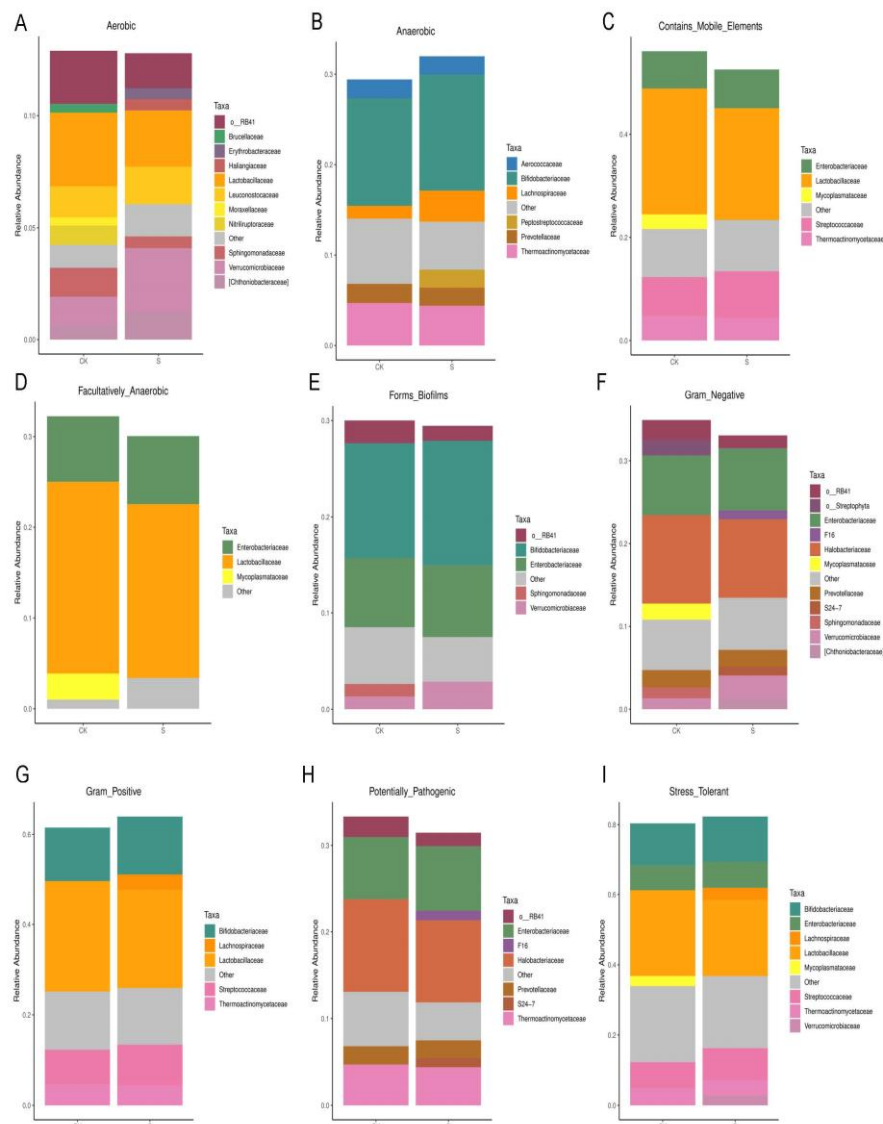


Figure 7. Relative abundance of nine archaea phenotypes at the phylum level predicted by BugBase function between CK and S groups. (A) Aerobic, (B) anaerobic, (C) mobile element containing, (D) facultatively anaerobic, (E) biofilm forming, (F) gram-negative, (G) gram-positive, (H) potentially pathogenic, (I) stress tolerant

