



The responses of a soil bacterial community under saline stress are associated with Cd availability in long-term wastewater-irrigated field soil

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HIGHLIGHTS

- Soil salinity increased Cd availability in Cd-contaminated soil due to long-term wastewater irrigation.
- Saline stress significantly changed major soil bacteria in Cd-contaminated soil.
- Bacterial community structure is associated with soil Cd availability under saline stress.
- Seasonal variation played a critical role in altering soil physiochemical characteristics and shaping bacterial communities.

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ABSTRACT

Long-term wastewater irrigation impacts soil geochemical properties (salinity, pH and soil aggregates) and promotes the bioaccumulation of heavy metals to plants, which may change soil function, decrease soil health, decrease the productivity of farmland, and even cause land degradation. In this study, we explored the impact of saline stress on soil bacterial communities and Cd availability in long-term wastewater-irrigated field soil. Different amounts of saline stress by adding 0.1, 0.3 and 0.5% salts in a fixed proportion (NaCl: Na₂SO₄: NaHCO₃: Na₂CO₃ = 1:9:9:1) were applied in a 1-year, multiple cropping system with winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.) in wastewater-polluted soil. Increased salinity in the soil increased Cd availability: compared with CK (no extra salts addition), addition of 0.5% mixed salts significantly increased soil available Cd up by 67.5% and grain Cd concentration by 43.7%, and adding 0.5% salts also resulted in the increases of soil pH (-0.5 unit) and electric conductivity (97.4%). Soil saline stress significantly changed major soil microbes in Cd-contaminated soil. Increased saline stress enriched taxa in the Bacillaceae, Staphylococcaceae and Pseudomonadaceae bacterial families, while one family within Proteobacteria (Sphingomonadaceae) was the most sensitive biomarker, based on Cd contamination without saline stress in CK-treated soils. Structural equation modeling (SEM) analysis revealed that soil saline stress induced an increase in soil Cd availability that was regulated by the bacterial community.

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1. Introduction

Agricultural irrigation with wastewater is inevitable, due to severe water scarcity in arid, semiarid and dry areas around the world, unfortunately the use of untreated wastewater is becoming an urgent problem especially in developing countries. Globally, at least 18 million hectares of land are irrigated by treated, untreated

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or diluted wastewater (Brzezinska et al., 2011; Cirelli et al., 2012; Verbyla et al., 2016). Wastewater irrigation has the advantage of transferring fertilizing elements (e.g. nitrogen, phosphorous, potassium), low molecular weight organic compounds, and meso/micro-nutrients into agricultural soil, all of which are essential nutritive substances for plants and soil organisms to grow (Pedrero et al., 2010; Gatta et al., 2015). More importantly, wastewater irrigation allows for disposal of treated effluents in an economical and environmentally-friendly way (Gatta et al., 2015). However, in addition to nutrients, most wastewaters contain many heavy metals, pathogenic bacteria and viruses (Stevik et al., 2004), and therefore, the environmental issues and health concerns associated with wastewater re-use cannot be ignored. Wastewater irrigation may adversely affect not only the soil structure, soil quality, and crop development but also the surrounding environment, ecological safety and human health (Awasthi et al., 2016).

Wastewater irrigation could transfer different types of metals such as Fe, Zn, Pb, Ni, Cd, and Cu, which can gradually accumulate in soil especially with long-term application. These metals are not only phytotoxic and easily accumulated in the edible parts of leafy vegetables, they also disturb soil microbial communities (Becerracastro et al., 2015). Saha et al. (2015) reported that the concentrations of heavy metals in sewage-irrigated vegetables crossed the safe limits for human consumption. Similarly, Singh et al. (2010) found that continuous application of wastewater for more than 20 years led to accumulation of heavy metal(loid)s (Cd, Cr, Cu, Ni, Pb, and Zn) in the soil and the concentrations of Cd, Pb, and Ni in all tested vegetables exceeded the critical levels. Metal accumulation in soil after wastewater irrigation was found to be associated with phosphatase activity and soil ATP content, and to suppress or alter the diversity and activity of arbuscular mycorrhizal fungi and metal resistant bacteria (Becerracastro et al., 2015).

In addition to heavy metals, high levels of dissolved inorganic substances like soluble salts are also contained in wastewater; total dissolved salts in treated wastewater can range from 200 to 3000 mg/L (Muyen et al., 2011; Ganjegunte et al., 2018). Therefore, wastewater irrigation has the potential to promote soil salinization. Salts that accumulate through evapotranspiration to a high level in soil could impose various stresses to plant growth, such as hyper-osmotic stress, oxidative stress and ion toxicity (Nakayama et al., 2006; Sou/Dakouré et al., 2013). In sodic soils, frequent ionic exchange of Na^+ and H^+ causes the dissociation of water in soil solution, resulting in increased levels of NaOH in the medium; as a consequence, the soil pH may increase to values above 10.5 (Sou/Dakouré et al., 2013). Soil pH exhibited consistent negative correlations with soil structure, and high soil pH results in the dissolution of organic matter, the disintegration of soil aggregates, and increases the extent of soil compaction (Regelink et al., 2015). These effects further alter soil microbial structure and function due to limiting air permeability and oxygen availability (Gómez-Acata et al., 2016). These structural disturbances lead to crusting and sealing on the surface of soils with high salt and pH levels, which negatively influences emergence of seedlings and growth of plant roots, ultimately leading to difficulties in planting crops (Sou/Dakouré et al., 2013). In addition, it has been reported that soil salinity affect the mobility of heavy metals. For example, when the base ions of Ca^{2+} and Mg^{2+} are more concentrated, competition with bivalent metal ions like Pb^{2+} and Cd^{2+} could be promoted, leading to the easy release of these toxic ions from soil aggregates into plant tissues (Sruthi et al., 2017). Cadmium (Cd) is considered one of the most severe environmental pollutants and has had negative impacts on the human food chain and human health, because Cd is highly toxic (Pal et al., 2017). The increase/decrease of soil Cd availability could determine the toxicity of Cd to plants and

affect the transfer of Cd from soil to the human food chain. Therefore, it is critical to study the change of soil Cd availability under saline stress in a long-term wastewater-irrigated field.

Because the growth and activity of microorganisms are sensitive to changes in soil structure and properties including nutrients, pH, temperature, and water content, the characteristics of soil bacterial community (diversity and composition) can be considered bio-indicators of soil health (Zhang et al., 2015; Gómez-Acata et al., 2016; Wang et al., 2019). The influences of wastewater irrigation on soil bacterial communities have been widely reported (Wang et al., 2017; Ibekwe et al., 2018): soil physicochemical properties like increased pH, salinity, and high levels of heavy metals affect soil microbial community size, composition, structure, function and activity. However, limited information is available to address the influence of increased salinity on soil Cd availability due to long-term wastewater irrigation. The objectives of this study were: 1) to determine the response of soil Cd availability to different amounts of saline stress in long-term wastewater-irrigated field soil; 2) to assess whether soil bacterial communities were directly affected by saline stress; and 3) to assess the relative contribution of soil structure and properties to the changes in soil bacterial activities and Cd availability.

2. Materials and methods

2.1. Study site and experimental setup

The study was conducted in the north China Plain, a major grain producing area in China, where wheat-maize double cropping is the common cropping system. The study site is located in eastern Baoding, Hebei province, China (38°76'N, 115°47'E), where sewage effluents originating from industrial wastewater and domestic sewage without proper purification treatment have been used for irrigation purposes since 1995 (Fig. 1A). This area has a temperate and continental monsoon climate (with warm and rainy summers and cold and dry winters) that is characterized by wide seasonal variation in annual average rainfall of 520 mm (80% of which falls between June and September) and temperature of 14.6 °C (Li et al., 2019). The soil in this area is mainly cinnamon soil (Argosols) according to World Reference Base for Soil Resources. Five soil cores (6 cm diameter) were taken from the plot (100 m²) as a composite sample from a 0–20 cm depth. Soil material was air-dried, thoroughly mixed and passed through a 2 mm mesh sieve. Soil characteristics at the beginning of the experiment (May 2016) are displayed in Table 1. The Cd background value in soil was 1.32 mg/kg, which significantly exceeds, by more than double, the concentration limit (0.6 mg/kg) listed in the Environmental Quality

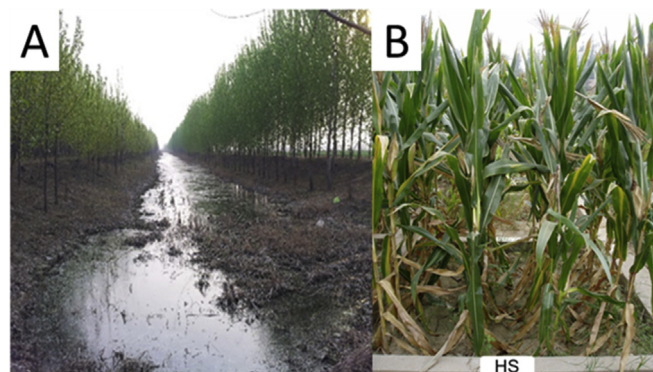


Fig. 1. The wastewater site for agricultural irrigation (A); and the growing maize in HS treatment (B). (HS: 0.5% salts addition (high stress)).

