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Effects of Lake Sediment on Soil Properties, Crop Growth, and the phoD-Harboring Microbial Community

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Abstract: Removal of lake sediment has been shown to be an effective method for lake restoration. High phosphorus (P) content makes it possible for lake sediment to provide fertility for agricultural production. However, little research has focused on the responses of the soil-phosphorus-related microbial community to the sediment-derived fertilizer enriched in phosphorus content. The phoD-harboring gene, important to the global phosphorus cycle, encodes alkaline phosphatase hydrolyzing organic P in soil. Accordingly, a plot experiment was performed to compare the effects of four different fertilization treatments—no-fertilizer control (CK), 50% chemical fertilization with compressed sediment (CS), 50% chemical fertilization with original lake sediment (S), and conventional chemical fertilization treatment (CT)—on the phoD gene community using QPCR and high-throughput sequencing analysis. Relationships among soil physicochemical properties, phoD-harboring microbial community abundance and composition were also evaluated. Results showed that compared to CT, CS significantly increased soil organic matter (SOM) content by 20.29%, and S enhanced the humus content by 20.75% ($p < 0.05$). There was no significant influence on phoD gene microbial community richness (Chao1 and Sobs indexes) and diversity (Shannon index) between all treatments. The CS treatment significantly altered the phoD community structure and enhanced the Chinese cabbage yield by 40.19% ($p < 0.05$). Pearson analysis showed that phoD gene abundance (copy number) had significant and negative relationships with SOM, total nitrogen (TN), total phosphorus (TP), available nitrogen (AN), available phosphorus (AP), and the Chao1 index. Redundancy analysis showed that shifts in the phoD community structure were related to soil physicochemical properties (SOM, TN, TP, AN, AP, and humus) rather than soil pH. In conclusion, the compressed sediment can be used in farmland since it optimizes the phoD-harboring microbial community abundance, composition, and structure, and thus significantly increases the Chinese cabbage yield.

Keywords: lake sediment; phosphorus; Chinese cabbage; phoD gene; high-throughput sequencing

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1. Introduction

Phosphorus (P) is one of the major nutrients necessary for plants and also a crucial nutrient element in the farmland ecosystem [1]. However, the P utilization rate is only 5–25% in Chinese agricultural production [2]. Soil organic P accounts for 30–80% of the total P but cannot be directly used by plants [3]. It can only be converted into inorganic P through phosphatase and then used for growth and metabolism by plants [4]. Alkaline phosphatase (ALP) is an enzyme that hydrolyzes soil organic P to orthophosphate available for plants [5], mainly including phoA, phoX, and phoD [6]. Among them, phoA and phoX genes are mainly distributed in the aquatic environment, and phoD is usually found in terrestrial ecosystems [7]. Among ALP homologous genes, phoD is the most common gene in 16s rRNA metagenomic datasets and has become an important indicator of the soil P

cycle [6,8]. Under the condition of low P, the phoD gene expression is induced to increase the phosphate absorption and transport enzymes, thus accelerating the transformation process of soil organic P. In contrast, under the condition of sufficient P, the expression of the phoD gene is inhibited, and the ALP activity is decreased, which is conducive to the stable accumulation of organic P [6]. Luo et al. [9] found that long-term organic and inorganic fertilizer applications significantly increased the phoD gene abundance, while a single application of inorganic fertilizer showed the opposite behavior. They also noticed that the content of available phosphorus (AP) was increased in long-term fertilizer treatment, which significantly reduced the abundance and diversity index of the phoD microbial community. Several reports have indicated that ALP activity is related to phoD gene abundance and community structure [5,6]. Factors such as soil pH [10,11], land use [12], and vegetation [13] might significantly affect the phoD gene abundance and microbial community structure in the soil.

In recent years, with the excessive application of chemical fertilizers, nutrients in the soil have become more easily lost with water [14]. These extra nutrients tend to accumulate at the lake bottom and are at a risk of recycling to the above water body (i.e., internal nutrient loading), resulting in eutrophication [15]. There is in total around 200 million m^3 of sediment removed from water bodies in European nations each year [16]. Removal of sediment from lakes is effective for lake control and restoration [17]; it also can provide fertility for agricultural production because of the relatively high contents of nutrients such as nitrogen (N) and P [18]. Canet et al. [19] found that silt from the lake bottom enhanced the lettuce yield but did not affect the output of tomatoes. Kazberuk et al. [20] suggested that the yield of mustard was significantly increased by adding 5% of the dam sediment, but the contents of heavy metals in soil also increased. Previous studies have mainly focused on the impact of sediment on plant yield, soil nutrients, and heavy metal content, but there are few reports on the impact of sediment application on the phoD community.

In this study, high-throughput pyrosequencing and QPCR were used to assess the soil phoD community abundance and composition based on a plot trial with lake sediment addition. The aim of this research was to clarify the impact of lake sediment addition on soil biochemical properties, crop growth, and phoD gene microbial communities, and to provide scientific support for the efficient and ecological utilization of lake sediment with respect to a stable or increasing crop yield.