We also clarify the relationship between top ten archaea and physicochemical properties of rhizosphere fly ash by correlation analysis (Fig. 9). The results showed that Hg was most positively correlated with *Acidobacteria* ($R=0.78$) and most negatively correlated with *Patescibacteria* ($R=-0.75$) (Fig. 9A). As was most positively correlated with *Acidobacteria* ($R=0.83$) and most negatively correlated with *Patescibacteria* ($R=-0.71$) (Fig. 9A). Cd was most positively correlated with *Chloroflexi* ($R=0.52$) and most negatively correlated with *Patescibacteria* ($R=-0.78$) (Fig. 9A). Cr was most positively correlated with *Chloroflexi* ($R=0.60$) and most negatively correlated with *Patescibacteria* ($R=-0.83$) (Fig. 9A). Mg was most significantly positively correlated with *Verrucomicrobia* ($R=0.71$) and most negatively correlated with *Proteobacteria* ($R=-0.60$) (Fig. 9B). TN was most significantly positively

correlated with *Bacteroidetes* ($R=0.49$) and most negatively correlated with *Proteobacteria* ($R=-0.26$) (Fig. 9B). P was most positively correlated with *Patescibacteria* ($R=0.52$) and most negatively correlated with *Chloroflexi* ($R=-0.77$) (Fig. 9B). AP was most positively correlated with *Patescibacteria* ($R=0.60$) and most negatively correlated with *Chloroflexi* ($R=-0.77$). OM was most positively correlated with *Patescibacteria* ($R=0.77$) and most negatively correlated with *Acidobacteria* ($R=-0.71$) (Fig. 9B). AK was most positively correlated with *Firmicutes* ($R=0.77$) and most negatively correlated with *Chloroflexi* ($R=-0.66$) (Fig. 9B). K was most positively correlated with *Firmicutes* ($R=0.71$) and most negatively correlated with *Planctomycetes* ($R=-0.83$) (Fig. 9B).

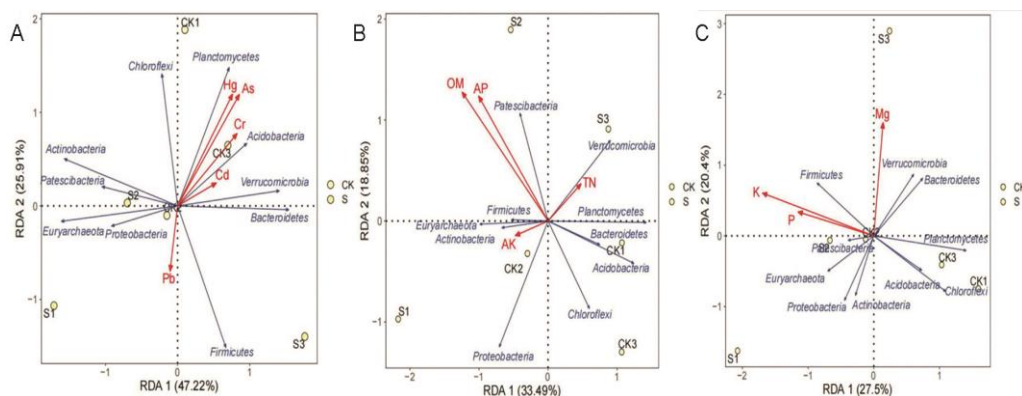


Figure 8. Redundancy analysis of physicochemical properties and 10 dominant archaea phyla between CK and S groups. (A) the relationship of heavy metals and top ten archaea; (B) the relationship of P, K, Mg and top ten archaea; (C) the relationship of TN, AK, OM and AP and top ten archaea

