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Effect of Lead Zirconate Titanate Bimorph on Soil Microorganisms: A Preliminary Study

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Abstract: Lead zirconate titanate (PZT) has been widely used because of its electrochemical effect, but its effect on soil microorganisms is rarely studied. In this study, laboratory soil microcosms with different soil moisture content and pH were established to explore the effects of the PZT-5H bimorph with different quantities and states on soil microorganisms after 49 days. Plate counting was used to study the number changes of soil bacteria, fungi and actinomycetes. Isothermal microcalorimetry was used to evaluate microbial activity. High-throughput sequencing was used to analyze soil microbial diversity and community structure. The results showed that the number and activity of microorganisms could be significantly promoted by two vibrating PZT bimorphs under the appropriate soil moisture content (20%) and pH (7). At the same time, it promoted the growth of non-dominant microorganisms and increased the diversity of microorganisms. These results indicate that it is possible for PZT bimorphs to be used in soil field.



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Keywords: lead zirconate titanate; High throughput sequencing; microcalorimetry; soil ecological impact

1. Introduction

Piezoelectricity was first discovered in 1880 [1]. The term “piezoelectricity” originates from “piezo” and “electricity”, in which “piezo” represents the application of a pressure and “electricity” corresponds to moving electrons [2,3]. Piezoelectric effect can be divided into positive piezoelectric effect and inverse piezoelectric effect, which reflects the mutual transformation of mechanical energy and electrical energy [4]. As a piezoelectric material, lead zirconate titanate (PZT) has been widely used in vibration control, energy collection, energy conversion, artificial intelligence, measurement of soil properties, and other fields due to its excellent electrical performance, fast response, and easy processing [5–9].

Electrochemical technology is one of the current research hotspots in the field of environmental protection [10]. In the field of soil remediation, electrochemical methods have also been well developed from single electrokinetic remediation to coupling with other technologies (such as electrokinetic microbial remediation) [11–14]. Electrochemical processes improve the bioavailability of pollutants by promoting the migration of nutrients, electron acceptors, and microorganisms in soil [15]. When the piezoelectric material is subjected to external stress or mechanical vibration, the ion displacement is induced, which leads to the change of unit cell dipole moment and produces net charge, thus forming piezoelectric potential on the material [16]. If the outer surface of the material is in contact with the dielectric at this time [17], the rearrangement of the charges will change the conductivity, which will strongly affect the electrochemical process [18]. The charge polarity on the surface of piezoelectric material will change with the direction of applied force. In theory, when one end of the PZT bimorph is fixed and the other end vibrates reciprocally under the action of force, the polarity of the charge received by the medium

contacting the piezoelectric material is constantly changing, which can avoid the extreme change of pH and keep the electrochemical effect for a long time. The electrochemical effect is widely used in the field of soil remediation as a means of bioaugmentation [19,20]. Hong et al. reported research on the degradation of organic pollutants by piezoelectric catalysis under ultrasound [21], extending the application of piezoelectric materials to the field of environmental remediation [22–24]. From the perspective of sustainable development, it is possible for PZT bimorphs to be used in soil field.

At present, scholars pay more attention to the performance improvement and structure innovation of PZT. However, in the process of application, the ecological effect of PZT on soil microorganisms is rarely studied. Before any kind of material is applied to soil, the effect on soil microorganism is an important evaluation index [25,26]. Therefore, based on the purpose of applying PZT bimorphs to the field of soil remediation, this study makes a preliminary exploration on evaluation of the positive or negative effects of PZT bimorphs on soil microorganisms from three aspects of microbial quantity, microbial growth activity, and microbial diversity. Isothermal microcalorimetry is an essential structural biology method developed in recent years to study thermodynamics and biokinetics [27]. It can continuously and accurately monitor and record the calorimetric curve of a change process and provide thermodynamic and kinetic information [28,29], becoming a new breakthrough in the study of microbial metabolism in soil systems [30,31]. High throughput sequencing technology has become the main method to study soil microbial diversity because of its high efficiency and accuracy [32,33]. In this study, plate counting method, isothermal microcalorimetry, and high-throughput sequencing method were used to investigate the effects of different quantities and states of PZT-5H bimorphs on soil microorganisms, so as to provide a theoretical basis for the application of PZT bimorphs in soil remediation in the future.