Table 1

Soil chemical and physical properties based on different saline stresses after long-term wastewater irrigation (CK: without saline stress (control), LS: 0.1% salts addition (low stress), MS: 0.3% salts addition (medium stress), and HS: 0.5% salts addition (high stress)) (these comparisons were done separately for the parameters in each column).

Treatment	pH (Water/soil = 2.5:1)	Cation Exchange Capacity (CEC) (cmol+/kg)	Organic C (g/kg)	Electric conductivity (EC) (mS/cm)	DTPA-Cd (mg/kg)	Cd concentration (mg/kg)
Before planting (May 2016)						
Original soil	7.78 ± 0.06 a	14.6 ± 0.48 a	7.86 ± 0.22 a	0.18 ± 0.01 a	0.43 ± 0.03 a	1.34 ± 0.10 a
After planting (Wheat season, May 2017)						
CK	7.76 ± 0.02 a	14.2 ± 0.55 a	7.90 ± 0.39 a	0.20 ± 0.01 a	0.39 ± 0.03 a	1.31 ± 0.09 a
LS	7.94 ± 0.07 b	12.4 ± 0.64 b	7.52 ± 0.16 b	2.38 ± 0.20 b	0.51 ± 0.05 b	1.33 ± 0.11 a
MS	8.18 ± 0.06 c	12.0 ± 0.32 cd	7.30 ± 0.42 bc	3.35 ± 0.12 c	0.60 ± 0.03 c	1.35 ± 0.26 a
HS	8.39 ± 0.05 e	11.2 ± 0.51 d	6.88 ± 0.24 c	4.89 ± 0.28 d	0.68 ± 0.02 e	1.28 ± 0.15 a
After planting (Maize season, October 2017)						
CK	7.71 ± 0.03 a	14.6 ± 0.55 a	7.82 ± 0.51 a	0.22 ± 0.01 a	0.40 ± 0.06 a	1.36 ± 0.22 a
LS	7.99 ± 0.07 b	13.6 ± 0.64 a	7.73 ± 0.42 a	2.15 ± 0.12 e	0.48 ± 0.04 ab	1.40 ± 0.19 a
MS	8.12 ± 0.03 c	12.9 ± 0.32 ab	7.69 ± 0.32 a	3.02 ± 0.15 b	0.54 ± 0.03 bc	1.27 ± 0.09 a
HS	8.29 ± 0.02 d	12.1 ± 0.29 c	7.12 ± 0.13 b	3.50 ± 0.22 c	0.62 ± 0.02 d	1.33 ± 0.08 a

Standard for Soils of China, GB15618-2018.

Because soil saline stress was not achieved in the study site, in order to illustrate the possible effect of continuous wastewater irrigation caused saline stress on soil Cd availability, the saline condition in nature was simulated based on the classification of saline soil in the north China plain. Four treatments (three replicates each) were implemented in 12 plots ($2 \times 2 \text{ m}^2$ each) under a multiple cropping of winter wheat (*Triticum aestivum* L.) and summer maize (*Zea mays* L.). Each plot was separated by cement dividers at 40 cm depth (10 cm aboveground and 30 cm belowground) to avoid irrigation water mixing and cross-contamination of treatments. Plots were arranged in a randomized complete block design. Treatments consisted of soil without saline stress (control, CK), 0.1% salts addition (low stress, LS), 0.3% salts addition (medium stress, MS), and 0.5% salts addition (high stress, HS). The following neutral and alkali salts were mixed in a fixed proportion ($\text{NaCl} : \text{Na}_2\text{SO}_4 : \text{NaHCO}_3 : \text{Na}_2\text{CO}_3 = 1:9:9:1$, $\text{pH} = 8.8$). It should be noted that in this study, the treatments about CK and saline soils both without the presence of Cd were not set up due to the experimental site already polluted by Cd, the corresponding pot experiment in greenhouse is suggested to be further conducted. The soil content of almost 900 kg in each plot was estimated and used to calculate the amount of salt addition. Salts were first dissolved in well water, then evenly irrigated into the soils of each plot with moisture at 70% of their water-holding capacity, and the depth of soil mixture with salts was about 20 cm. The salts were evenly homogenized into the soil by subsequent tossing. The salts were applied in May 2016. After then, well water was applied in this experiment rather than wastewater. All plots were uncultivated to equilibrate for a period of one cropping season (May–October 2016) before the salts contained in the plot were remixed and used for the experiments. During this period, the irrigation of these plots was scheduled by the flooding method with the application of well water; irrigation times were dependent on local precipitation and evaporation. Usually, plots were irrigated 2–3 times in the wheat season and 1–2 times for the maize season. The average volume of well water for each irrigation was $0.2\text{--}0.4 \text{ m}^3/\text{plot}$. The characteristics of sewage wastewater historically used for irrigation and well water for irrigation in this study (mg/L) are displayed in Table 2.

Table 2

Characteristics of sewage wastewater (shown in Fig. 1A) historically used for irrigation and well water for irrigation in this study (mg/L).

Characteristic	pH	CO_3^{2-}	HCO_3^-	Cl^-	Na	Mg	Fe	Mn	Zn	Cu	Pb	Ni	COD	BOD5	Cd
Sewage wastewater	7.73	1.03	6.26	5.32	1.58	1.86	96	45	3.8	2.6	1.9	3.2	212	105	0.05
Well water	7.04	0.37	2.15	1.85	0.01	0.79	12	21	1.5	0.9	0.1	0.1	20.6	13.6	0.002

Ridging was conducted before planting. Direct drilling was used to sow winter wheat seeds in the ridge around October 10, 2016; plants were harvested in late May of 2017. In early June, summer maize seeds were sown along the ridge in holes spaced 20 cm apart between the wheat rows and harvested in early October. Maize growth is displayed in Fig. 1B. Wheat/maize planting density and cultivation management (i.e. fertilizer, pesticide or herbicide applications) was consistent with the practices of local farmers.

After wheat and maize were harvested in May 25, 2017 and October 8, 2017, respectively, wheat and maize grains were washed with tap water to remove any adhering soil particles and rinsed with deionized water. They were dried in an oven (60°C) until a constant weight was reached, and the grain weight was recorded. The grain samples were ground into fine powder and passed through a 0.25 mm sieve for analyses. Soil samples from 5 cores (5-cm diameter) from the 0–15 cm layer in each plot were taken using a stainless steel auger and homogenized. Soil samples were then transported to the laboratory on ice, sieved (2 mm), and stored at room temperature for chemical analysis and at -70°C for molecular analysis.

2.2. Analyses

2.2.1. Soil and plant tissue Cd

The strong acid-extractable Cd concentration in the soil before and after planting was measured by digesting approximately 1 g of air-dried soil with 4.5 ml HCl (64.3%), 1.5 ml HNO_3 (21.5%) and 1 ml H_2O_2 (14.2%) in a Teflon bomb placed in a microwave digestion apparatus (Milestone MLS 1200 Mega). A similar procedure was used to digest grains but without adding HCl. The amount of diethylenetriamine pentaacetate (DTPA)-extractable Cd in soils was determined using 0.005 mol/L DTPA + 0.01 mol/L CaCl_2 + 0.1 mol/L triethanolamine, pH 7.30 at a soil-to-solution ratio (w/v) of 1:2. Quality assurance and control of the analyses were ensured by using sample replicates, reference material (National Research Center for Certified Reference Materials, China) and blanks. The Cd concentrations in both the digest and extract of grain and soil samples were determined using a PerkinElmer 1100B atomic absorption spectrometer. Several soil physiochemical

properties were determined according to Chinese standard methods given in [Table 1](#). Soil pH and EC were measured in a soil-water suspension with a 1:5 (w/w) soil:water ratio. Soil organic matter content was determined by the potassium dichromate external-heating method. Cation-exchange capacity (CEC) was measured using the barium chloride method. Soil base ions (Na^+ , K^+ , Mg^{2+}) and inorganic anions (Cl^-) concentrations were measured by inductively coupled plasma-atomic emission spectrometry.