2. Materials and Methods

2.1. Site Description

The experiment was performed in the greenhouse test plot of Maqiao Youran Agricultural Base, China (30°59′44″ N, 121°20′39″ E). In this area, the average annual temperature and precipitation were 15.8 °C and 1178.0 mm, respectively. The soil texture was sandy loam, and the planting crop was *Brassica chinensis* L. The initial soil physicochemical characteristics in the test plots were: pH value of 8.47, total nitrogen (TN) value of 1.35 $g \cdot kg^{-1}$, total phosphorus (TP) value of 1.25 $g \cdot kg^{-1}$, soil organic matter (SOM) value of 31.26 $g \cdot kg^{-1}$.

2.2. Experimental Design

Brassica chinensis L. was planted and harvested on 1 March 2021 and 2 May 2021, respectively. Four treatments were set for the test: no fertilizer (CK), 50% chemical fertilization with compressed sediment (CS), 50% chemical fertilization with original lake sediment (S), and conventional inorganic fertilizer treatment (CT). Each treatment was performed in four replicates in a random block design. The planting area was 20 m^2. Based on the local regime, the pure amounts of N, P, and potassium (K) were the same in every fertilized treatment, which were N value of 375 kg/ha, P (P_2O_5) value of 225 kg/ha, and K (K_2O) value of 225 kg/ha, respectively. The original lake sediment was derived from Xuanmiaoguan lake in Yichang, Hubei Province, China and underwent sedimentation for 10~20 min and mechanical compression to form the compressed sediment (water content = 40%). The properties of the original lake sediment and the compressed sediment

used in the experiment are shown in Table S1, and the content of heavy metals were all below the control standards for pollutants in sludges from agricultural use in China (GB 4284-84). In the trial test, the original lake sediment and compressed sediment accounted for half of the P supply in the S and CS treatments, respectively. The remaining nutrients in the experiment were supplemented by urea, $Ca(H_2PO_4)_2$, and K_2SO_4. All the fertilizers were used as base fertilizer before planting.

2.3. Sampling and Measurements

After crop harvest, the 0–20 cm layer of soil samples were collected using a five-point sampling method with a soil sampler of each plot and mixed together. After removing the dead leaves, stones, and roots, the soil samples were kept in sterilized bags and then brought back to the laboratory for study. One portion of the samples was ground to 0.25 mm in a sieve after natural air drying to determine the physicochemical properties, and the other portion was kept at −80 °C for QPCR analysis and high-throughput sequencing of the phoD-harboring microbial community.

The SOM was analyzed by potassium dichromate oxidation [21]. The soil pH was determined by potentiometry (water: soil = 2.5:1) [22]. The TN content was evaluated by the Kjeldahl method [21]. The soil AN was determined by the alkali diffusion method [23]. The soil TP was determined by the sulfuric acid–perchloric acid digestion method [24]. The soil AP was determined by the $NaHCO_3$–Mo-Sb anti spectrophotometric technique [25]. The measurement of humus content was according to Wu et al. (2020) [26].

2.4. Microbial Analysis

Soil DNA was extracted using an Omega E.Z.N.A.® Soil DNA Kit (D5625-02) following the manufacturer's instructions. The concentration and purity of the isolated DNA were measured using a spectrophotometer (RS232G, Eppendorf, Germany). The primer pairs for the phoD gene were ALPS-F73 (5′-CAGTGGGACGACCACGAGGT-3′) and ALPS-1101 (5′-GAGGCCGATCGGC-ATGTCG-3′) [27]. The QPCR quantitative tests were run in duplicates, and the amplifications were performed with a total volume of 20 μL, which contained 4 μM of respective primer, 1 μL DNA template (approximately 20 ng), and SYBR real-time PCR premix (Takara, Dalian, China). The thermal conditions were: 95 °C for 5 min, followed by 40 cycles of 95 °C for 5 s, and 60 °C for 40 s [28]. The standard curve was established using a serial dilution of purified plasmid DNA harboring phoD genes.

The PCR reactions were carried out in 25 μL mixtures containing 12.5 μL of Phusion Master Mix (New England Biolabs, Ipswich, MA, USA) (2×), 0.5 μL of each primer, 1 μL of DNA template, and 10.5 μL of H_2O. Samples were subjected to the following amplification program: 95 °C denaturation for 30 s, 40 cycles of 95 °C for 5 s and at 60 °C for 34 s, and a final extension at 72 °C for 7 min [28]. After quantification, the PCR amplicons were pooled in equal amounts ($ng \cdot \mu L^{-1}$), and paired-end 2 × 300 bp sequencing was performed using the Illumina NovaSeq platform at Allwegene Tech. (Beijing, China).

2.5. Statistical Analysis

To identify the significant differences between the average values of different treatments, one-way analyses of variance (ANOVA) with least significant difference (LSD) tests ($p < 0.05$) were performed using SPSS 26.0 software. Venn diagrams were generated by the Venn Diagram package (Adrian Dusa, University of Bucharest, Bucharest, Romina) of R (version 3.5.1) software. The phoD gene richness and diversity indexes (Chao1 and observed species; PD_whole_tree and Shannon) were calculated using Mothur (version v.1.30.1) (Patrick Schloss, University of Michigan, Ann Arbor, MI, USA). Partial least squares discriminant analysis (PLS-DA) was performed to investigate the differences of phoD-harboring microbial community structure among the treatments using R software. Redundancy analysis (RDA) was employed to determine the relationships between the phoD-harboring microbial community composition and the soil properties using R software. Using the AMOS (IBM SPSS AMOS 25) (IBM, Armonk, NY, USA) software, structural

equation modelling (SEM) was established to evaluate the direct and indirect relationships among the diversity of the phoD-harboring microbial community, input of fertilizer and sediment, and soil physicochemical characteristics.