2. Materials and Methods

2.1. Soil Sampling

The alluvial soil samples were taken from grassland without artificial disturbance. The sampling site was located in Baodi District, Tianjin, China (39° 33'8" N, 117° 24'18" E). The sampling time occurred in April 2019. The S-type sampling method [34] was used to strip the surface cover and mix the soil sample after removing the stones and tree roots. The mixed soil sample was transported to the laboratory and stored at 4 °C until used. Soil pH value was detected by a pH-meter (PHS-3E). The pH value was determined by placing the pH electrode in the supernatant solution prepared by mixing 10.0 g soil and 25.0 mL deionized water. Organic matter was determined by titrating the samples in an acidic medium, with the end point followed by a redox reaction [35]. The soil moisture content was determined by the weighing method [36]. Soil used in the microcosm setup was measured to contain 12% of moisture content, 37.54 g/kg of organic matter, and weakly alkaline (7.1 ± 0.1).

2.2. Microcosms Assemblment

The PZT bimorph consists of five layers, including a copper elastic substrate in the middle, PZT-5H in the upper and lower layers and silver films on the surface with the same size as the PZT sheet. The sizes of the PZT sheet and the copper substrate were 60 mm × 30 mm × 0.1 mm and 80 mm × 33 mm × 0.6 mm, respectively. The two ends of the PZT bimorph were respectively fixed by an insulating base and an insulating clamp, the insulating base was fixed at the bottom center of the square rubber box (8 cm × 8 cm × 8 cm), and the insulating clamp was fixed on the stepping motor to ensure that the mechanical force of PZT bimorph was equal and the direction was opposite (Figure 1). Soil sieved through a 2 mm sieve was collected as described in Section 2.1 and 500 g was added to each box. Deionized water was used to adjust soil moisture content (MC). HCl (1 mol/L) solution and NaOH solution (1 mol/L) were used to adjust soil pH. The temperature was maintained at room temperature ($28 \text{ }^{\circ}\text{C} \pm 1 \text{ }^{\circ}\text{C}$). Considering the service life of the PZT

bimorph and the friction of the stepper motor, the reciprocating amplitude and period of the stepper motor were set as 4 mm and 2 s. Oscilloscope (UNI-T-UTD2102CEX) was used to monitor the output voltage–curve under this condition (Figure 2). The maximum output voltage of single-sided PZT was 2 V. Therefore, the maximum number of the PZT bimorph in the experiment was 2 [37,38]. Five soil microcosms were set up in each experiment. Group A was the blank control (without the PZT bimorph). Groups B, C, D, and E were fixed with one static PZT bimorph, two static PZT bimorphs, one vibrating PZT bimorph, and two vibrating PZT bimorphs, respectively. Through comprehensive consideration of relevant literature [39,40], the period of each experiment was determined as 49 days.