2.2.2. Water-stable soil aggregates

The physical procedure for aggregate separation was adopted from [Manna et al. \(2007\)](#) with modifications. Dry soil material was wet sieved to obtain different aggregate size fractions: large macroaggregates (>2 mm), macroaggregates (0.2–2 mm), microaggregates (0.02–0.2 mm), silt size particles (0.02–0.002 mm), and clay size particles (<0.002 mm). In brief, each equivalent of 50 g dry mass of fresh soil was saturated with 300 ml of deionized water and allowed to stand overnight, and then transferred to a nest of sieves with mesh sizes of 2 mm, 0.2 mm, 0.02 mm, and 0.002 mm. The soil-water suspension was dispersed by shaking the sieve 3 cm vertically 30 times in 2 min. Subsequently, the large macro-, macro- and micro-aggregate fractions retained on each sieve were collected. The small fractions (0.02–0.002 mm and <0.002 mm) were collected by centrifuging suspensions for 10 min at 3000 rpm. We explored the relationship between soil physicochemical properties, crop yield, Cd concentration in grain and Cd distribution in soil aggregates and bacterial communities using structural equation modeling (SEM).

2.2.3. DNA extraction and PCR amplification

Soil bacterial DNA was extracted from 0.5 g of frozen soil sample using a FastDNA SPIN Kit for Soil (MP Biomedicals; Solon, OH, USA) and the FastPrep-24 instrument (MP Biomedicals) according to the manufacturer's instructions. DNA quality and quantity was determined using a NanoDrop 2000 Spectrophotometer (Bio-Rad Laboratories Inc., USA.). Primers 341f (5'- CCTAYGGGRBGCASCAG-3') and 806r (5'- GGACTACNNGGTTWTCTAAT-3') were used to amplify the hypervariable region V4–V5 of the bacterial 16S ribosomal DNA (rDNA). All PCR reactions were carried out in triplicate and contained 10 μl of SYBR[®] Premix Ex Taq (Tli RNaseH Plus, 2 \times , Takara Bio, Japan), 1 μl of 10 mM each primer, 7 μl molecular biology-grade water, and 1 μl of 10-fold diluted DNA in a total 20 μl volume. The PCR conditions were an initial denaturation at 95 °C for 30 s (ramp rate of 4.4 °C/s), followed by 30 cycles of denaturation at 95 °C for 5 s, annealing at 60 °C for 30 s, 95 °C for 5 s and then elongation at 60 °C for 1 min, with a final extension at 50 °C for 30 s. Three replicate PCR products of the same sample were pooled and purified using an AxyPrepDNA purification kit (AXYGEN, Inc.). The purified amplicons (465-bp fragments) from all samples were submitted to Lingen Biotechnology Co., Ltd. (Shanghai, China) for paired-end sequencing on the Illumina HiSeq platform.

The QIIME software package (version 1.8.0) was used to analyze the raw Illumina HiSeq sequencing data ([Caporaso et al., 2010](#)). The reads were quality trimmed by discarding quality scores below 20 and sequence lengths below 400 bp. The unique sequences among these remaining reads were used to define operational taxonomic units (OTUs) using Usearch (version 7.1 <http://drive5.com/uparse/>) with a threshold of 97% similarity. The taxonomic identities of the phylotypes were determined using the Ribosomal Database Project (RDP) Classifier (version 2.2 <http://sourceforge.net/projects/rdpclassifier/>) at a confidence threshold of 70%.

2.3. Statistical analyses

Mothur (version 1.34.0) was used to generate hierarchical cluster dendrograms (with Bray-Curtis distance dissimilarities) and perform non-metric multidimensional scaling (NMDS) to compare the structures of bacterial communities across soil samples. Permutational multivariate analysis of variance (PERMANOVA) using the adonis function was applied to examine the influences of saline stress on soil bacterial community structures at the plot level. Linear discriminant analysis (LDA) effect size (LEfSe) (<http://huttenhower.sph.harvard.edu/galaxy>) was performed to identify specific phylotypes with different abundance responses to different amounts of saline stress. The Kruskal-Wallis rank sum test was applied to detect features with significantly different abundances of assigned taxa. All tests for significance were two-sided, and p values < 0.05 were considered statistically significant. In this study, the SEM framework was applied to investigate the direct and indirect effects of soil saline stress on Cd availability. The model fit was assessed by means of a Chi-square test; a non-significant Chi-square test ($0.05 \leq P \leq 1.00$) indicates a good fit of the model to the data. The strength of the modelled relationships between variables was quantified by the estimated coefficients and significance was assessed using z statistics ([Rao et al., 2018](#)). The coefficients of each path were calculated by the analysis of correlation matrices. Paths in this model were considered significant with a P -value < 0.05. SEM analyses were performed using AMOS 21.0 (Amos Development Corporation, Crawfordville, FL, USA). The correlations between the abundant bacterial phyla and soil properties were determined by the Mantel test, and redundancy analysis (RDA) was carried out using the R vegan package. SPSS v20.0 (SPSS Inc., USA) was used to perform one-way analysis of variance (ANOVA) with post hoc Tukey's honest significant difference (HSD) tests and to calculate Spearman's rank correlations.

3. Results

3.1. The effect of saline stress on soil Cd availability

3.1.1. Crop yield and grain Cd accumulation

In this study, we found that grain yield decreased while grain Cd concentration increased for both wheat and maize under saline stress compared to the control. For example, wheat grain yield under the HS treatment decreased up to 33.9% and grain Cd concentration increased to 43.7%, compared to CK ([Fig. 2](#)). The increase in salinity resulted in decreased grain yield and increased grain Cd concentration of wheat and maize: when the saline stress increased from LS to HS, grain yield decreased to 35.5% and 32.6%, respectively, and grain Cd concentration increased to 47.9% and 48.4%, respectively ([Fig. 2](#)). The saline stress resulted in significant changes in soil chemical properties when compared to CK ([Table 1](#)). Soil pH and electric conductivity increased but CEC and organic matter content decreased significantly in treatments when compared with CK. Seasonal variation along with planting different crops also altered soil properties. For example, the increased extent of soil pH and EC was slightly stronger in wheat season when compared with maize planting.

3.1.2. Water-stable soil aggregates

Distribution of particle-sized aggregates based on different saline stresses in wheat and maize seasons are shown in [Fig. 3A](#). Particle-size distributions were dominated by the main fractions of macro- and micro-aggregates, particularly for the fractions 0.2–2 mm, 0.02–0.2 mm and 0.02–0.002 mm in all treatments, which accounted for more than 89.5% of the total soil fractions for both wheat and maize seasons. In addition, the aggregate fractions

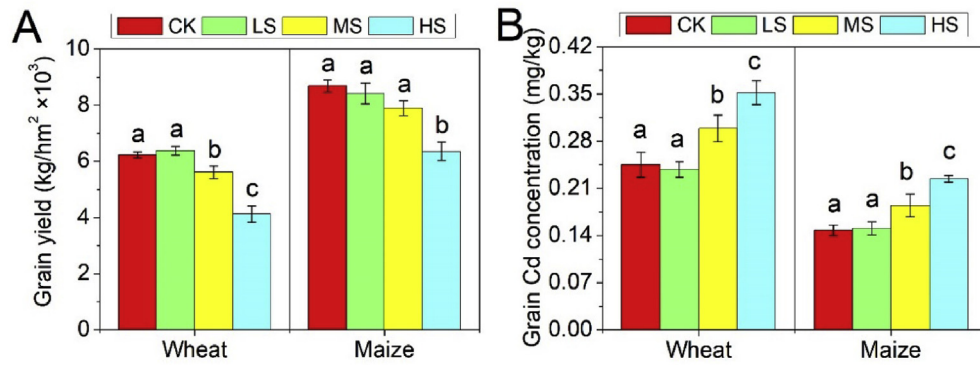


Fig. 2. Grain yield (A) and grain Cd concentration (B) in wheat and maize under different saline stresses. Error bars represent standard deviations, and bars with different letters are significantly different at $P < 0.05$. (CK: without saline stress (control), LS: 0.1% salts addition (low stress), MS: 0.3% salts addition (medium stress), and HS: 0.5% salts addition (high stress)).