3. Results and Discussion

3.1. Soil Properties

Fertilization significantly affected the soil pH with an increase and decrease in the CT and CS treatments, respectively (Table 1, $p < 0.05$). The SOM, TN, and AP contents were highest in the CS treatment and lowest in the CK treatment ($p < 0.05$), while similar and intermediate values were observed in the CT and S treatments. The AN content in the CT treatment (98.03 mg·kg^{-1}) was 1.70, 1.31, and 1.26 times more than those of the CK, S, and CS treatments, respectively ($p < 0.05$). Fertilizer addition (CT, S, and CS treatments) notably increased the TN, TP, and AP contents compared to the CK treatment ($p < 0.05$). The humus content increased significantly only in the S treatment (4.48 g·kg^{-1}) in comparison with CK and CT ($p < 0.05$).

Table 1. Soil properties under different fertilization treatments.

Items	pH	SOM (g·kg^{-1})	TN (g·kg^{-1})	TP (g·kg^{-1})	Humus (g·kg^{-1})	AN (mg·kg^{-1})	AP (mg·kg^{-1})
CK	8.44 ± 0.06ab	30.36 ± 0.88c	1.33 ± 0.04b	1.19 ± 0.21b	3.41 ± 0.25b	57.6 ± 2.92c	24.64 ± 1.97b
CS	7.77 ± 0.04c	49.26 ± 2.89a	1.84 ± 0.19a	1.87 ± 0.22a	4.06 ± 0.16ab	77.89 ± 6.64b	46.30 ± 2.45a
S	8.23 ± 0.22b	37.68 ± 3.25b	1.64 ± 0.06a	1.90 ± 0.03a	4.48 ± 0.46a	74.55 ± 6.13b	44.66 ± 2.16a
CT	8.52 ± 0.04a	40.95 ± 5.18b	1.73 ± 0.08a	1.83 ± 0.06a	3.71 ± 0.18b	98.03 ± 1.6a	45.11 ± 1.34a

Note: The values present the average ± standard deviation ($n = 4$). CK: no fertilizer application; CS: 50% chemical fertilization with compressed sediment; S: 50% chemical fertilization with original lake sediment; CT: conventional treatment; SOM: soil organic matter; TN, total nitrogen; TP: total phosphorus; AN: available nitrogen; AP: available phosphorus. Different lowercase letters indicate significant differences between treatments analyzed by ANOVA using LSD test ($p < 0.05$).

3.2. Crop Yield and Growth-Related Traits

Fertilization significantly improved the yield and quality of Chinese cabbage (Table 2). Fertilizer application (CT, S, and CS treatments) resulted in 2.25~3.15 times the vegetable yield of that in the CK treatment, among which the production in the CS treatment was the highest (3992.67 kg·667 m^{-2}) ($p < 0.05$), and no significant difference was observed between the CT and S treatments. The S and CS treatments exhibited more obvious enhancement in plant height than the CK and CT treatments (Table 2, $p < 0.05$), but there were no differences between them. Similarly, lake-sediment-derived substances (S and CS treatments) increased the chlorophyll contents to 34.93 mg·kg^{-1} and 36.27 mg·kg^{-1}, respectively. Different fertilizers did not change the p-TN content (1.98~2.06 g·kg^{-1}); the highest p-TP contents were in the S and CS vegetables, followed by that of the CT sample (0.30 g·kg^{-1}) ($p < 0.05$). The CK vegetable had the lowest p-TP content (0.20 g·kg^{-1}), which was almost 1.53~1.76-fold lower than that in the other treatments.

The application of chemical fertilizer or organic matter is a widely used agricultural practice to improve the soil P supply [29]. The S and CS sediments have a relatively neutral pH (7.30~7.45) (Table S1), which might influence the soil pH to a certain degree. The CS possesses nutrients to an extent almost 10-fold higher than S, so they had similar contents of TN, TP, AN, and AP in the soil, but not SOM content. Adding fertilizers significantly increased the storage (TP) and availability (AP) of P (Table 1). There was no significant difference of TP content in CT, S, and CS treatments; this may correspond to the same concentration of total P applied in the three treatments. However, the p-TP contents in the S and CS vegetables were 13.33~16.67% higher than in the CT treatment ($p < 0.05$, Table 2). Regarding the crop yield, the CS treatment had the highest P apparent utilization efficiency.

Table 2. The yield and quality of Chinese cabbage under different treatments.