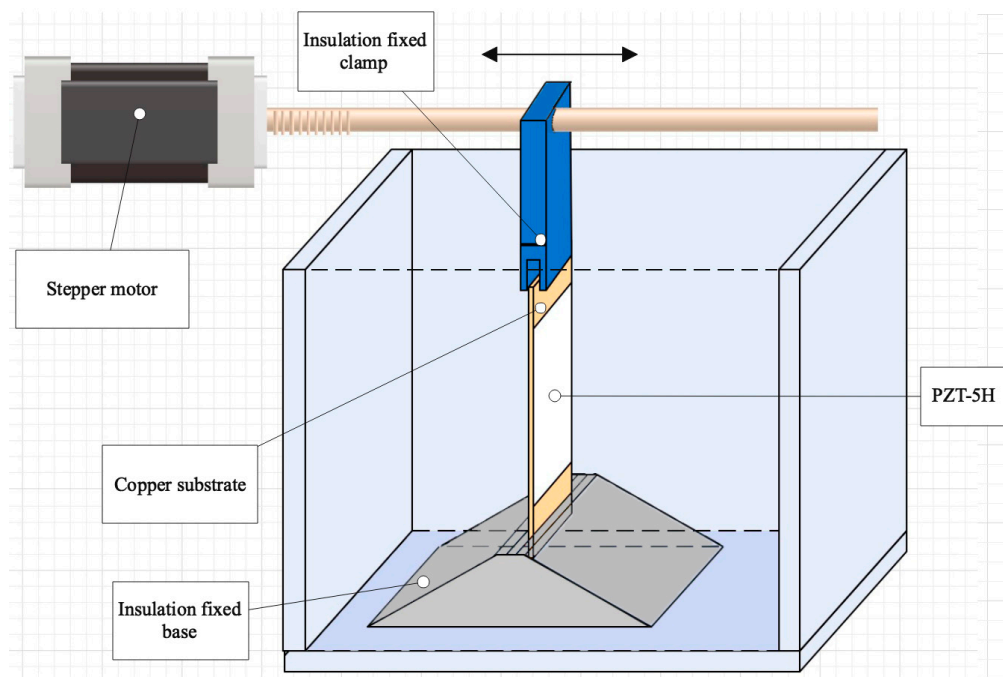


Figure 1. Experimental device chart.

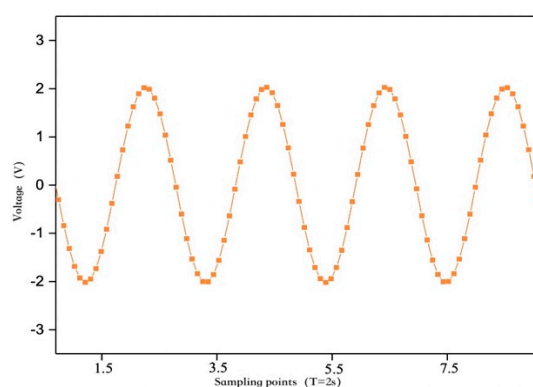


Figure 2. Output voltage–time curve of single-side PZT (smoothed by Origin 10.5.1).

2.3. Biological Analyses

2.3.1. Count of Bacteria, Fungi, and Actinomycetes

The experiment was divided into two parts. In part one, without changing other properties of the original soil, the soil content of water was adjusted to 15%, 20%, and 30% [41,42]. Part two was based on part one; the soil content of water was set to 20%; at the same time, soil pH was adjusted to 6, 7, and 8, and other properties remained unchanged. The number of culturable microorganisms in the soil was determined by the plate counting method [43]. Soil bacteria, actinomycetes, and fungi were cultured in a beef extract peptone

medium, Gaoshi No.1 medium, and Martin medium, respectively [44–47]; 10 g soil samples from different microcosms were taken out and put into the above liquid medium. Bacteria were cultured at 37 °C for 24 h, fungi at 25 °C for four days, and actinomycetes at 28 °C for 7 days. After cultured, three kinds of the liquid medium were serially diluted with sterilized water and then coated on the corresponding solid medium plate. The experiment was repeated three times.

2.3.2. Microcalorimetric Analysis

Based on the result of Section 2.3.1, the soil content of water was set to 20%; at the same time, the number of PZT bimorphs is determined to be 2 and the state is vibration. Soil pH was adjusted to 6 (G), 7 (I), and 8 (K). At the same time, the control group F, H, and J were set respectively. Tam III isothermal microcalorimeter (TA instruments, New Castle, DE, USA) [48] was used to reflect the effect of PZT bimorph on soil microbial growth activity under different pH and MC conditions. The microcalorimeter was adjusted to 28 °C. Under aseptic condition, 1.0 g dry soil sample passing through 1 mm fine sieve was put into the sample bottle; 0.2 mL of a mixed solution containing 5.0 mg glucose and 5.0 mg ammonium sulfate was added into the sample bottle. After being sealed, it was placed in a microcalorimeter for determination. Four thermodynamic parameters to characterize the microbial growth were analyzed after each power–time curve was obtained: growth rate constant (k), maximum thermal power (P_{peak}), time to reach the maximum peak (T_{peak}), and total heat dissipation (Q_{total}). P_{peak} and T_{peak} were obtained directly from the power–time curve. To obtain k , the power–time curve in the logarithmic growth stage was fitted in the thermokinetic equation: $\ln P_t = \ln P_0 + kt$, where t is the time, and P_0 is the power at the beginning of the exponential growth phase. Q_{total} is the sum of metabolic processes that occur during substrate consumption.