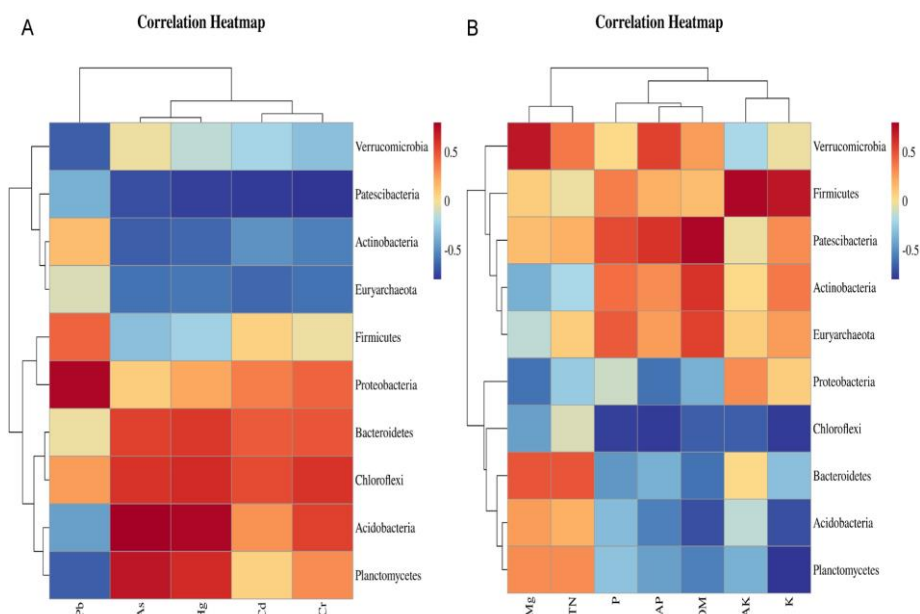


Figure 9. Heat map of correlation clustering between physicochemical properties and 10 dominant archaea phyla in CK and S groups. (A) Correlation clustering of heavy metals and

top ten archaea. (B) Correlation clustering of P, K, Mg, TN, AK, OM and AP and top ten archaea

Discussion

Over the years, people have been exploring the utilization and treatment of fly ash, and most of them are used as filling materials in cement or building materials (Zyryanov and Zyryanov, 2010). Actually, fly ash can also be used to improve agricultural biomass and yield of amendment, thus it can increase the biomass of plants (He et al., 2017; Yu et al., 2019). However, the heavy metal elements in fly ash not only cause water pollution, but also affect human health (Ma et al., 2017). Phytoremediation is a green and sustainable physical and chemical remediation method for contaminated soil (Correa-Garcia et al., 2018). The ability of plants to tolerate and accumulate heavy metals is important for phytoremediation (Luo et al., 2017). In view of this, phytoremediation that could reduce heavy metal content in fly ash (Gerhardt et al., 2009; Nouri et al., 2009). In our study, Pb, Cr, Cd, and as were detected in the vegetative tissues of *X. sorbifolia* after planting, indicating that *X. sorbifolia* can absorb heavy metal elements in fly ash. Jing et al. (2007) showed that metal can be translocated from the root to aerial tissues (stems and leaves). Many studies also show that heavy metals elements in fly ash can be accumulated in plants (Raj and Maiti, 2020; Sahoo et al., 2020; Varshney et al., 2020).

Our study found that the content of Hg was also significantly reduced, but it was not found in the tissues of *X. sorbifolia*. Studies have shown that soil microorganisms could adsorb and degrade heavy metals, so we speculated that it might because soil microorganisms could adsorb Hg (Gavrilescu, 2004). The heavy metal elements in fly ash can be transported to the *X. sorbifolia*, and some studied also showed the ways to reduce heavy metal elements in fly ash, but the process is complicated (Meer and Nazir, 2017). Therefore, Planting *X. sorbifolia* would be an environmentally friendly and simple way to reduce the content of heavy metals elements in fly ash, and then reduce the harm of fly ash to the environment and organisms.

Fly ash also contains a series of basic elements for plant growth (He et al., 2017), and some elements are generally low in solubility (Belviso et al., 2015). Our study also showed that planting *X. sorbifolia* increased the contents of P, K and Mg elements in fly ash, which we suspected was due to some rhizosphere microorganisms converting some elements that could not be directly used by plants into elements that could be used by plants. For example, potassium solubilizing microorganisms (KSMs) can dissolve insoluble potassium (K) into soluble potassium to promote plant growth and yield (Meena et al., 2014). There are also soil microorganisms that produce a range of phosphatases, capable of utilizing various forms of soil organophosphorus and effectively releasing orthophosphates from soil organophosphorus (Richardson and Simpson, 2011).