Items	Yield ($kg \cdot 667m^{-2}$)	Plant Height (cm)	Chlorophyll ($mg \cdot kg^{-1}$)	p-TN ($g \cdot kg^{-1}$)	p-TP ($g \cdot kg^{-1}$)
CK	1266.55 ± 45.13c	11.3 ± 0.88c	28.85 ± 0.91c	1.19 ± 0.08b	0.20 ± 0.01c
CS	3992.67 ± 85.27a	23.38 ± 0.56a	34.93 ± 0.47ab	2.04 ± 0.06a	0.34 ± 0.01a
S	2835.2 ± 108.69b	23.74 ± 1.04a	36.27 ± 1.32a	1.98 ± 0.16a	0.35 ± 0.00a
CT	2847.95 ± 105.48b	19.46 ± 1.38b	33.18 ± 1.57b	2.06 ± 0.12a	0.30 ± 0.01b

Note: p-TN: TN content in Chinese cabbage; p-TP: TP content in Chinese cabbage. Dates in the table are Mean ± SE; Different letters in the same column indicate a significant difference ($p < 0.05$).

3.3. phoD-Harboring Microbial Abundance Analysis

In the case of phosphorus-derived organic materials as an alternative to chemical fertilizers, it is important to understand how these organic materials affect soil phoD microbes, since they could accelerate the mineralization of organophosphates. CK soil had the highest phoD gene abundance (5.85×10^7 copy·g^{-1} soil) (Figure 1a). Fertilization led to a substantial 2.02~2.95-fold reduction in the phoD gene abundance compared to CK ($p < 0.05$), with no significant differences between the CT, S, and CS treatments.

In this study, a total of 1,198,288 clean data were obtained, and subsequently 9761 OTUs were generated. After rarefication, 9280 OTUs remained in the soil samples. The level of Good's coverage per sample was >97%, suggesting that the majority of phoD gene diversity in the sample soils was captured. The total OTUs in the CK, CT, S, and CS soils were 3893, 4966, 5320, and 4523, respectively (Figure 1b). The shared OTUs of the four treatments were 1158; the unique OTUs were 1641, 450, 1051, and 914, respectively. The shared OTUs mainly belonged to p_unidentified, Actinobacteria, and Proteobacteria. Although the CK soil had the lowest total OTUs (3893), it exhibited the highest number of unique OTUs (1641). The S and CS treatments alleviated the decrease in unique OTUs compared to the CT treatment. Therefore, fertilization regimes reshaped the phoD gene microbial community.

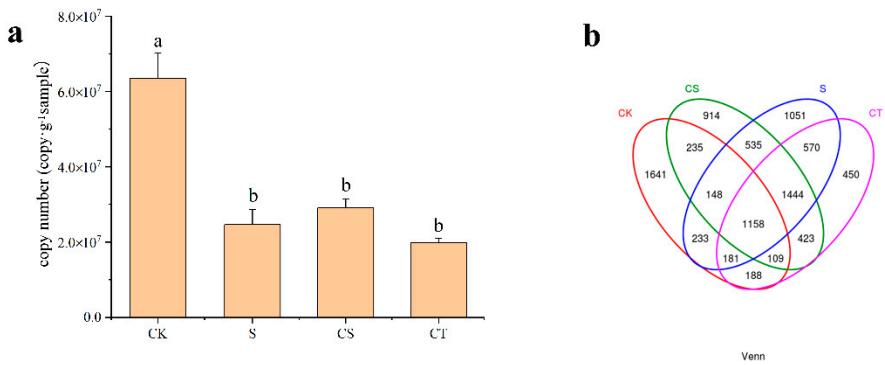


Figure 1. (**a**) Abundance of microbial phoD gene (copy number) quantified by QPCR. (**b**) Unique and shared OTUs among the different treatments by Venn analysis. Different lowercase letters indicate a significant difference between treatments ($p < 0.05$).

The high phoD gene abundance was thought to be caused by the available P deficiency, so microbes need to increase the high expression of genes related to P transportation and absorption in order to facilitate plant uptake. In addition, it has been well proved that low available P favors the synthesis of phosphatases [30], which was also identified by the Pearson analysis (Table 3). However, in the CT treatment, the P input comprised mainly small molecules which could be directly absorbed and utilized by plants, so the expression of phoD was definitely inhibited. Organic fertilizer, rich in C substrate but low in available P, probably favored the proliferation of some phoD-harboring species to mineralize organic phosphonate, thus increasing the phoD gene abundance (Figure 1a). Luo et al. [9] reported that chemical-only fertilization had the lowest phoD gene abundance,

and organic–inorganic mixed fertilization would significantly increase it when compared with the control. However, Chen et al. reported that phoD gene abundance was highest in organic–inorganic mixed soil samples, and lowest in inorganic chemical fertilization [29]. Such conflicting results regarding the exogenous organic substance may be due to fertilization regime, crop type, and intrinsic soil property. Long-term soil with no P had the lowest phoD microbial diversity and total bacterial diversity [31]. Long-term utilization of chemical fertilizers would inhibit the growth of the phoD bacterial community [9,32]. Such a phenomenon was not noticeable in this short-term trial assay, and the temporal variation needs further analysis.