2.3.3. High Throughput Sequencing

Based on the result of Section 2.3.1, the soil MC was determined to be 20%, and the number of vibrating PZT bimorphs was 2. The soil (pH = 7) without PZT bimorph was used as control group (S1). The condition of experimental groups was as follows: pH = 8 (S2) and pH = 7 (S3), and high-throughput sequencing analysis was conducted after 49 days. After mixing the soil evenly, the soil (10 g) from each soil chamber was removed and stored in dry ice and sent to Shanghai Meiji Biomedical Technology Co., Ltd. IlluminaMiSeq high-throughput sequencing technology has been used to analyze microbial diversity and structural characteristics [49,50]. The amplified region was V3–V4 of 16S rDNA gene. 338F_806R was the forward and reverse primer [51]. The length of the amplified fragment was 468 bp, the sequencing method was PE300, the original sequence number was $148,818 \times 2$, and the total base number was 89,588,436 bp. After optimization, the effective sequence number was 148,818 and the effective base number was 61,928,949 bp. The 16S rRNA gene sequences were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession number PRJNA707201.

2.4. Data Analysis and Processing

Graphpad Prism 8 and Origin 10.5.1 were used for statistical analysis. In high-throughput sequencing analysis, all sequences were classified as OTU (operational taxonomic units) with 97% similarity (Uparse vesion 7.1). Sequence similarity $\geq 97\%$ was classified as one OTU unit. RDP classifier Bayes algorithm (RDP Classifier version 2.2) was used to classify the OTU representative sequences with 97% similarity level. The community species composition of each sample was counted at the taxonomic level of Phylum, Class, and Genus (Silva Release119, Unite Release 6.0, GeneBank Release 7.3). The community bar chart was constructed by R language package, and the Venn diagram was constructed by online R language package (<http://bioinformatics.psb.ugent.be/webtools/Venn/>, accessed on 17 November 2020). The circos diagram was drawn by Perl package.

2.5. Statistical Analysis

Statistical procedures were performed with the SPSS 19.0 software. Data were expressed as the means with standard deviation (SD). Significant differences of means for all treatments were judged by t test. The statistical significance level was set at $p < 0.05$.

3. Results

3.1. Response of Bacteria, Fungi, and Actinomycetes to the PZT Bimorph

The effect of PZT bimorphs on the number of microorganisms in microcosms with different MC is shown in Figure 3a–c. Compared with group A, microbial numbers in group B and group C had no significant difference. There was no significant difference between group E and group D, but both groups showed significant differences compared with group A. Group E showed obvious growth-promoting effect in soil with MC = 20%. The number of bacteria, fungi, and actinomycetes increased by 14.61%, 23.28%, and 15.91%, respectively, after 49 days.