Some studies have shown that adding fly ash to soil can change the microbial activity in soil (Nayak et al., 2015; Leclercq-Dransart et al., 2019), further to increase the onion yield (Parab et al., 2015). However, there is few research on the microbial diversity of fly ash by planting plants in fly ash. Microorganisms can affect the physical and chemical properties of soil and nutrients needed for crop cultivation (Jiang et al., 2017). In this study, 16S rRNA and Illumina MiSeq sequencing were analyzed to reveal that the planting of *X. sorbifolia* changed the microbial diversity and community structure of

fly ash rhizosphere. Previous studies have shown that different vegetation types have different effects on soil microbial diversity (Millard and Singh, 2009). Our study found that planting *X. sorbifolia* in fly ash reduced the biodiversity of *X. sorbifolia* in fly ash. However, *Firmicutes* were the first dominant phylum in fly ash. After *X. sorbifolia* was planted, the abundance of *Firmicutes* increased to a certain extent. *Firmicutes* had high resistance to heavy metal elements (Fajardo et al., 2019), thus it can drive efficient soil respiration (Cleveland et al., 2006), inhibit bacterial wilt (Lee et al., 2021), and are positively correlated with plant health (Xiong et al., 2015). *Euryarchaeota* is an important and ubiquitous archaea that contributes significantly to the global energy cycle (Hu et al., 2013), and participates in the nitrogen cycle and the carbon fixed (Ren et al., 2018), whose abundance is increased. In the soil contaminated with long-term high concentration of heavy metals, *Proteobacteria* are the most abundant phylum soil microorganisms, which not only interact with each other, but also interact with the surrounding environment (Lin et al., 2021). We guess that the reason for the decrease of *Proteobacteria* abundance is the decrease of heavy metal elements in fly ash (Lin et al., 2021). In addition, in the RDA analysis, *Firmicutes* and *Euryarchaeota* were positively correlated with K, P, organic matter and available phosphorus. This also proves that the improvement of soil fertility after planting *X. sorbifolia* is related to the change of archaea community structure.

BugBase's prediction of biolevel trait coverage has higher sensitivity and can clearly reflect changes in microbial phenotypes. We found that BugBase provides easily interpretable biological properties and greatly improves the power and interpretability of microbiome studies (Ward et al., 2017). Among them, anaerobic bacteria play a role in soil disinfection and can eliminate soil-borne phytopathogens in agriculture (Ueki et al., 2018). Gram-positive bacteria play a role in resistance to drought stress, and the abundance of archaea with pathogenic potential has decreased (Su et al., 2020). In short, after planting *X. sorbifolia* in fly ash, the content of heavy metals in fly ash decreased, and the fertility of fly ash increased. Although the soil archaea diversity of fly ash decreased, the abundance of some beneficial archaea increased, which provided some ideas for the improvement and utilization of fly ash.

Conclusion

After planting *X. sorbifolia* on the fly ash, the heavy metal elements were detected in the main root, fibrous root, stem and leaf of *X. sorbifolia*, and the content of heavy metal elements in fly ash decreased. The contents of P, K, Mg, total nitrogen, available phosphorus, available potassium, the organic matter in fly ash increased after planting *X. sorbifolia*, which elevated the fertility of fly ash. The planting of *X. sorbifolia* can change the archaea structure and composition of rhizosphere fly ash, and the number of OTU and microbial diversity of archaea decreased. However, the abundance of beneficial bacteria such as *Firmicutes* and *Euryarchaeota* increased after planting *X. sorbifolia*. BugBase function prediction showed that the number of aerobic, mobile element containing, facultatively anaerobic, biofilm forming, potentially pathogenic decreased, whereas the abundance of anaerobic, gram-negative, gram-positive, stress tolerant species increased after planting *X. sorbifolia*. Planting *X. sorbifolia* on fly ash has certain effect on the repair and utilization of fly ash.

Acknowledgement. Thanks to Ms. Zehui Liu for her help during the experiment.

Funding. This work was financially supported by the National Natural Science Foundation of China (52071192, 51804191), Key R & D projects in Datong City (2019023), the Doctoral Research Initiation Foundation project of Shanxi Datong University (2019-B-03), Shanxi New Carbon Functional Materials Engineering Research Center, the Shanxi 1331 Project Foundation for Graphene Industrialization Application Technology of Collaborative Innovation Center, the Platform and Base Project of Datong (2020190).