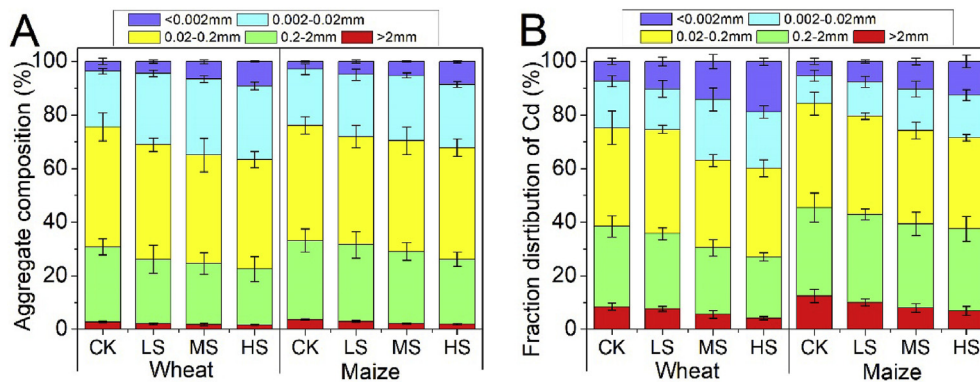


Fig. 3. Aggregate composition (A) and fractional distribution of Cd in aggregates (B) based on different saline stresses in both wheat and maize seasons. Vertical bars represent the standard deviation ($n = 3$). (CK: without saline stress (control), LS: 0.1% salts addition (low stress), MS: 0.3% salts addition (medium stress), and HS: 0.5% salts addition (high stress)).

were strongly modified by saline stress. The proportions of silt and clay size fractions (0.02–0.002 mm and <0.002 mm) increased significantly under all treatments when compared with CK, but the fractions of >2 mm and 0.2–2 mm decreased. Different responses of soil aggregate composition between wheat and maize seasons were observed; there were more macroaggregates but fewer microaggregates in the summer maize season compared to winter wheat season. Similarly, Cd was mainly distributed in the fractions of macro- and micro-aggregates in all treatments, and saline stress affected the fractional distribution of Cd in soil aggregates when compared with CK (Fig. 3B). The translocation of Cd from large size aggregates (eg. > 2 mm and 0.2–2 mm) into clay/silt size ones (eg. <0.002 mm and 0.002–0.02 mm) was observed under different amounts of saline stress.

3.2. The effect of saline stress on soil bacterial community structure

NMDS ordinations showed that the bacterial communities were significantly separated by different levels of saline stress (Fig. 4A). The marked structural rearrangement of soil bacterial microbiota was further confirmed by the PERMANOVA analysis (Table 3). In addition, statistical differentiation of bacterial community structure was also evident between seasonal turnover and wheat vs. maize planting (Fig. 4B and Table 3).

In this study, the dominant phyla Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Bacteroidetes and Planctomycetes were identified according to soil bacterial analysis (Fig. S1). These phyla have been described as common inhabitants of non-saline soils (Li

et al., 2016). Summer maize and winter wheat samples differed in community structure corresponding approximately to phylum level. For example, an increase of Actinobacteria was observed in winter wheat samples, which was compensated by a corresponding decrease of Acidobacteria and Proteobacteria. Similarly, there was a significant change in the abundant phyla between CK and saline treatments. For example, the increases of Proteobacteria and Actinobacteria were observed in saline-treated samples, while corresponding decreases of Saccharibacteria and Acidobacteria were displayed. The bacterial community analysis also identified the dominant genera with an average abundance of >1% (Fig. S2). These organisms could be related to dynamically biogeochemical cycling in stressed soils.

LEfSe was performed to identify the abundances of species of core microbiota responding to different amounts of saline stress in Cd-contaminated soil. In this study, the LEfSe algorithm detected 28 differentially abundant phylotypes in the wheat season as active biomarkers, which separated the soil bacterial community among saline treated soils and reflected the differences in abundance among these analyzed bacterial communities with statistical and biological significance (Fig. 5). Only 10 phylotypes at the family level with LDA scores >3 were identified in soil planted with summer maize, indicating that compared to the soils with repeated freezing and thawing in winter, a lesser impact on soil bacterial microbiota was observed in a warm, wet summer season (Fig. 5). It also can be seen that in CK-treated soils, some taxonomic families were enriched, like Sphingomonadaceae, Streptomycetaceae, Blasrocattellaceae, Gemmatimonadaceae. While in HS-treated soils,

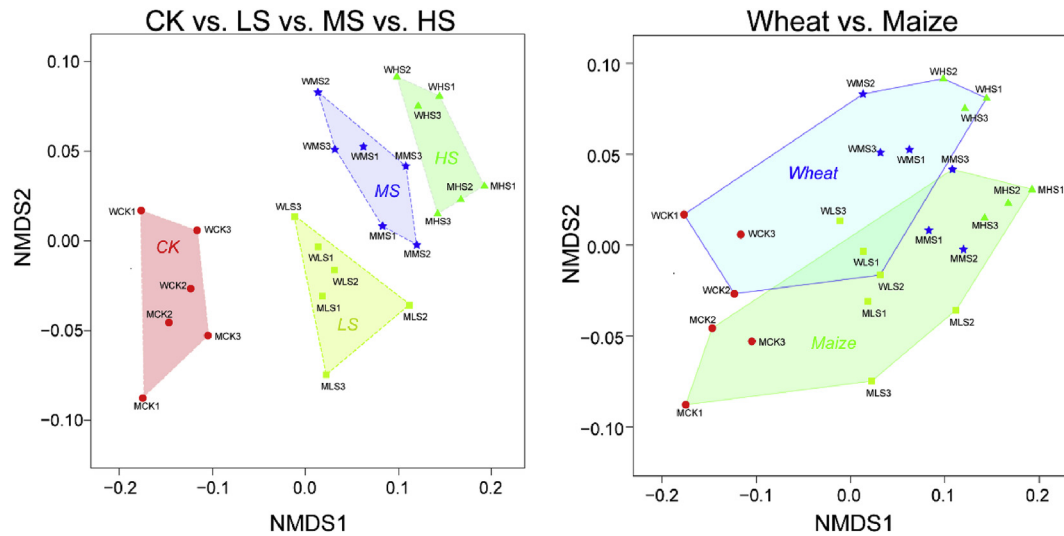


Fig. 4. Non-metric multidimensional scaling (NMDS) representation of soil bacterial communities based on different saline stresses in both wheat and maize seasons. The filled polygons indicate a significant influence of saline treatment or seasonal change on bacterial community structure in soils (assessed by permutational multivariate analysis of variance (PERMANOVA) see Table 3). (CK: without saline stress (control), LS: 0.1% salts addition (low stress), MS: 0.3% salts addition (medium stress), and HS: 0.5% salts addition (high stress)).

Table 3

Effect of saline stress and seasonal change on bacterial community structure assessed by PERMANOVA analysis (CK: without saline stress (control), LS: 0.1% salts addition (low stress), MS: 0.3% salts addition (medium stress), and HS: 0.5% salts addition (high stress)).

Factor	F	R ²
Saline stress		
CK vs. LS	3.21**	0.22
CK vs. MS	4.62**	0.29
CK vs. HS	6.53***	0.41
LS vs. MS	3.05**	0.20
LS vs. HS	3.89**	0.24
MS vs. HS	2.02 ^a	0.13
Seasonal change with crop rotation		
Winter wheat vs. Summer maize	1.96 ^a	0.11

^a $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

the taxonomic groups were discovered as high-dimensional biomarkers, including Bacillaceae, Halomonadaceae, Acidimicrobiaceae, Pseudomonadaceae etc. (Fig. 5).

3.3. Correlation analyses of soil variables, bacterial community and Cd availability

To better integrate the complex interrelationships among soil variables, bacterial community and Cd availability, structural equation modeling was used. Bacterial abundance was characterized by Chao index, diversity was characterized by Shannon index. Soil saline stress was represented by soil properties pH, EC and organic C. The latent variables (denoted in blue rectangles in Fig. 6) were successfully represented as soil saline stress, bacterial community and Cd availability. Results showed that soil saline stress had a significantly direct effect on the bacterial community and Cd availability, and bacterial composition was driven by direct negative effects from saline stress and soil available Cd. Soil salinity exerted a positive effect on Cd availability.