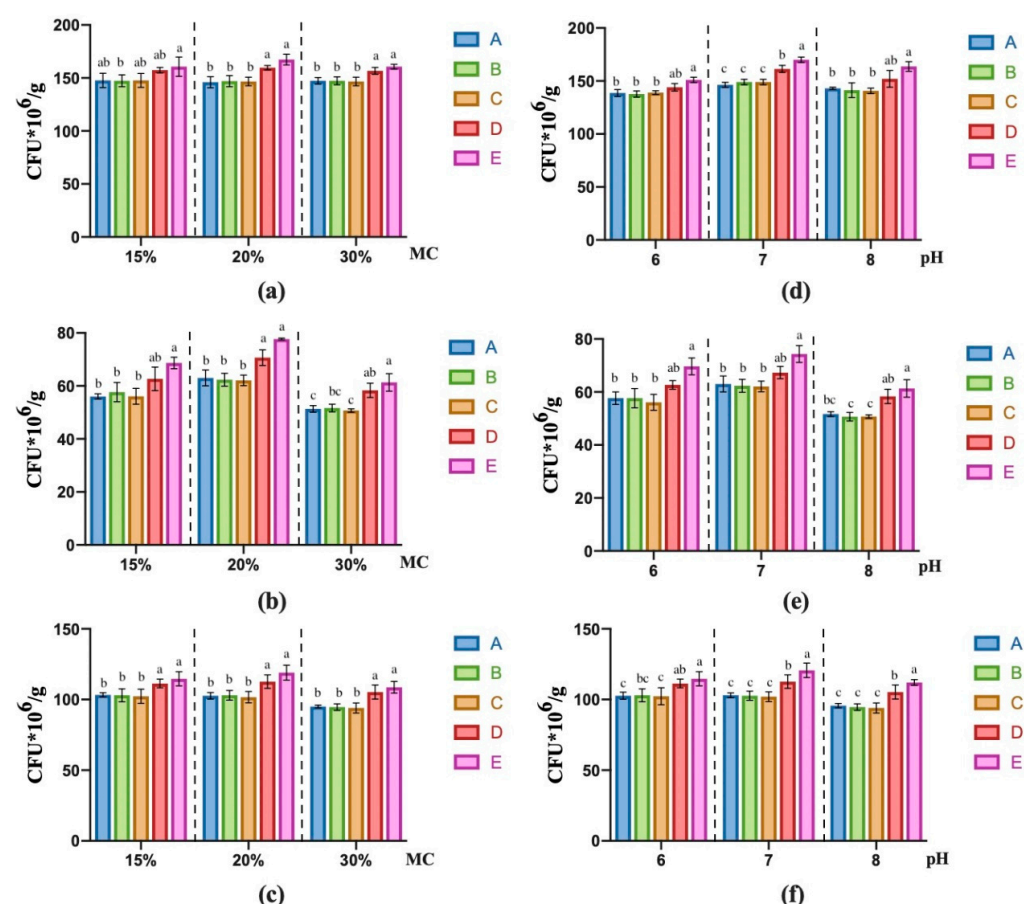


Figure 3. Effects of the Lead zirconate titanate (PZT) bimorph on soil microbial population with different moisture content (MC) and pH for 49 days: (a,d) Bacteria; (b,e) fungi; (c,f) actinomycetes.

The effect of PZT bimorphs on the number of microorganisms in microcosms with different pH is shown in Figure 3d–f. Microbial numbers in group B and group C had no significant change compared with group A. There was no significant difference between group E and group D when pH = 6. For bacteria and actinomycetes, group D and group E showed significant differences at pH = 7 and 8. Group E showed obvious growth-promoting effect in soil with pH = 7 and pH = 8 after 49 days. The quantity of bacteria, fungi, and actinomycetes increased by 16.17%, 17.99%, and 17.15% when pH = 7, respectively. The number of bacteria, fungi, and actinomycetes increased by 14.45%, 18.71%, and 17.07%, when pH = 8, respectively.

3.2. Isothermal Microcalorimetric Analysis

Group E (MC = 20%), which had the greatest influence on microbial number, was selected for microcalorimetric analysis. Adaptation, proliferation, logarithmic growth, delay, nutrient depletion, endogenous metabolism, typical decline, and incubation period were included in each curve in Figure 4. There was only one peak power in the power-time curve of each soil sample. The thermodynamic parameters of the microcalorimetry curve were shown in Table 1. T_{peak} , k , P_{peak} , and Q_{total} of all soil microcosms containing vibrating PZT bimorphs increases compared with the control group. P_{peak} in a short time with high k , which means that they have high metabolic activity [41,42]. Q_{total} has a high correlation with soil microbial community composition [52–54]. When pH = 7 or 8, P_{peak} and Q_{total} change obviously. When pH = 7, the increasing rates of P_{peak} and Q_{total} were 20.63% and 33.66%, respectively. When pH = 8, the increasing rates of P_{peak} and Q_{total} were 51.13% and 30.85%, respectively. The results showed that, in a word, the vibration PZT bimorph promoted the growth activity and quantity of soil microorganisms, which led to the increase of heat release, but also prolonged the slow growth period and logarithmic period of microorganisms.