Table 3. Pearson correlations between phoD gene abundance, soil properties, and α diversity under different treatments.

	phoD	pH	SOM	TN	AN	TP	AP	Humus	Chao1	Shannon
PhoD	1									
pH	0.193	1								
SOM	−0.534 *	−0.662 **	1							
TN	−0.683 *	−0.500 *	0.772 **	1						
AN	−0.712 *	0.045	0.496	0.670 **	1					
TP	−0.888 **	−0.369	0.630 **	0.700 **	0.599 *	1				
AP	−0.885 **	−0.398	0.718 **	0.835 **	0.689 **	0.869 **	1			
Humus	−0.471	−0.493	0.375	0.371	0.110	0.599 *	0.592 *	1		
Chao1	−0.628 **	−0.379	0.431	0.399	0.371	0.561 *	0.682 **	0.422	1	
Shannon	−0.084	−0.221	0.073	−0.083	−0.056	−0.057	0.033	−0.146	0.628 **	1

Note: *: correlation is significant at the 0.05 level; **: correlation is significant at the 0.01 level.

3.4. phoD Microbial Community α and β Diversity Analysis

The Chao1, Observed_species (Sobs), PD_whole_tree, and Shannon indexes were calculated to analyze the phoD microbial α diversity (Figure 2). For Chao1 and Sobs indexes, S and CS treatments showed significant increases compared with the unfertilized soil (CK) ($p < 0.05$). The CT treatment had lower microbial richness, but there was no significant difference between the CT, S, and CS treatments. The microbial community diversity index, PD_whole_tree, was higher under fertilizer application treatments than the CK; however, it is noteworthy that there was no notable difference between the CT, S, and CS treatments (Figure 2c). Fertilization, or not, had no effect on the Shannon index (Figure 2d).

The PLS-DA revealed a good model to differentiate the CK from other fertilization treatments at the OTU level, with a total explanatory degree of 26.79% (Figure 3). Unfertilized soil (CK) and fertilized soil (CT, S, and CS) were separated by the PC1 axis (17.75%). The PC2 axis distinguished the CS treatment from the CT and S treatments, with an interpretation of 9.04%. Samples of the CT and S treatments gathered together, indicating a similar phoD microbial community structure in their soil samples. Furthermore, PERMANOVA showed a notable structural difference between the treatments with $p = 0.001$ (data not shown). In the present study, the compressed sediment application significantly affected the phoD gene community structure in the vegetable soil.

Chen et al. [33] reported that long-term P fertilizer input enhanced phoD gene diversity. However, in the present study, only the S treatment increased the PD_whole_tree compared with the CK, and fertilization showed no influence on the Shannon index (Figure 2). A contradictory finding concluded that no detectable effects were observed on soil microbial abundance and diversity after repeated applications of sediments for two seasons; at least, such effects on soil microbial ecology variation seemed to be more remarkable in long-term fertilization experiments [34]. Sapp et al. [35] in a short-term greenhouse trial concluded that digestate application decreased the bacterial community diversity. Similarly, in the present study, the original lake/compressed sediment attenuated the decrease in phoD gene microbial richness and diversity.

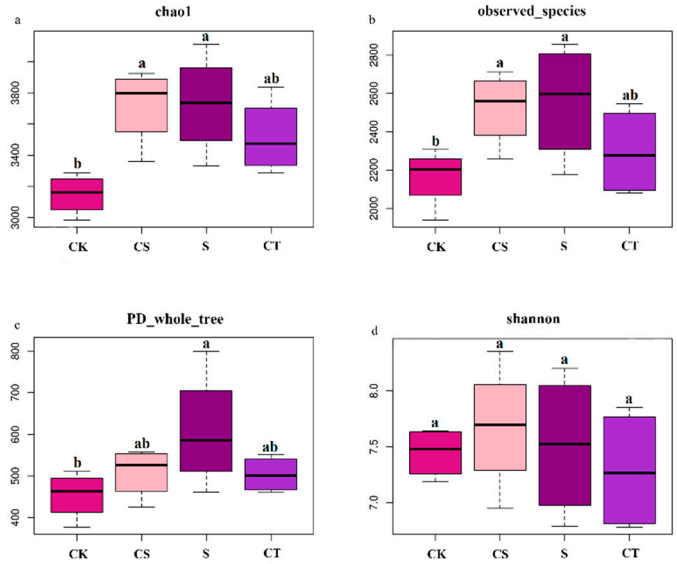


Figure 2. Comparison of the estimated OTU α-diversity indexes ((**a**): chao1, (**b**): observed_species, (**c**): PD_whole_tree, (**d**): shannon) of the phoD microbial community in different fertilizer treatments. Different lowercase letters indicate a significant difference between treatments ($p < 0.05$).