The Mantel test was performed to analyze the correlation between environmental variables and bacterial community structure in Cd-polluted soils. The results showed that pH, EC, organic C, concentrations of soil Na⁺, Mg²⁺ and Cd in <0.002 mm aggregate

($P < 0.05$) were the most influential environmental factors to drive the changes in bacterial community composition (Fig. 7). It is fair to say that these parameters played active roles in shaping the indigenous bacterial communities, namely the change of soil properties disturbed soil bacterial activities. For example, the intracellular pH of most microorganisms is usually neutral within 1 pH unit, this increase in pH is sufficient to impose a stress and likely influences the bacterial diversity. It seems that Cd concentration in <0.002 mm aggregate was strongly positively correlated with some base ions, such as Na⁺, K⁺, Cl⁻ and Mg²⁺ as shown in RDA analysis, indicating the content of soil available Cd might be increased due to the addition of sodium salts. Especially a closer correlation of Cd²⁺ concentration with bacterial community in HS treated soils than others was observed. Likewise, some salinity tolerant Cd-resistant archaea such as Actinobacteria, Proteobacteria might have become more dominant in saline stress treated soils.

4. Discussion

In this study, the composition and structure of bacterial communities were associated with soil Cd availability under different saline stresses: soil Cd availability was affected by saline stress, soil salinity was responsible for shaping bacterial communities. Usually soil bacterial communities play critical roles in maintaining soil health and quality, as they are involved in most nutrient transformations in soil. Changes in abiotic and biotic environmental variables are usually responsible for shaping microbiota structures. For example, salinity changes soil physicochemical properties and alters the osmotic strength of the soil solution, thus adversely affecting soil bacterial communities and their activities (Zhang et al., 2016), similar to heavy metals in the soil.

After saline stress, soil Cd availability increased, indicated by significant decreases in crop grain yield, increases in extractable Cd, grain Cd concentration, and the accumulation of Cd into clay/silt size aggregates. Saline soils are believed to negatively affect plant growth, because excessive salt ions in the rhizosphere could reduce the uptake of nitrogen, phosphorus, and potassium by crops (Sivanadanam et al., 2011). An excess of sodium ions also results in weak photosynthesis due to stomatal closure and thus restricts carbon dioxide uptake, and inhibits cell division and expansion

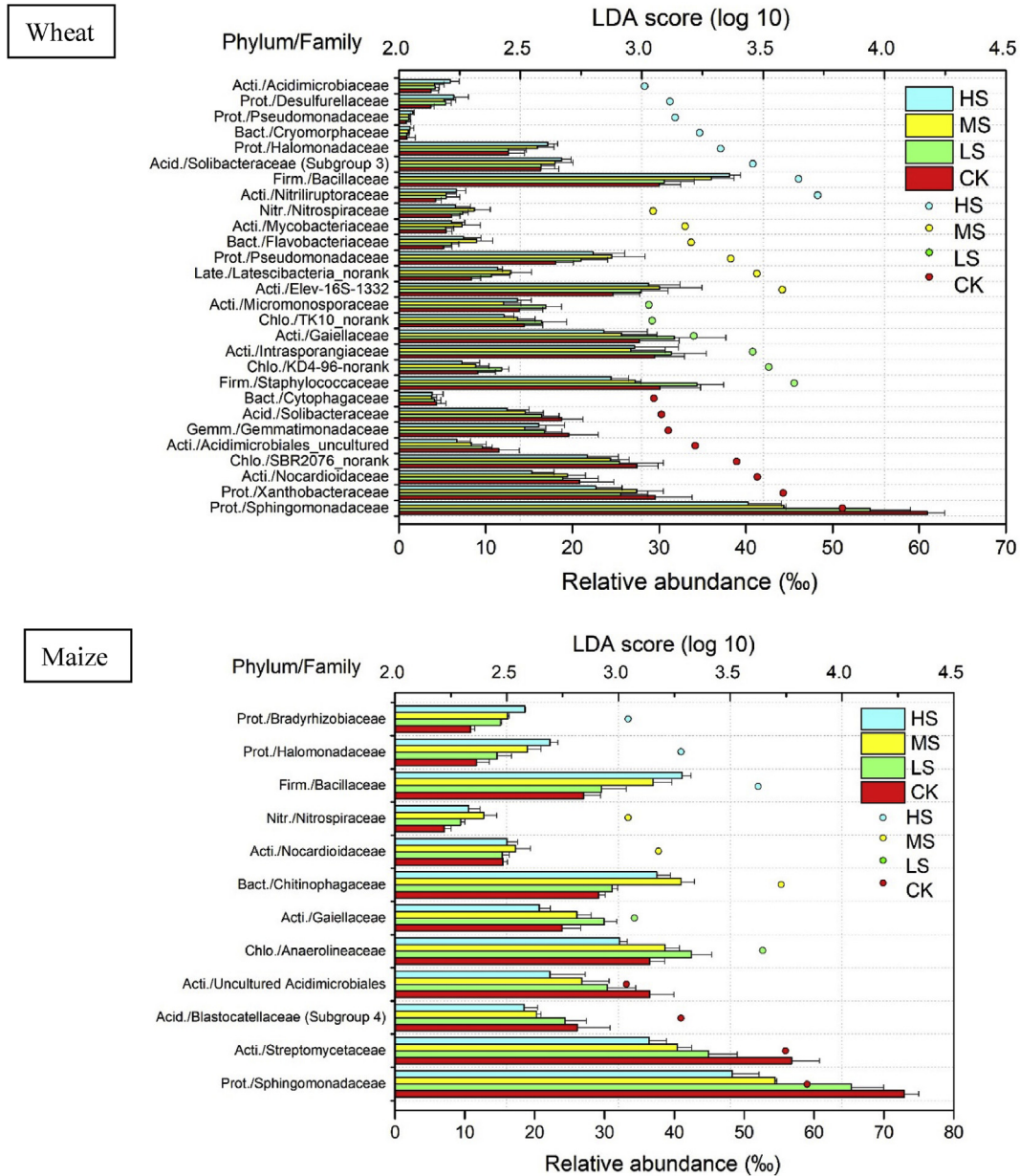


Fig. 5. Key phylotypes of soil bacterial microbiota responding to different amounts of saline stress in the wheat season and maize season identified by linear discriminant analysis (LDA) effect size (LEfSe). The colored circle displays significantly ($p < 0.05$) abundant and enriched bacterial communities with LDA score > 3 in treatments. The histogram shows the relative abundance of families. The error bars represent the standard deviation ($n = 3$). Abbreviations: Acid., Acidobacteria; Prot., Proteobacteria; Bact., Bacteroidetes; Firm., Firmicutes; Acti., Actinobacteria; Nitr., Nitrospirae; Late., Latescibacteria; Chlo., Chloroflexi; Gemm., Gemmatimonadetes. (CK: without saline stress (control), LS: 0.1% salts addition (low stress), MS: 0.3% salts addition (medium stress), and HS: 0.5% salts addition (high stress)).

(Zhu et al., 2001). In this study, soil Cd availability was significantly increased as the strength of salinity increased, which can probably be explained by the following reasons: first, with an increase in the concentration of Cl^- or SO_4^{2-} in Cd-contaminated soil, uncharged species and negatively charged complexes that could weaken the electrostatic interaction to Cd could form in the soil, and thus increase the freedom of Cd in soil. Second, high ionic strength induced by salt addition could enhance the competition ability of base ions eg. Na^+ in soil binding sites which already were occupied by Cd (Li et al., 2018).