REFERENCES

- [1] Belviso, C., Cavalcante, F., Di Gennaro, S., Palma, A., Ragone, P., Fiore, S. (2015): Mobility of trace elements in fly ash and in zeolitised coal fly ash. – *Fuel* 144: 369-379.
- [2] Bolger, A. M., Lohse, M., Usadel, B. (2014): Trimmomatic: a flexible trimmer for Illumina sequence data. – *Bioinformatics* 30(15): 2114-20.
- [3] Chappidi, S., Villa, E. C., Cantarel, B. L. (2019): Using Mothur to Determine Bacterial Community Composition and Structure in 16S Ribosomal RNA Datasets. – *Curr Protoc Bioinformatics* 67(1): e83.
- [4] Chernov, T. I., Tkachakhova, A. K., Kutovaya, O. V. (2015): Assessment of diversity indices for the characterization of the soil prokaryotic community by metagenomic analysis. – *Eurasian Soil Science* 48(4): 410-415.
- [5] Cleveland, C. C., Nemergut, D. R., Schmidt, S. K., Townsend, A. R. (2006): Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. – *Biogeochemistry* 82(3): 229-240.
- [6] Correa-Garcia, S., Pande, P., Seguin, A., St-Arnaud, M., Yergeau, E. (2018): Rhizoremediation of petroleum hydrocarbons: a model system for plant microbiome manipulation. – *Microb Biotechnol* 11(5): 819-832.
- [7] Dubois, P. C. et al. (2010): Multiple common variants for celiac disease influencing immune gene expression. – *Nat Genet* 42(4): 295-302.
- [8] Dzantor, K., Adeleke, E., Kankarla, V., Ogunmayowa, O., Hui, D. (2015): Using Coal Fly Ash Agriculture: Combination of Fly Ash and Poultry Litter as Soil Amendments for Bioenergy Feedstock Production. – *Coal Combustion and Gasification Products* 7(1): 33-39.
- [9] Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., Knight, R. (2011): UCHIME improves sensitivity and speed of chimera detection. – *Bioinformatics* 27(16): 2194-200.
- [10] Edgar, R. C. (2013): UPARSE: highly accurate OTU sequences from microbial amplicon reads. – *Nat Methods* 10(10): 996-8.
- [11] Fajardo, C., Costa, G., Nande, M., Botías, P., García-Cantalejo, J., Martín, M. (2019): Pb, Cd, and Zn soil contamination: Monitoring functional and structural impacts on the microbiome. – *Applied Soil Ecology* 135: 56-64.
- [12] Gavrilescu, M. (2004): Removal of Heavy Metals from the Environment by Biosorption. – *Engineering in Life Sciences* 4(3): 219-232.
- [13] Gerhardt, K. E., Huang, X.-D., Glick, B. R., Greenberg, B. M. (2009): Phytoremediation and rhizoremediation of organic soil contaminants: Potential and challenges. – *Plant Science* 176(1): 20-30.
- [14] Ghazali, N., Muthusamy, K., Wan Ahmad, S. (2019): Utilization of Fly Ash in Construction. – *IOP Conference Series: Materials Science and Engineering* 601(1): 012023.
- [15] Grice, E. A., Kong, H. H., Conian, S., Deming, C. B., Davis, J., Young, A. C., Bouffard, G. G., Blakesley, R. W., Murray, P. R., Green, E. D., Turner, M. L., Segre, J. A. (2009): Topographical and temporal diversity of the human skin microbiome. – *Science* 324(5931): 1190-2.