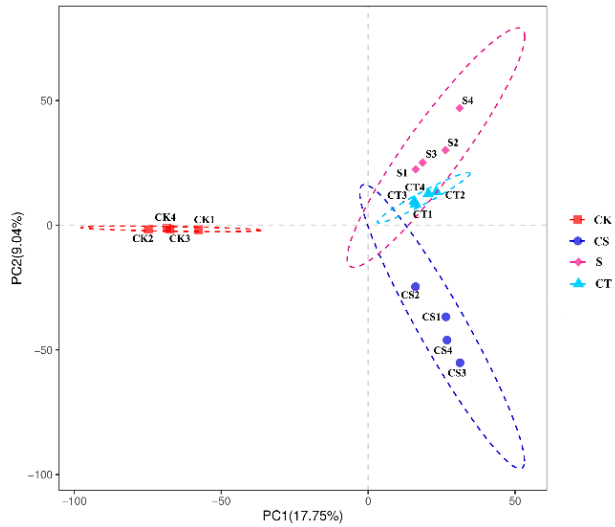
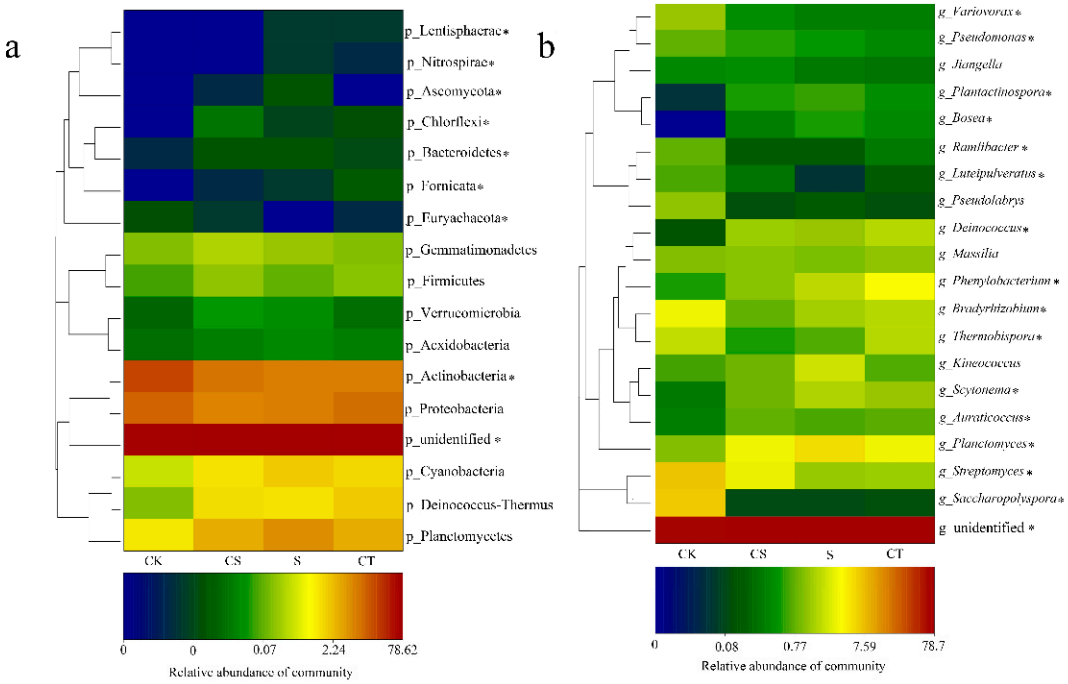


Figure 3. Analysis of the phoD microbial community structure in different fertilizer treatments by PLS-DA.

3.5. Comparison of phoD Microbial Community Composition among the Different Treatments

Fertilization did not change the composition of the phoD microbial community, but significantly affected its relative abundances (Figure 4). The species across all soil samples were classified into 17 phyla, with the predominated phyla p_unclassified, Actinobacteria, and Proteobacteria occupying 66.86~78.62%, 7.36~19.99%, and 1.12~5.85%, respectively. The minimal phyla were Nitrospirae, Lentisphaerae, Ascomycota, Euryarchaeota, and Fornicata. One-way ANOVA of the 17 phyla taxa showed that the relative abundances of nine bacterial phyla were markedly influenced by the fertilization regime compared with CK ($p < 0.05$). Compared to the CT treatment, the relative abundances of Lentisphaerae, Nitrospiare, and Fornicata were significantly decreased in the CS treatment; on the contrary, Ascomycota and Chloroflexi contents were increased ($p < 0.05$). Ascomycota, Bacteroidetes, and Fornicata were distinctly affected in the S soil compared with the CT treatment ($p < 0.05$) (Figure 4c). The relative abundances of dominant genera are shown in Figure 4b,d, and the genera with an abundance of less than 1% was classified as "Others". The top five dominant genera

were g_unidentified, Planctomyces, Streptomyces, Bradyrhizobium, and Phenylobacterium. In comparison with the CK treatment, fertilization treatments (CT, S, and CS) increased the relative abundances of Plantactinospora, Bosea, Phenylobacterium, Scytonema, Auraticoccus, Planctomyces, and g_unidentified ($p < 0.05$). Vriovorax, Pseudomonas, Ramlibacter, Luteipulveratus, Deinococcus, Bradyrhizobium, Thermobispora, Streptomyces, and Saccharopolyspora contents were decreased by fertilizer application ($p < 0.05$). The S and CS treatments significantly decreased the contents of Phenylobacterium (47.04% and 68.45%, respectively) and Bradyrhizobium (18.48% and 55.26%, respectively), compared with the CT treatment ($p < 0.05$).