Salinization negatively affects the stability of soil aggregates and soil structure, leading to an increase of soil compaction, loss of soil permeability and reduction of hydraulic conductivity

(Becerracastro et al., 2015). Salinization could induce the dispersion of clay, result in aggregate slaking or soil swelling and cause a collapse of soil structure (Sou/Dakouré et al., 2013). In addition, soil aggregates are not stable under saline conditions, probably because the abundance of Na^+ may replace divalent cations breaking aggregates, which may explain why the saline stress promoted the disintegration of large size aggregates and the formation of small ones (Fig. 3). Notably, the increase of Cd distribution in the size fraction of small aggregates and decrease in the size fraction of large aggregates could promote the transfer of toxic heavy metals from soil into plant tissues. Because fine soil aggregates have a higher affinity for heavy metals compared to large aggregates and a greater ability to move from one place to another because of their

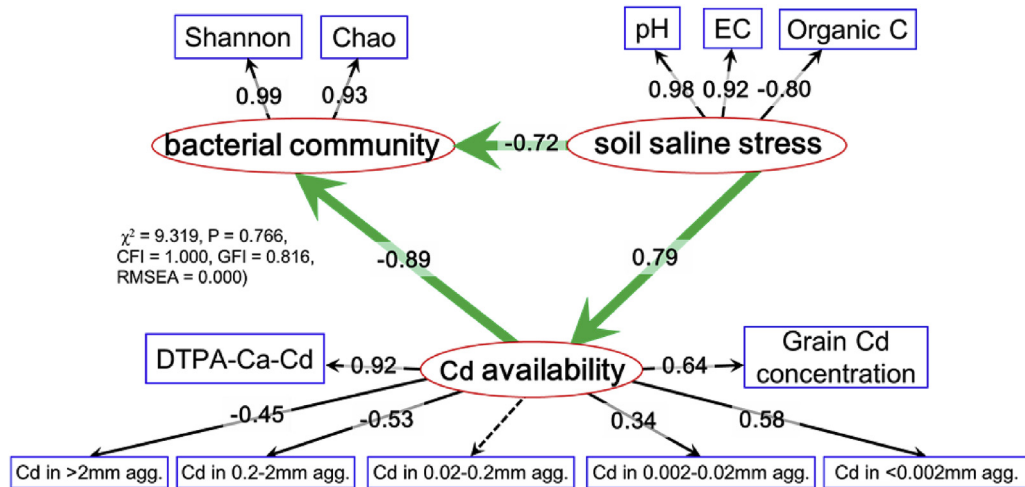


Fig. 6. Structural equation model (SEM) showing the relationship among soil variables, bacterial community and Cd availability (chi-squared test statistics (χ^2) = 9.319, $P = 0.766$, comparative fit index (CFI) = 1.000, goodness-of-fit index (GFI) = 0.816, root square mean errors of approximation (RMSEA) = 0.000). A significant correlation is represented by a solid arrow (P -value < 0.05), and a nonsignificant relationship is indicated by a dashed arrow. The numbers adjacent to arrows are standardized path coefficients. The arrow direction implied the flow of the causal effect.

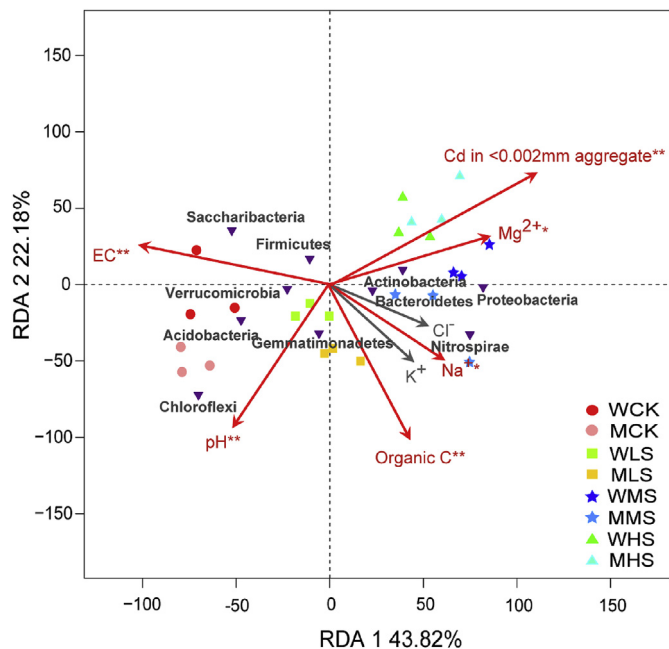


Fig. 7. Redundancy analyses (RDA) of the correlations between soil environmental variables and bacterial community composition. The red arrows indicate that the relevant parameters had a strong and significant impact on the microbial community composition (* $P \leq 0.05$, ** $P \leq 0.01$, Two-way ANOVA). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

smaller size, they are more likely to be transported into plant tissues along with the heavy metals (Wang et al., 2019).

It has been widely accepted that changes in environmental stresses are responsible for shaping microbiota structures and different bacterial communities play critical roles in specific ecosystem functions (Sharma et al., 2006; Liu et al., 2010). Our results showed that saline stress significantly changed major soil microbes when compared with CK in Cd-contaminated soil according to the NMDS ordinations and PERMANOVA analysis (Fig. 3, Table 3). This occurred because salinization and Cd conditions in the soil likely facilitated the dominance of stress-tolerant bacteria.

Consistent with our results, previous studies have indicated that soil salinization (increase of soluble salts concentration) interfere not only with plant growth and productivity but also with soil microbiota, because soil salinization is usually associated with the increase of electrical conductivity and excessive salinity could impose hyperosmotic and oxidative stresses (Becerracastro et al., 2015).

The soil bacterial community was greatly responsive to saline stress, but such responses differed among taxa. For example, Proteobacteria and Actinobacteria were the two most abundant phyla, because they generally serve as the active bacterial fraction in heavy metal-polluted soils; Proteobacteria are identified as the most stress-tolerant microorganisms in heavily contaminated soils (Burkhardt et al., 2011; Li et al., 2016). Of particular interest is one specific family within Proteobacteria, Sphingomonadaceae, which was the most sensitive biomarker responding to Cd contamination in CK soils. It has been reported that multiple heavy metal oxidase genes encoded by Proteobacteria are involved in heavy metal resistance (Altimira et al., 2012). For example, *Sphingomonas* sp. BSAR-1, which expresses high levels of alkaline phosphatase, displays great potential to bioprecipitate Cd present in soils and resist its phytotoxicity (Nilgiriwala et al., 2008). Similarly, it was found that the *Sphingomonas* sp. strains O12 and A32 possessed plasmids containing heavy metal-resistance genes (Altimira et al., 2012). Certain strains of Proteobacteria are usually related to respiratory and dissimilatory nitrate-reducing communities in saline soil (Alcantara-Hernandez et al., 2009; Zhang et al., 2016).

Soil bacterial communities are greatly influenced by salinity as well as other abiotic and biotic environmental variables such as pH (Hu et al., 2019). Compared to CK, the abundance of the phylum Acidobacteria decreased with the increase in soil saline stress strength. Usually, lower soil pH leads to greater expected abundance of Acidobacteria (Lauber et al., 2009; Li et al., 2016). In addition, Acidobacteria and Chloroflexi are generally classified as slow-growing oligotrophs that thrive in soils with less available nutrients (Fierer et al., 2007; Eo and Park, 2016). An interesting feature of the obtained sequences belonging to the Actinobacterial family Nitriliruptoraceae was the most sensitive biomarker responsive to high salt stress (refer to HS treatment); species of *Nitriliruptor* were found to exhibit a haloalkaliphilic capacity, presumably better adapted with high alkali environments (pH,

9.35–9.5) (Sorokin et al., 2009). Similarly, in the family Bacillaceae, strains in the genus *Bacillus* were found to be the most tolerant, showing great tolerance to different metal ions (Fe, Cd, Zn, Co, Ni and Cu) (Moreno et al., 2012). *Bacillus* species are well known for their ability to produce many useful enzymes and antibiotic substances, which play critical roles in maintaining soil health (Kuramshina et al., 2016). Among the bacterial families, *Salinococcus* and *Pseudomonas* belonging to the families Staphylococcaceae and Pseudomonadaceae, respectively, were found to have strong alkali adaptability and could be regarded as alkaline tolerant bacteria (Paul et al., 2016). Together, these results indicated that different amounts of saline stress greatly influenced the diversity and structure of the soil bacterial communities in Cd-contaminated soil.

We observed moderate shifts in the relative abundances of individual groups but no significant switches between dominant and rare taxa from the winter wheat season to the summer maize season. In addition, microbial activity was strongly correlated with soil temperature and soil respiration, which greatly differed between summer and winter sampling (Kuffner et al., 2012). It also should be noted that usually 40–70% of the soil bacteria are associated with stable macroaggregates and clay particles with sizes lower than 20 µm. Therefore, the interference on the formation of soil aggregates due to seasonal variation is expected to modify soil microhabitats (Becerracastro et al., 2015).

