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Research Article

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How silicon fertilizer improves nitrogen and phosphorus nutrient availability in paddy soil?

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Abstract: In order to reveal the mechanism of silicon (Si) fertilizer in improving nitrogen (N) and phosphorus (P) nutrient availability in paddy soil, we designed a series of soil culture experiments by combining application of varying Si fertilizer concentrations with fixed N and P fertilizer concentrations. Following the recommendations of fertilizer manufacturers and local farmers, we applied Si in concentrations of 0, 5.2, 10.4, 15.6, and 20.8 μg/kg. At each concentration of added Si, the availability of soil N and P nutrients, soil microbial activity, numbers of ammonia-oxidizing bacteria and P-decomposing bacteria which means that the organic P is decomposed into inorganic nutrients which can be absorbed and utilized by plants, and urease and phosphatase activity first increased, and then decreased, as Si was added to the soil. These indicators reached their highest levels with a Si application rate of 15.6 μg/kg, showing values respectively 19.78%, 105.09%, 8.34%, 73.12%, 130.36%, 28.12%, and 20.15% higher than those of the controls. Appropriate Si application (10.4 to 15.6 μg/kg) could significantly increase the richness of the soil microbial community involved in cycling of N and P nutrients in the soil. When the Si application rate was 15.6 μg/kg, parameters for characterizing microbial abundance such as sequence numbers, operational taxonomic unit (OTU) number, and correlation indices of microbial community richness such as Chao1 index, the adaptive coherence estimator (ACE) index, Shannon index, and Simpson index all reached maximum values, with amounts increased by 14.46%, 10.01%, 23.80%, 30.54%, 0.18%, and 2.64%, respectively, compared with the control group. There is also a good correlation between N and P mineralization and addition of Si fertilizer. The correlation coefficients between the ratio of available P/total P (AP/TP) and the number of ammonia-oxidizing bacteria, AP/TP and acid phosphatase activity (AcPA), AP/TP and the Shannon index, the ratio of available N/total amount of N (AN/TN) and the number of ammoniated bacteria, and AN/TN and AcPA were 0.9290, 0.9508, 0.9202, 0.9140, and 0.9366, respectively. In summary, these results revealed that enhancement of soil microbial community structure diversity and soil microbial activity by appropriate application of Si is the key ecological mechanism by which application of Si fertilizer improves N and P nutrient availability.

Key words: Silicon; Paddy soil; Nitrogen and phosphorus nutrient availability; Microbial community structure nutrient

1 Introduction

Numerous studies have shown that silicon (Si) has a positive effect on soil nutrient utilization (Eneji et al., 2008; Mehrabanjoubani et al., 2015; Pati et al., 2016; Schaller et al., 2016). In recent years, many studies have confirmed that the application of Si improves the utilization of soil nutrients by both plants and microbes. For example, Neu et al. (2017) have shown that Si application at concentrations of 10.4–15.6 μg/kg can effectively increase grain yield and fertilizer utilization rate, while excessive Si application (concentrations higher than 20.8 μg/kg) has adverse effects on plant growth. Schaller et al. (2012) have also found that Si application can affect the growth of reeds. Other researchers have pointed out that Si not only improves the efficiency of nitrogen (N)-utilization, but also participates in the process of phosphorus (P)-metabolism in plants (Saudy and Mubarak, 2015; Neu et al., 2017; Liao et al., 2020). With regard to the underlying mechanisms Si-driven mobilization of P from soil

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minerals, recent research has shown that silicic acid competes with P for binding soil minerals and that increasing Si availability leads to strong mobilization of P from soil minerals (Schaller et al., 2019; Hömberg et al., 2020). It can be concluded that application of Si plays an important role in improving soil nutrient availability. However, the specific mechanisms underlying the effects of Si in soil-nutrient dynamics are still poorly misunderstood.