c

	CK	CS	S	CT
p__Lentisphaerae	b	b	a	a
p__Nitrospirae	b	b	a	a
p__Ascomycota	c	b	a	c
p__Chloroflexi	b	a	b	b
p__Bacteroidetes	b	b	a	b
p__Fornicata	d	c	b	a
p__Euryarchaeota	a	ab	c	bc
p__Gemmatimonadetes	a	a	a	a
p__Firmicutes	a	a	a	a
p__Verrucomicrobia	a	a	a	a
p__Acidobacteria	a	a	a	a
p__Actinobacteria	a	b	b	b
p__Proteobacteria	a	a	a	a
p__unidentified	b	a	ab	ab
p__Cyanobacteria	a	a	a	a
p__Deinococcus-Thermus	a	a	a	a
p__Planctomycetes	a	a	a	a

d

	CK	CS	S	CT
g__Variovorax	a	b	b	b
g__Pseudomonas	a	ab	ab	b
g__Jiangella	a	a	a	a
g__Plantactinospora	b	a	a	a
g__Bosea	b	a	a	a
g__Ramlibacter	a	b	b	b
g__Luteipulveratus	a	ab	b	ab
g__Pseudolabrys	a	a	a	a
g__Deinococcus	a	b	b	b
g__Massilia	a	a	a	a
g__Phenylobacterium	c	b	b	a
g__Bradyrhizobium	a	c	bc	ab
g__Thermobispora	a	b	ab	ab
g__Kineococcus	a	a	a	a
g__Scytonema	b	ab	a	a
g__Auraticoccus	b	a	ab	ab
g__Planctomyces	b	ab	a	ab
g__Streptomyces	a	b	b	b
g__Saccharopolyspora	a	b	b	b
g__unidentified	b	a	ab	ab

Figure 4. Comparison of the phoD microbial community composition in different treatments at the phylum (**a**) and genus levels (**b**). Different letters in the same column indicate a significant difference ($p < 0.05$) ((**c**): at phylum level, (**d**): at genus level).

Many micoorganisms can degrade and transform nutrients from soil organic matter, improve soil quality, and increase crop yield [36]. The dominant phoD-harboring microbes include Actinobacteria, Proteobacteria, Planctomycetes, and Cyanobacteria, which was in accordance with some other studies [9,37]. Generally, the variation in relative abundances of the phoD gene was not notable between the CT, S, and CS treatments, suggesting that the phoD-containing bacteria were not as sensitive to phosphate fertilizer as the 16S rRNA

bacteria. The P fertilizer, soil types, and experimental conditions may explain the divergent results reported in the various studies [38].

Ragot et al. [10] emphasized that Bradyrhizobium and Streptomyces were the dominant genera of phoD-harboring microbes and are not affected by changes in environmental conditions. Bradyrhizobium and Streptomyces were both monitored in this study, but their relative abundances were significantly decreased by fertilization (Figure 4d), which was in accordance with the relatively low copy number of the phoD gene in the CT, S, and CS treatments (Figure 1a). Bradyrhizobium is a symbiotic N_2 fixer and may play important roles in coupling the soil N and P cycles. Some α-Proteobacteia (e.g., Bradyrhizobium) increased ALP activity and P transport rates as a response to P stress, so CK had the highest relative abundance of Bradyrhizobium (3.26%). Furthermore, Bradyrhizobium may fix N in the air to avoid N limitation in the CK and CT treatments. Streptomyces is closely related with P transformation, particularly mineralizing organic P and phosphate solubilization [39]. Their relative abundance seemed to sensitively respond to P limitation, at least in the present study (Figure 4d). Pseudomonas content was significantly decreased by inorganic fertilizer (CT treatment) (Figure 4d); bacteria belonging to this genus usually contribute to mineralizing organophosphorus and thus play an important role in promoting plant growth. Phenylobacterium was also known as a N-fixer bacterium, belonging to the Proteobacteria phylum; CT significantly increased its relative abundance compared to CK and the other fertilizer addition treatments (S and CS). Therefore, future study should focus on the investigation of N cycling and P turnover under various conditions.

In relation to the phoD microbial community composition tested in the fertilization application and their agricultural importance, most dominant groups were found to have unknown or not well-reported roles in the soil P cycle and/or crop growth, such as the "unidentified" species shown in Figure 4a,b. This might embody the need for a deeper sequencing depth between metagenomics and phoD alleles with more comprehensive primers. The ALP activity and phoD expression should be considered in future research.

3.6. Comparison of phoD Microbial Community Composition among the Different Treatments

Correlations were investigated between soil properties and the phoD gene communities (Table 3, Figure 5). Results showed that phoD gene copy number was significantly and negatively correlated with SOM*, TN*, AN*, TP**, AP**, and phoD gene microbial community richness (Chao1**) (Table 3, * $p < 0.05$, ** $p < 0.01$). On the other hand, phoD gene richness also positively correlated with soil TP ($p < 0.05$) and AP ($p < 0.01$), while phoD gene community diversity (Shannon index) only positively correlated with the Chao1 index ($p < 0.01$) and had no correlations with soil properties and phoD gene abundance. Soil pH showed no obvious correlations with phoD gene α diversity (Chao1 and Shannon), which was in contrast with Chen et al. reports [29,33].