The hypothesis of this study was that bacterial community structure might be associated with soil Cd availability under different saline stresses. Our SEM results further showed that alterations in soil saline stress were important for regulating the bacterial community and changing Cd availability. This is a noteworthy finding and indicates that long-term wastewater irrigation that brings more neutral or alkali salts and heavy metals into the soil could change soil physicochemical properties (pH, EC, organic C or soil aggregate stability) and bacterial community structure; these corresponding soil environmental changes could further increase the concentration of liberated Cd in soil and promote its transfer from soil to plant tissues (Table 1, Figs. 3 and 5). Our previous study found that pH has been identified as one of the most influential environmental factors driving the changes in bacterial community composition. Increased soil pH is generally beneficial for remediating Cd-polluted soil in agricultural production, through reducing extractable Cd and the exchangeable Cd fraction (Yuan et al., 2018). However, the increase of electrical conductivity in soil solution was reported to positively correlate with DTPA-extractable Cd (Khanmirzaei et al., 2013).

5. Conclusions

In this study, increased soil salinity was found to enhance Cd availability in Cd-contaminated soil due to long-term wastewater irrigation. Addition of 0.5% mixed salts significantly increased soil DTPA-Cd up by 67.5% and grain Cd concentration by 43.7%, when compared with CK (no extra salt addition). Soil saline stress resulted in shifts of major soil microbes in Cd-contaminated soil. Increased saline stress enriched the taxa in the Bacillaceae, Staphylococcaceae and Pseudomonadaceae families, while a specific family within Proteobacteria (Sphingomonadaceae) was the most sensitive biomarker, only responding to Cd contamination without saline stress in CK-treated soils. Based on SEM analysis, soil saline stress increased soil Cd availability that further regulated the bacterial community. Seasonal variation played a critical role in altering soil physicochemical characteristics and shaping bacterial communities.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2019.124372>.

References

- Alcántara-Hernández, R.J., Valenzuela-Encinas, C., Marsch, R., Dendooven, L., 2009. Respiratory and dissimilatory nitrate-reducing communities from an extreme saline alkaline soil of the former lake Texcoco (Mexico). *Extremophiles Life Under Extreme Conditions*, 13 (1), 169–178. <https://doi.org/10.1007/s00792-008-0207-1>.
- Altimira, F., Yáñez, C., Bravo, G., González, M., Rojas, L., Seeger, M., 2012. Characterization of copper-resistant bacteria and bacterial communities from copper-polluted agricultural soils of central Chile. *BMC Microbiol.* 12, 193. <https://doi.org/10.1186/1471-2180-12-193>.
- Awasthi, A.K., Zeng, X., Li, J., 2016. Environmental pollution of electronic waste recycling in India: a critical review. *Environ. Pollut.* 211, 259–270. <https://doi.org/10.1016/j.envpol.2015.11.027>.
- Becerracastro, C., Lopes, A.R., Vazmoreira, I., Silva, E.F., Manaia, C.M., Nunes, O.C., 2015. Wastewater reuse in irrigation: a microbiological perspective on implications in soil fertility and human and environmental health. *Environ. Int.* 75 (4), 117–135. <https://doi.org/10.1016/j.envint.2014.11.001>.
- Brzezinska, M., Sokolowska, Z., Alekseeva, T., Alekseev, A., Hajnos, M., Szarlip, P., 2011. Some characteristics of organic soils irrigated with municipal wastewater. *Land Degrad. Dev.* 22 (6), 586–595. <https://doi.org/10.1002/ldr.1036>.
- Burkhardt, E., Bischoff, S., Akob, D., Büchel, G., Küsel, K., 2011. Heavy metal tolerance of Fe(III)-reducing microbial communities in contaminated creek bank soils. *Appl. Environ. Microbiol.* 77 (9), 3132–3136. <https://doi.org/10.1128/AEM.02085-10>.
- Caporaso, J., Bittinger, K., Bushman, F., Desantis, T., Andersen, G., Knight, R., 2010. PYNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* 26, 266–267. <https://doi.org/10.1093/bioinformatics/btp636>.
- Cirelli, G.L., Consoli, S., Licciardello, F., Aiello, R., Giuffrida, F., Leonardi, C., 2012. Treated municipal wastewater reuse in vegetable production. *Agric. Water Manag.* 104 (1), 163–170. <https://doi.org/10.1016/j.agwat.2011.12.011>.
- Eo, J., Park, K.C., 2016. Long-term effects of imbalanced fertilization on the composition and diversity of soil bacterial community. *Agric. Ecosyst. Environ.* 231, 176–182. <https://doi.org/10.1016/j.agee.2016.06.039>.
- Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil bacteria. *Ecology* 88, 1354–1364. <https://doi.org/10.1890/05-1839>.
- Ganjegunte, G., Ulery, A., Niu, G., Wu, Y., 2018. Treated urban wastewater irrigation effects on bioenergy sorghum biomass, quality and soil salinity in an arid environment. *Land Degrad. Dev.* 29 (3), 534. <https://doi.org/10.1002/ldr.2883>.
- Gatta, G., Libutti, A., Gagliardi, A., Beneduce, L., Brusetti, L., Borruso, L., et al., 2015. Treated agro-industrial wastewater irrigation of tomato crop: effects on qualitative/quantitative characteristics of production and microbiological properties of the soil. *Agric. Water Manag.* 149, 33–43. <https://doi.org/10.1016/j.agwat.2014.10.016>.
- Gómez-Acata, E., Selene, Valencia-Becerril, I., Valenzuela-Encinas, C., Velásquez-Rodríguez, A., Socorro, Navarro-Noya, Ebenezer, Y., Montoya-Ciriaco, N., Suárez-Arriaga, M., Cecilia, Rojas-Valdez, A., Reyes-Reyes, B., Gabriel, Luna-Guido, M., Dendooven, L., 2016. Deforestation and cultivation with maize (*Zea mays* L.) has a profound effect on the bacterial community structure in soil. *Land Degrad. Dev.* 27 (4), 1122–1130. <https://doi.org/10.1002/ldr.2328>.
- Hu, M., Sun, W., Krumins, V., Li, F., 2019. Arsenic contamination influences microbial community structure and putative arsenic metabolism gene abundance in iron plaque on paddy rice root. *Sci. Total Environ.* 649, 405–412. <https://doi.org/10.1016/j.scitotenv.2018.08.388>.
- Ibekwe, A.M., Gonzalez-Rubio, A., Suarez, D.L., 2018. Impact of treated wastewater for irrigation on soil microbial communities. *Sci. Total Environ.* 622–623, 1603–1610. <https://doi.org/10.1016/j.scitotenv.2017.10.039>.
- Khanmirzaei, A., Bazargan, K., Moezzi, A.A., Richards, B.K., Shahbazi, K., 2013. Single and sequential extraction of cadmium in some highly calcareous soils of southwestern Iran. *J. Soil Sci. Plant Nutr.* 13 (ahead), 153–164. <https://doi.org/10.4067/S0718-95162013005000014>.
- Kuffner, M., Hai, B., Rattei, T., Melodelima, C., Schloter, M., Zechmeister-Boltenstern, S., et al., 2012. Effects of season and experimental warming on the bacterial community in a temperate mountain forest soil assessed by 16s rRNA gene pyrosequencing. *FEMS Microbiol. Ecol.* 82 (3), 551–562. <https://doi.org/10.1111/j.1574-6941.2012.01420.x>.