- [16] Hall, M., Beiko, R. G. (2018): 16S rRNA Gene Analysis with QIIME2. – *Methods Mol Biol* 1849: 113-129.
- [17] He, Y., Luo, Q., Hu, H. (2012): Situation Analysis and Countermeasures of China's Fly Ash Pollution Prevention and Control. – *Procedia Environmental Sciences* 16: 690-696.
- [18] He, H., Dong, Z., Peng, Q., Wang, X., Fan, C., Zhang, X. (2017): Impacts of coal fly ash on plant growth and accumulation of essential nutrients and trace elements by alfalfa (*Medicago sativa*) grown in a loessial soil. – *J Environ Manage* 197: 428-439.
- [19] Hu, H.-W., Zhang, L.-M., Yuan, C.-L., He, J.-Z. (2013): Contrasting Euryarchaeota communities between upland and paddy soils exhibited similar pH-impacted biogeographic patterns. – *Soil Biology and Biochemistry* 64: 18-27.
- [20] Izsák, J., Pavoine, S. (2012): Links between the species abundance distribution and the shape of the corresponding rank abundance curve. – *Ecological Indicators* 14(1): 1-6.
- [21] Jiang, J., Song, Z., Yang, X., Mao, Z., Nie, X., Guo, H., Peng, X. (2017): Microbial community analysis of apple rhizosphere around Bohai Gulf. – *Sci Rep* 7(1): 8918.
- [22] Jing, Y. D., He, Z. L., Yang, X. E. (2007): Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. – *J Zhejiang Univ Sci B* 8(3): 192-207.
- [23] Kim, B. R., Shin, J., Guevarra, R., Lee, J. H., Kim, D. W., Seol, K. H., Lee, J. H., Kim, H. B., Isaacson, R. (2017): Deciphering Diversity Indices for a Better Understanding of Microbial Communities. – *J Microbiol Biotechnol* 27(12): 2089-2093.
- [24] Leclercq-Dransart, J., Demuyne, S., Bidar, G., Douay, F., Grumiaux, F., Louvel, B., Pernin, C., Lepître, A. (2019): Does adding fly ash to metal-contaminated soils play a role in soil functionality regarding metal availability, litter quality, microbial activity and the community structure of Diptera larvae? – *Applied Soil Ecology* 138: 99-111.
- [25] Lee, S. M., Kong, H. G., Song, G. C., Ryu, C. M. (2021): Disruption of Firmicutes and Actinobacteria abundance in tomato rhizosphere causes the incidence of bacterial wilt disease. – *ISME J* 15(1): 330-347.
- [26] Li, J., Zu, Y.-G., Fu, Y.-J., Yang, Y.-C., Li, S.-M., Li, Z.-N., Wink, M. (2010): Optimization of microwave-assisted extraction of triterpene saponins from defatted residue of yellow horn (*Xanthoceras sorbifolia* Bunge.) kernel and evaluation of its antioxidant activity. – *Innovative Food Science & Emerging Technologies* 11(4): 637-643.
- [27] Lin, H., Liu, C., Li, B., Dong, Y. (2021): *Trifolium repens* L. regulated phytoremediation of heavy metal contaminated soil by promoting soil enzyme activities and beneficial rhizosphere associated microorganisms. – *J Hazard Mater* 402: 123829.
- [28] Liu, Y., Huang, Z., Ao, Y., Li, W., Zhang, Z. (2013): Transcriptome analysis of yellow horn (*Xanthoceras sorbifolia* Bunge): a potential oil-rich seed tree for biodiesel in China. – *PLoS One* 8(9): e74441.
- [29] Luo, Q., Wang, S., Sun, L. N., Wang, H. (2017): Metabolic profiling of root exudates from two ecotypes of *Sedum alfredii* treated with Pb based on GC-MS. – *Sci Rep* 7: 39878.
- [30] Ma, S.-H., Xu, M.-D., Qiqige, Wang, X.-H., Zhou, X. (2017): Challenges and Developments in the Utilization of Fly Ash in China. – *International Journal of Environmental Science and Development* 8(11): 781-785.
- [31] Magoc, T., Salzberg, S. L. (2011): FLASH: fast length adjustment of short reads to improve genome assemblies. – *Bioinformatics* 27(21): 2957-63.
- [32] Meena, V. S., Maurya, B. R., Verma, J. P. (2014): Does a rhizospheric microorganism enhance K⁺ availability in agricultural soils? – *Microbiological Research* 169(5-6): 337-347.
- [33] Meer, I., Nazir, R. (2017): Removal techniques for heavy metals from fly ash. – *Journal of Material Cycles and Waste Management* 20(2): 703-722.
- [34] Millard, P., Singh, B. K. (2009): Does grassland vegetation drive soil microbial diversity? – *Nutrient Cycling in Agroecosystems* 88(2): 147-158.