The first two axes of RDA explained 49.54% and 9.50% of the total variation (Figure 5). The phoD gene community of the CK soil was separated from the other fertilized soils (CT, S, and CS) along the RDA1 axis. However, the CS samples could not differentiate from the CT and S samples by the RDA2 axis. The structure of the phoD gene community was strongly correlated with TP (r2 = 0.702, $p = 0.001$) and AP (r2 = 0.827, $p = 0.001$), and to a lesser extent with SOM (r2 = 0.523, $p = 0.007$), TN (r2 = 0.544, $p = 0.012$), AN (r2 = 0.487, $p = 0.009$), and humus (r2 = 0.458, $p = 0.019$) except soil pH (r2 = 0.123, $p = 0.431$) (Table S2). Soil AP was strongly correlated with the RDA2 axis, indicating that the phoD bacterial community structure was mainly related to available nutrient changes caused by P inputs. Environmental factors played more roles in the phoD microbial community structure of fertilized treatments (CT, S, and CS) than in the CK treatment.

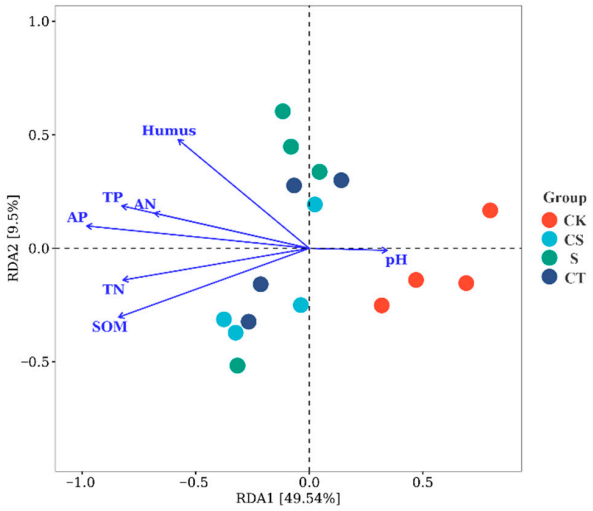


Figure 5. Ordination plots by RDA at the phylum level to explore the relationships between the phoD community and soil properties. Samples from different fertilization treatments were marked with different colors.

3.7. Structural Equation Model Analysis of the phoD Microbial Communities

The SEM analysis quantified the impact of each environmental factor and were expressed as path coefficients (Figure 6). Fertilizers had significant effects on all the indicators (SOM, TN, AN, TP, and AP) with the path coefficients ranging from 0.770 to 0.851, except in pH and humus. Original lake sediment could indirectly improve the phoD gene diversity by increasing the humus content (path coefficient = 0.678). Compressed sediment had negative correlations with pH and AN with path coefficients of −0.554 and −0.387, respectively, and was positively correlated with the SOM content (path coefficient = 0.409). The original lake sediment and compressed sediment both had no notable influence on the AP and TP contents, though they were intended to be used to reduce the inorganic P input. The pH and humus were significantly and positively correlated with phoD gene diversity, with path coefficients of 0.585 and 0.296, respectively. The AP and TP were negatively correlated with phoD gene diversity, with the path coefficients of −0.832 and −0.462, respectively. Different fertilization treatments regulated the variation in phoD gene diversity mainly by changing the soil pH, humus, TP, and AP.

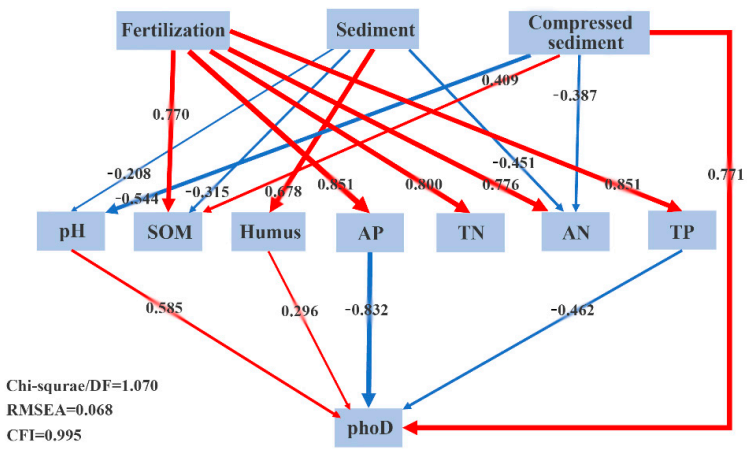


Figure 6. Structural equation model (SEM) analysis of the influences of fertilization on phoD abundance as mediated by soil physicochemical properties. Numbers adjacent to arrows are standardized path coefficients. Red and blue arrows indicate positive and negative effects with significance, respectively.

Many studies have emphasized that pH is associated with microbial and phoD communities and could be reflected as a strong indicator [10,11,29]. In our present study, pH exerted insignificant effects on phoD gene abundance, microbial community diversity, and structure. In addition, whether the dominant microbial community from the original lake/compressed sediment survived and thus affected the native microbial population of the soil was not analyzed in the present study. Bacteria and soil properties always altered by temporal and spatial variations [9].

4. Conclusions

The compressed sediment addition could enhance Chinese cabbage yield and increase the P cycling through the promotion of the soil phoD-harboring microbial community. Compressed sediment addition is a feasible technical means to improve the soil P supply and promote crop growth. The optimal addition quantity of compressed sediment in various agricultural fields will be tested in a further study.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12122065/s1, Table S1: Basic properties of the original lake sediment and compressed sediment used in the experiment; Table S2: Monte Carlo permutation test of soil phoD microbial communities and soil properties.

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