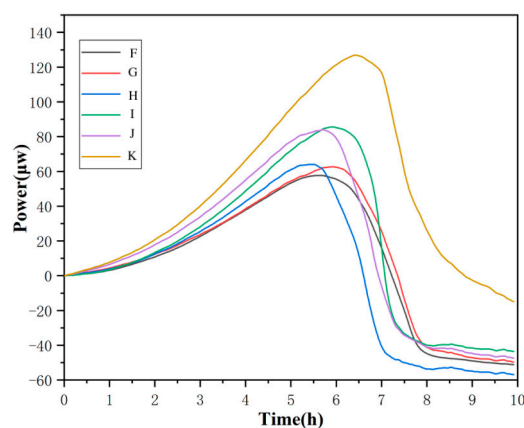


Figure 4. Power-time curves of different pH treated for 49 days.

Table 1. Thermodynamic parameters of soil under different treatment conditions.

Group	P_{peak} (μ W)	T_{peak} (h)	Q_{total} (J)	k (h^{-1})
F	57.73	5.64	0.57	0.1882 ± 0.0047
G	62.73	5.93	0.60	0.2445 ± 0.0036
H	64.05	5.42	0.63	0.2485 ± 0.0010
I	85.61	5.93	0.76	0.2718 ± 0.0058
J	83.99	5.71	0.94	0.2648 ± 0.0078
K	126.93	6.43	1.23	0.3153 ± 0.0061

3.3. Microcalorimetric Analysis

Rank abundance curve is one of the ways to analyze microbial diversity. The abundance of species is reflected by the width of the curve. The higher the species richness, the larger the range of the curve on the horizontal axis. The smoother the curve, the more uniform the species distribution [55,56]. On the OTU level, the species richness and evenness of S3 group were the best, while those of S2 were the worst, shown Figure 5.

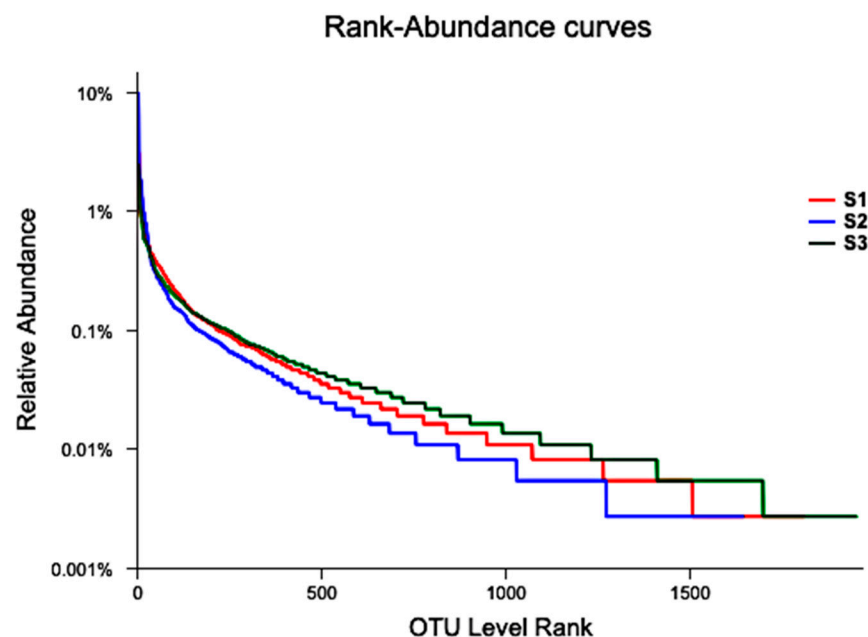


Figure 5. Rank-abundance curve on the OTU level.

The community bar chart can directly show the microbial types of each soil sample at a certain taxonomic level (phylum, class, genus) and the relative abundance of various microorganisms in each soil sample (Figure 6). The columns with different colors represent different microorganisms, and the length of columns represents the proportion of microorganisms. At the phylum level, the dominant phyla of S1 and S3 were *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, and *Acidobacteria*. However, the proportion of *Proteobacteria* in S3 increased significantly (from 26% to 35.1%), while *Actinobacteria* decreased significantly (from 36.1% to 26.9%), indicating that vibrating PZT bimorphs promoted the growth and reproduction of *Proteobacteria*, but inhibited that of *Actinobacteria*. In S2, *Acidobacteria* was not dominant, while *Firmicutes* was the most advantageous (27.2%). At the class level, the microbial composition of S3 is more uniform than that of S1. Compared with S1, *Actinobacteria* and *Bacilli* of S2 showed significant changes. *Actinobacteria* decreased from 36.1% to 25%, and *Bacilli* increased from 0.8% to 25.1%. Compared with S1, *Actinobacteria* decreased from 36.1% to 26.8% in S3, and others increased from 6.9% to 9.2%. At the genus level, the composition ratio of the three groups was very similar. Nevertheless, *Bacillus* accounted for the largest proportion in S2 (22.6%). Others in S3 (52.6%) accounted for more than others in S1 (44.1%) and S2 (33.8%). It was revealed that vibrating PZT bimorphs increased the diversity of microorganisms.