- [35] Mitteer, D. R., Greer, B. D., Randall, K. R., Briggs, A. M. (2020): Further Evaluation of Teaching Behavior Technicians to Input Data and Graph Using GraphPad Prism. – Behav Anal (Wash D C) 20(2): 81-93.
- [36] Nayak, A. K., Raja, R., Rao, K. S., Shukla, A. K., Moharthy, S., Shahid, M. (2015): Effect of fly ash application on soil microbial response and heavy metal accumulation in soil and rice plant. – Ecotoxicol Environ Saf 114: 257-62.
- [37] Nouri, J., Khorasani, N., Lorestani, B., Karami, M., Hassani, A. H., Yousefi, N. (2009): Accumulation of heavy metals in soil and uptake by plant species with phytoremediation potential. – Environmental Earth Sciences 59(2): 315-323.
- [38] Nozawa, S., Hakoda, A., Sakaida, K., Suzuki, T., Yasui, A. (2005): Method Performance Study of the Determination of Total Nitrogen in Soy Sauce by the Kjeldahl Method. – Analytical Sciences 21(9): 1129-1132.
- [39] Parab, N., Sinha, S., Mishra, S. (2015): Coal fly ash amendment in acidic field: Effect on soil microbial activity and onion yield. – Applied Soil Ecology 96: 211-216.
- [40] Raj, D., Maiti, S. K. (2020): Risk assessment of potentially toxic elements in soils and vegetables around coal-fired thermal power plant: a case study of Dhanbad, India. – Environ Monit Assess 192(11): 699.
- [41] Ram, L. C., Srivastava, N. K., Jha, S. K., Sinha, A. K., Masto, R. E., Selvi, V. A. (2007): Management of lignite fly ash for improving soil fertility and crop productivity. – Environ Manage 40(3): 438-52.
- [42] Ram, L. C., Masto, R. E. (2014): Fly ash for soil amelioration: A review on the influence of ash blending with inorganic and organic amendments. – Earth-Science Reviews 128: 52-74.
- [43] Ren, M., Zhang, Z., Wang, X., Zhou, Z., Chen, D., Zeng, H. (2018): Diversity and Contributions to Nitrogen Cycling and Carbon Fixation of Soil Salinity Shaped Microbial Communities in Tarim Basin. – Front Microbiol 9: 431.
- [44] Richardson, A. E., Simpson, R. J. (2011): Soil microorganisms mediating phosphorus availability update on microbial phosphorus. – Plant Physiol 156(3): 989-96.
- [45] Sahoo, C. R., Sahu, R. K., Padhy, R. N. (2020): Growth, yield and element-analysis of rice (*Oryza sativa* L.), grown in soil amended with fly ash and municipal biosolids. – Paddy and Water Environment 19(1): 149-157.
- [46] Schönegger, D., Gómez-Brandón, M., Mazzier, T., Insam, H., Hermanns, R., Leijenhorst, E., Bardelli, T., Fernández-Delgado Juárez, M. (2018): Phosphorus fertilising potential of fly ash and effects on soil microbiota and crop. – Resources, Conservation and Recycling 134: 262-270.
- [47] Socolar, J. B., Gilroy, J. J., Kunin, W. E., Edwards, D. P. (2016): How Should Beta-Diversity Inform Biodiversity Conservation? – Trends Ecol Evol 31(1): 67-80.
- [48] Su, Y. G., Liu, J., Zhang, B. C., Zhao, H. M., Huang, G. (2020): Habitat-specific environmental factors regulate spatial variability of soil bacterial communities in biocrusts across northern China's drylands. – Sci Total Environ 719: 137479.
- [49] Sunjidmaa, D., Batdemberel, G., Takibai, S. (2019): A Study of Ferrospheres in the Coal Fly Ash. – Open Journal of Applied Sciences 09(01): 10-16.
- [50] Tadesse, A. W., Gereslassie, T., Yan, X., Wang, J. (2019): Determination of Heavy Metal Concentrations and Their Potential Sources in Selected Plants: *Xanthium strumarium* L. (Asteraceae), *Ficus exasperata* Vahl (Moraceae), *Persicaria attenuata* (R.Br) Sojak (Polygonaceae), and *Kanahia laniflora* (Forssk.) R.Br. (Asclepiadaceae) from Awash River Basin, Ethiopia. – Biol Trace Elem Res 191(1): 231-242.
- [51] Thind, H. S., Yadvinder, S., Bijay, S., Varinderpal, S., Sharma, S., Vashistha, M., Singh, G. (2012): Land application of rice husk ash, bagasse ash and coal fly ash: Effects on crop productivity and nutrient uptake in rice–wheat system on an alkaline loamy sand. – Field Crops Research 135: 137-144.