As the main driving force of nutrient-cycle in the soil–plant system, soil microorganisms not only drive the transformation and cycling of soil organic matter and inorganic nutrients, but also serve as a nutrient reservoir. As important contributors to the decomposition and synthesis of organic matter (an important source of soil nutrient reserves), soil microorganisms participate in the transformation and cycling of soil nutrients. In terms of metabolism, microorganisms are one of the most active involvements in the decomposition of soil organic matter. Biogeochemical cycling of mineral nutrients is the foundation of soil nutrient cycling (carbon (C), N, P, etc.) (Zhong et al., 2010; Chen et al., 2015; Wang et al., 2015). Organic fertilizer is first decomposed, then dissolved, and finally transformed into soil by microorganisms. Some organic compounds are nutrients for microbial activities, while others are absorbed and utilized by plants (Wang et al., 2015). According to recent research, different types of fertilizers and methods of fertilization can affect soil nutrient status and plant growth, with consequences for floristic abundance (abundance of microbial flora), community structure, and biological functioning of soil microorganisms (Wei et al., 2013). Conversely, the floristic abundance, community structure, and biological function of soil microorganisms can also affect soil nutrient status and plant growth (He et al., 2008; Shen et al., 2010). Previous studies also indicated that application of N and P can affect the dominant microbial community in soil, transforming the microbial community from fungus-dominated to bacteria-dominated (Waldrop et al., 2004; Cusack et al., 2011).

Soil microbial biomass, community structure diversity, and activity of metabolic enzymes are known to be important indicators of soil quality and soil ecosystem health; researchers have found that a combination of P fertilizer and organic fertilizer promotes soil microbial activity and functional diversity, and improves soil microbial community structure, thereby improving biological functioning and productivity of soil (Chen et al., 2015). P is an important factor in controlling soil microbial biomass and microbial diversity (He et al., 2008; Zhong et al., 2010). Application of N can significantly reduce the ratio of fungi to bacteria in soil microorganisms, increase soil microbial enzyme activity, and enhance decomposition activity in some microorganisms (Su et al., 2014). Other studies have also shown that different fertilizers can affect the activity of soil enzymes; for instance, application of N significantly increases cellulase and phenoloxidase activity in soil (Waldrop et al., 2004; Zhong et al., 2010; Li et al., 2012). As key members of the plant soil ecosystem, soil microorganisms participate in all aspects of plant–soil interactions, including mineral nutrient cycling, soil-structure optimization, plant pest control, and plant growth and development (Mendes et al., 2011; Schnitzer et al., 2011; Schneider et al., 2012; Meng et al., 2017). It can be seen that different application modes of N fertilizer and P fertilizer affect soil fertility and productivity by affecting microbial community structure and soil enzyme activity in soil ecosystems. However, up to now, few studies have reported changes in soil microbial community structure and soil ecosystem after the combined application of Si fertilizer and N and P fertilizers, as well as the ecological mechanisms underlying the enhanced soil available nutrient content and utilization efficiency of N fertilizer and P fertilizer arising from the application of N fertilizer and P fertilizer in combination with Si fertilizer.

Therefore, we hypothesize that the mechanism of Si-driven improvement of soil nutrient availability may be related to microbial action in soil. A series of soil culture experiments were designed to study the effects of application of different levels of Si and a particular concentration of N and P fertilizers on soil microbial activity, soil microbial quantity, soil microbial community structure, and the activity of important metabolic enzymes related to soil N and P metabolisms, and quantify soil available N and P proportions, in order to explore the mechanism of Si improvement of soil nutrient availability and the correlation between Si and microbial activity in paddy soil.

2 Materials and methods

2.1 Soil

The paddy soils used in this research were sampled from rice fields in Zengjia Village, Sixi Town,

Shanggao County, Yichun City, Jiangxi Province, China (E 115°07′04.41″, N 28°16′15.79″). This area belongs to a typical subtropical monsoon climate, with an average annual temperature of 17.6 ℃, an average annual precipitation of 1718.4 mm, and an average frost-free period of 276 d. It includes a rice planting area in the middle and lower reaches of the Yangtze River. Its native materials consist mainly of Quaternary red soil and river and lake sediments.

In the field, the study area was divided into 16 plots, each with an area of 16 m^2 (4 m×4 m) and a 0.2-m distance between adjacent plots. A total of 5 kg soil samples were taken from each plot. The random sampling method was used to collect mixed soil samples at depths of 0–20 cm; some of these soil samples were dried naturally to determine their basic physicochemical properties. Other samples were for pre-incubation. The basic physicochemical properties of the soil are shown in Table 1.