The venn diagram can directly reflect the difference of microbial community structure composition and find the “core microbial community” in these environments [57]. On the OTU level, the core groups of the three groups were very obvious (Figure 7a). The common OTUs in S1 and S3 groups were the most, while those in S1 and S2 groups were the least. S3 had the most OTUs, while S2 had the least. At the same time, S2 was also the sample with the least number of OTUs.

The Circos diagram was used to visualize the distribution proportion of dominant species in each sample (Figure 7b). The composition and proportion of dominant genera in S1 and S3 groups were similar. In S2 group, *Bacillus* accounted for the largest proportion (22.6%), which was different from S1 (0.4%) and S3 (0.3%) groups. The abundance of others in S3 group was higher than that in S1 group and S2 group, so the richness of S3 was better than the other two samples.

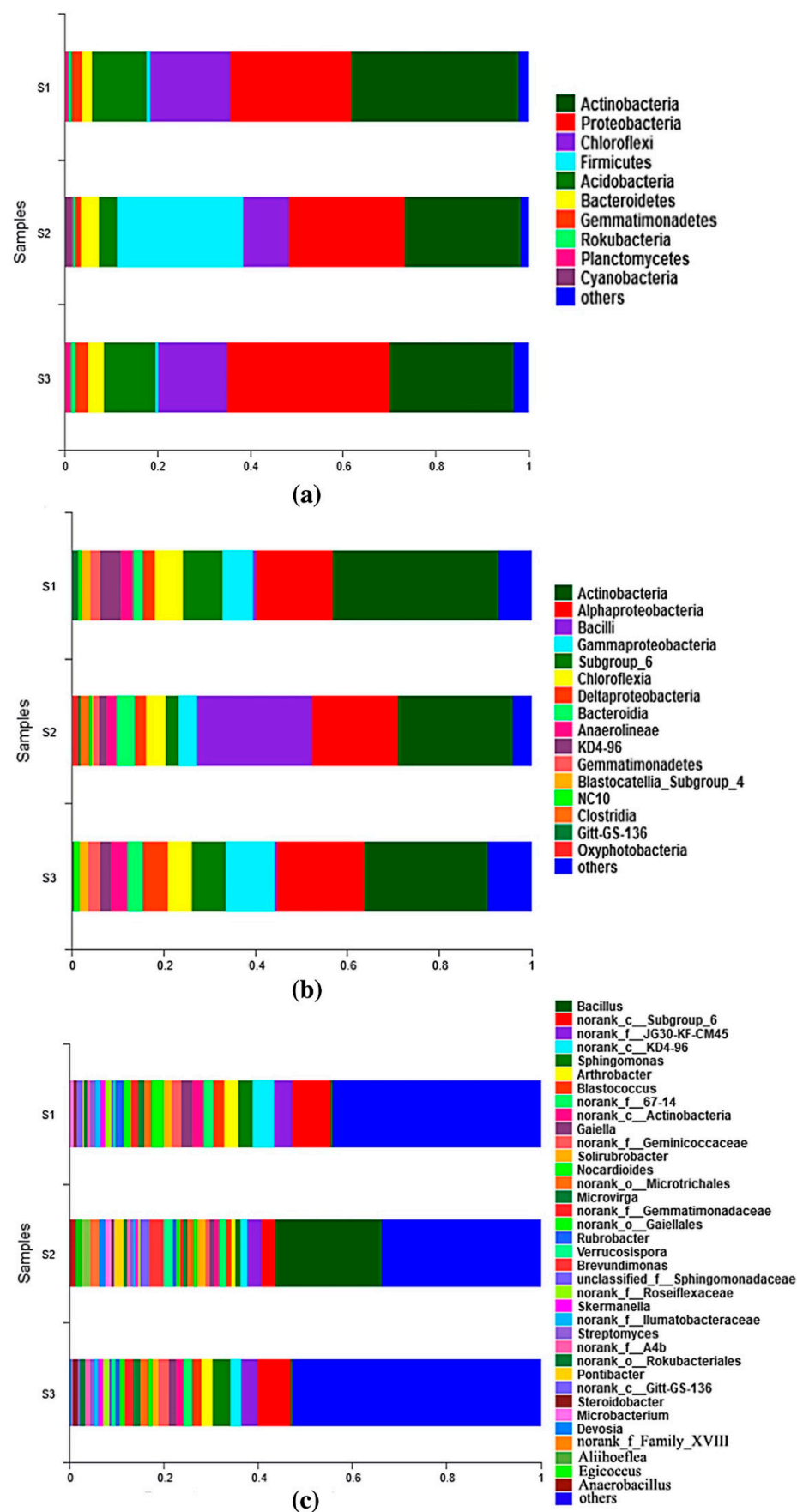


Figure 6. Bar chart of community at (a): phylum, (b): class, and (c) genus level.

stability. Because the original soil is weakly alkaline, when $\text{pH} = 6$, the activation effect of PZT bimorphs on microorganisms is not obvious compared with other groups, and it may even accelerate the death of intolerant microorganisms.

Fan et al. [69] found that the application of the electric field helps to maintain the uniformity of microbial community distribution. The main microbial composition of S1 and S3 was similar, but the proportion of major microorganisms in S2 changed obviously at the phylum level. At the class level, the vibrating PZT bimorph can make the soil microbial community more uniform. The vibrating PZT bimorph promoted the growth and reproduction of some non-dominant microorganisms at the genus level. When the pH value was close to the original soil, the vibrating PZT bimorph would increase the microbial richness on the premise of protecting the microbial genus structure through the analysis of high-throughput sequencing. However, when the $\text{pH} = 8$, although the addition of vibrating PZT bimorph can increase the number and activity of microorganisms, the structure of microorganisms still changed.