- [52] Ueki, A., Kaku, N., Ueki, K. (2018): Role of anaerobic bacteria in biological soil disinfection for elimination of soil-borne plant pathogens in agriculture. – *Applied Microbiology and Biotechnology* 102(15): 6309-6318.
- [53] Varshney, A., Mohan, S., Dahiya, P. (2020): Growth and antioxidant responses in plants induced by heavy metals present in fly ash. – *Energy, Ecology and Environment* 6(2): 92-110.
- [54] Wang, Y., Sheng, H. F., He, Y., Wu, J. Y., Jiang, Y. X., Tam, N. F., Zhou, H. W. (2012): Comparison of the levels of bacterial diversity in freshwater, intertidal wetland, and marine sediments by using millions of illumina tags. – *Appl Environ Microbiol* 78(23): 8264-71.
- [55] Wang, Z., Deguchi, Y., Watanabe, H., Kurose, R., Yan, J., Liu, J. (2015): Improvement on Quantitative Measurement of Fly Ash Contents Using Laser-Induced Breakdown Spectroscopy. – *Journal of Flow Control, Measurement & Visualization* 03(01): 10-21.
- [56] Wang, N., Sun, X., Zhao, Q., Yang, Y., Wang, P. (2020): Leachability and adverse effects of coal fly ash: A review. – *J Hazard Mater* 396: 122725.
- [57] Ward, T., Larson, J., Meulemans, J., Hillmann, B. (2017): BugBase predicts organism-level microbiome phenotypes. – *Genome Biology*.
- [58] Willis, A. D. (2019): Rarefaction, Alpha Diversity, and Statistics. – *Front Microbiol* 10: 2407.
- [59] Xiong, W., Zhao, Q., Zhao, J., Xun, W., Li, R., Zhang, R., Wu, H., Shen, Q. (2015): Different continuous cropping spans significantly affect microbial community membership and structure in a vanilla-grown soil as revealed by deep pyrosequencing. – *Microb Ecol* 70(1): 209-18.
- [60] Yu, C. L., Deng, Q., Jian, S., Li, J., Dzantor, E. K., Hui, D. (2019): Effects of fly ash application on plant biomass and element accumulations: a meta-analysis. – *Environ Pollut* 250: 137-142.
- [61] Zhang, S., Zu, Y. G., Fu, Y. J., Luo, M., Liu, W., Li, J., Efferth, T. (2010): Supercritical carbon dioxide extraction of seed oil from yellow horn (*Xanthoceras sorbifolia* Bunge.) and its anti-oxidant activity. – *Bioresour Technol* 101(7): 2537-44.
- [62] Zhang, W., Jiao, L., Liu, R., Zhang, Y., Ji, Q., Zhang, H., Gao, X., Ma, Y., Shi, H. N. (2018): The effect of exposure to high altitude and low oxygen on intestinal microbial communities in mice. – *PLoS One* 13(9): e0203701.
- [63] Zu, Y., Zhang, S., Fu, Y., Liu, W., Liu, Z., Luo, M., Efferth, T. (2009): Rapid microwave-assisted transesterification for the preparation of fatty acid methyl esters from the oil of yellow horn (*Xanthoceras sorbifolia* Bunge.). – *European Food Research and Technology* 229(1): 43-49.
- [64] Zyryanov, V. V., Zyryanov, D. V. (2010): Complex Processing of Pulverized Fly Ash by Dry Separation Methods. – *Journal of Environmental Protection* 1(03): 293-301.