- Kuramshina, Z.M., Smirnova, Y.V., Khairullin, R.M., 2016. Increasing *triticum aestivum*, tolerance to cadmium stress through endophytic strains of *bacillus subtilis*. Russ. J. Plant Physiol. 63 (5), 636–644. <https://doi.org/10.1134/S1021443716050083>.
- Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol. 75 (15), 5111–5120. <https://doi.org/10.1128/AEM.00335-09>.
- Li, X., Zhang, W., Liu, T., Chen, L., Chen, P., Li, F., 2016. Changes in the composition and diversity of microbial communities during anaerobic nitrate reduction and Fe(II) oxidation at circumneutral pH in paddy soil. Soil Biol. Biochem. 94, 70–79. <https://doi.org/10.1016/j.soilbio.2015.11.013>.
- Li, S., Wang, M., Zhao, Z., Ma, C., Chen, S., 2018. Adsorption and desorption of Cd by soil amendment: mechanisms and environmental implications in field-soil remediation. Sustainability 10, 2337. <https://doi.org/10.3390/su10072337>.
- Li, S., Wang, M., Zhao, Z., Li, X., Han, Y., Chen, S., 2019. Alleviation of cadmium phytotoxicity to wheat is associated with Cd re-distribution in soil aggregates as affected by amendments. RSC Adv. 8, 17426. <https://doi.org/10.1039/c8ra03066a>.
- Liu, X., Wu, B., Chen, B., Wen, J., Renman, R., Yao, G., et al., 2010. Bioleaching of chalcocite started at different pH: response of the microbial community to environmental stress and leaching kinetics. Hydrometallurgy 103 (1), 1–6. <https://doi.org/10.1016/j.hydromet.2010.02.002>.
- Manna, M.C., Swarup, A., Wanjari, R.H., Mishra, B., Shahi, D.K., 2007. Long-term fertilization, manure and liming effects on soil organic matter and crop yields. Soil Tillage Res. 94 (2), 397–409. <https://doi.org/10.1016/j.still.2006.08.013>.
- Moreno, M.L., Piubeli, F., Bonfá, M.R.L., García, M.T., Durrant, L.R., Mellado, E., 2012. Analysis and characterization of cultivable extremophilic hydrolytic bacterial community in heavy-metal-contaminated soils from the Atacama desert and their biotechnological potentials. J. Appl. Microbiol. 113 (3), 550–559. <https://doi.org/10.1111/j.1365-2672.2012.05366.x>.
- Muyen, Z., Moore, G.A., Wrigley, R.J., 2011. Soil salinity and sodicity effects of wastewater irrigation in south east Australia. Agric. Water Manag. 99 (1), 33–41. <https://doi.org/10.1016/j.agwat.2011.07.021>.
- Nakayama, H., Horie, T., Yonamine, I., Shinmyo, A., Yoshida, K., 2006. Improving salt tolerance in plant cells. Plant Biotechnol. 22 (5), 477–487. <https://doi.org/10.5511/plantbiotechnology.22.477>.
- Nilgiriwala, K.S., Alahari, A., Rao, A.S., Apte, S.K., 2008. Cloning and overexpression of alkaline phosphatase PhoK from *Sphingomonas* sp. strain BSAR-1 for bio-precipitation of uranium from alkaline solutions. Appl. Environ. Microbiol. 74, 5516–5523. <https://doi.org/10.1128/AEM.00107-08>.
- Pal, S., Mukherjee, S., Bhattacharyya, N., 2017. Microorganism and agricultural based biosorbents towards removal of cadmium from waste-water: an overview. Recent Pat. Biotechnol. 11 (3), 204–217. <https://doi.org/10.2174/1872208311666170220121535>.
- Paul, D., Kumbhare, S.V., Mhatre, S.S., Chowdhury, S.P., Shetty, S.A., Marathe, N.P., et al., 2016. Exploration of microbial diversity and community structure of lonar lake: the only hypersaline meteorite crater lake within basalt rock. Front. Microbiol. 6 (44), 1553. <https://doi.org/10.3389/fmicb.2015.01553>.
- Pedrero, F., Kalavrouziotis, I., Alarcón, J.J., Koukoulakis, P., Asano, T., 2010. Use of treated municipal wastewater in irrigated agriculture-review of some practices in Spain and Greece. Agric. Water Manag. 97 (9), 1233–1241. <https://doi.org/10.1016/j.agwat.2010.03.003>.
- Rao, Z.X., Huang, D.Y., Wu, J.S., Zhu, Q.H., Zhu, H.H., Xu, C., et al., 2018. Distribution and availability of cadmium in profile and aggregates of a paddy soil with 30-year fertilization and its impact on Cd accumulation in rice plant. Environ. Pollut. 239, 198–204. <https://doi.org/10.1016/j.envpol.2018.04.024>.
- Regelink, I.C., Stoof, C.R., Roussea, S., Weng, L., Lair, G.J., Kram, P., et al., 2015. Linkages between aggregate formation, porosity and soil chemical properties. Geoderma 247–248, 24–37. <https://doi.org/10.1016/j.geoderma.2015.01.022>.
- Saha, S., Hazra, G.C., Saha, B., Mandal, B., 2015. Assessment of heavy metals contamination in different crops grown in long-term sewage-irrigated areas of Kolkata, West Bengal, India. Environ. Monit. Assess. 187 (1), 4087–4099. <https://doi.org/10.1007/s10661-014-4087-9>.
- Sharma, S., Szele, Z., Schilling, R., Munch, J.C., Schloter, M., 2006. Influence of freeze-thaw stress on the structure and function of microbial communities and denitrifying populations in soil. Appl. Environ. Microbiol. 72 (3), 2148–2154. <https://doi.org/10.1128/AEM.72.3.2148-2154.2006>.
- Singh, A., Sharma, R.K., Agrawal, M., Marshall, F.M., 2010. Risk assessment of heavy metal toxicity through contaminated vegetables from waste water irrigated area of Varanasi, India. Trop. Ecol. 51 (2), 375–387. <https://doi.org/10.1016/j.fct.2009.11.041>.
- Sivanadanam, V., Neelamegam, R., Chellappan, K.P., 2011. Salinity effects: iii. on nitrogen, phosphorus and potassium (NPK) uptake in rhizophora apiculata blume and acanthus ilicifolius linn seedlings. Plant Arch. 11, 141–146.
- Sorokin, D.Y., van Pelt, S., Tourova, T.P., Evtushenko, L.I., 2009. *Nitriliruptor alkaliphilus* gen. nov. sp. nov. a deep-lineage haloalkaliphilic actinobacterium from soda lakes capable of growth on aliphatic nitriles, and proposal of *nitriliruptoraceae* fam. nov. and *nitriliruptorales* ord. nov. Int. J. Syst. Evol. Microbiol. 59 (Pt 2), 248–253. <https://doi.org/10.1099/ijs.0.002204-0>.
- Sou/Dakouré, M.Y., Mermoud, A., Yacouba, H., Boivin, P., 2013. Impacts of irrigation with industrial treated wastewater on soil properties. Geoderma 200–201 (6), 31–39. <https://doi.org/10.1016/j.geoderma.2013.02.008>.
- Sruthi, P., Shackira, A.M., Puthur, J.T., 2017. Heavy metal detoxification mechanisms in halophytes: an overview. Wetl. Ecol. Manag. 25, 129–148. <https://doi.org/10.1007/s11273-016-9513-z>.
- Stevik, T.K., Aa, K., Ausland, G., Hanssen, J.F., 2004. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. Water Res. 38 (6), 1355–1367. <https://doi.org/10.1016/j.watres.2003.12.024>.
- Verbyla, M.E., Symonds, E.M., Kafle, R.C., Cairns, M.R., Iriarte, M., Mercado, A., et al., 2016. Managing microbial risks from indirect wastewater reuse for irrigation in urbanizing watersheds. Environ. Sci. Technol. 50 (13), 6803–6813. <https://doi.org/10.1021/acs.est.5b05398>.
- Wang, J., Niu, W., Zhang, M., Li, Y., 2017. Effect of alternate partial root-zone drip irrigation on soil bacterial communities and tomato yield. Appl. Soil Ecol. 119, 250–259. <https://doi.org/10.1016/j.apsoil.2017.06.032>.
- Wang, M., Chen, S., Han, Y., Chen, L., Wang, D., 2019. Responses of soil aggregates and bacterial communities to soil-Pb immobilization induced by biofertilizer. Chemosphere 220, 828–836. <https://doi.org/10.1016/j.chemosphere.2018.12.214>.
- Yuan, C., Liu, T., Li, F., Liu, C., Yu, H., Sun, W., Huang, W., 2018. Microbial iron reduction as a method for immobilization of a low concentration of dissolved cadmium. J. Environ. Manag. 217, 747–753. <https://doi.org/10.1016/j.jenvman.2018.04.023>.
- Zhang, Y., Cao, C., Guo, L., Wu, Q., Cui, Z., 2015. Soil properties, bacterial community composition, and metabolic diversity responses to soil salinization of a semiarid grassland in northeast China. J. Soil Water Conserv. 70 (2), 110–120. <https://doi.org/10.2489/jswc.70.2.110>.
- Zhang, W., Cao, J., Zhang, S., Wang, C., 2016. Effect of earthworms and arbuscular mycorrhizal fungi on the microbial community and maize growth under salt stress. Appl. Soil Ecol. 107, 214–223. <https://doi.org/10.1016/j.apsoil.2016.06.005>.
- Zhu, J.K., 2001. Plant salt tolerance. Trends Plant Sci. 6 (2), 66–71. [https://doi.org/10.1016/S1360-1385\(00\)01838-0](https://doi.org/10.1016/S1360-1385(00)01838-0).