Bioremediation is a sustainable remediation technology that rectifies and re-establishes the natural soil condition [70]. At present, many scholars try to use the electric field to strengthen the remediation of contaminated soil by microorganisms, including oil pollution [71], heavy metal pollution [72], organic pollution [73], and so on. Its shortcomings include the loss of microbial viability during inoculation, eventual cell death after inoculation, and long repair time [74]. According to the research in this paper, the vibrating PZT bimorph can activate the microorganisms in soil and increase the biodiversity. With the help of vibrating PZT bimorphs, it is possible to keep the activity of inoculated microorganisms and make them adapt to the soil environment quickly. At the same time, the proportion of unknown microorganisms which originally accounted for a small proportion increased due to the activation of vibrating PZT bimorphs, and there may be some microorganisms which can transform pollutants efficiently and adapt to vibrating PZT bimorphs. Future research will be based on this as a starting point.

5. Conclusions

The laboratory studies indicated that the number of bacteria, fungi, and actinomycetes all increased under the action of vibrating PZT bimorph under different MC and pH conditions. When $\text{MC} = 20\%$, the increase of microbial number was the largest. The effect is more obvious when the number of PZT bimorphs is 2 with different soil pH . Under different pH conditions, the vibrating PZT bimorph can promote the growth activity of microorganisms, but the promotion effect is more obvious with $\text{pH} = 7$ and 8. Meanwhile, vibrating PZT bimorphs can increase the microbial diversity. However, when $\text{pH} = 8$, the structure of the microbial system would change significantly.

In conclusion, the effect of the PZT bimorph on soil microorganisms has been preliminarily explored. Our studies suggest that the number, activity, and abundance of soil microorganism can be improved by vibrating PZT bimorph under suitable MC and pH conditions. Piezoelectric materials are developing in the direction of non-toxic and efficient conversion efficiency, and their electrical parameters will become more controllable. In the future research, piezoelectric materials have the possibility to become a sustainable in-situ soil remediation method.

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