Table 1 Basic physicochemical properties of the used soil

Properties	Value
Total nitrogen (g/kg)	3.41
Alkali-hydrolyzable nitrogen (mg/kg)	235.08
Total phosphorus (mg/kg)	662.15
Available phosphorus (mg/kg)	67.51
Available potassium (mg/kg)	192.61
Organic material (g/kg)	47.55
pH	4.97

2.2 Soil treatments and incubation conditions

For pre-incubation, we placed the soil samples (equivalent to 150 g dry air weight apiece) into a series of 250 mL beakers, then adjusted the soil samples to equivalent moisture levels according to flooding conditions, simulating flooding in rice planting, and covered each sample with plastic film. To mimic flooded-paddy conditions, we added water to each beaker until the water was 2 cm above the surface of the soil; we then incubated the beakers at 25 ℃ for two weeks. Humidity was kept constant throughout the entire process by adding distilled and sterilized water regularly.

After pre-incubation, a total of 75 pre-incubated beakers (5 treatments×3 replicates×5 stages) were prepared to adapt to different nutrient treatments. The N fertilizer was urea, with N content of 46.4% (Shanxi Tianze Coal Chemical Industry Group Co., Ltd., China). As phosphate fertilizer, we used superphosphate produced with P_2O_5 content of 16.3% (Hunan Yonghe Phosphate Fertilizer Factory Co., Ltd., China). For potassium (K) fertilizer, we used potassium chloride (KCl) with K_2O content of 60.2% (Sinochem Chemical fertilizer Co., Ltd., China). The Si fertilizer tested in this experiment was a broad-spectrum type, with Si content of 26.4% (Wuhan Tuli Fertilizer Industry Co., Ltd., China). The standard application concentrations of N, P, and K for each beaker were 106.40, 39.18, and 51.05 μg/kg, respectively. Each treatment was repeated three times. In addition, an appropriate amount of Si fertilizer was added as the experimental group. With the non-Si fertilizer group as control, five different concentrations of pure Si were designed and calculated based on the recommendations of fertilizer manufacturers and local farmers and the content of pure Si in fertilizer: control check (CK, without additional Si), S1 (Si, 5.2 μg/kg), S2 (Si, 10.4 μg/kg), S3 (Si, 15.6 μg/kg), and S4 (Si, 20.8 μg/kg). All soils (including the control) were adjusted to replicate the same flooding conditions (water level 2 cm higher than the soil water interface). Next, all beakers were covered with plastic plates with small holes and incubated in the dark at 25 ℃ for 30 d. The water was kept constant by adding distilled water regularly throughout the culture process. After 30 d, soil samples were taken out and analyzed for various parameters.

All results were the average values of three repeated determinations, expressed as oven soil weight (105 ℃, 24 h).

2.3 Soil assay

The basic physical and chemical properties and N and P contents of soil samples were determined using standard soil agrochemical analysis methods (Bao, 2011). Urease content in soil was determined by the indophenol blue colorimetric method and P content was determined by the *p*-nitrobenzene phosphate method (Guan, 1986). The numbers of ammonifying bacteria and phosphatolytic bacteria were determined by the dilution plate counting method (Li et al., 1996). The activity of soil microorganisms was determined by the fluorescein diacetate (FDA) hydrolysis method (Liu et al., 2009).

The community structure of soil microbes was determined with high-throughput sequencing (Shannon, 1948a, 1948b; Simpson, 1949; Chao, 1984; Chao and Yang, 1993; Blaxter et al., 2005). Total microbion DNA was extracted with a DNA extraction kit (Axygen Company, Hangzhou, China). The quality of DNA

extraction was detected by 0.8% agarose gel electrophoresis. DNA was quantified using an ultraviolet (UV) spectrophotometer. Soil bacteria amplified the target fragment with common primers of the v3–v4 region of the 16S ribosomal RNA (rRNA) gene. The amplification product was detected by 2% agarose gel electrophoresis; the target fragment was cut and recovered using a gel recovery (Axygen). Fluorescence quantitative analysis was performed on the recovered products of polymerase chain reaction (PCR) amplification with fluorescence quantitative instrument. The X-ray fluorescence intensity of the sample was determined and compared with that of the standard sample; samples were mixed in proportion according to the quantitative fluorescence results and the quantities required for sequencing each sample. An TruSeq Nano DNA LT Library Prep kit (Illumina Company, Shanghai, China) was used to prepare the sequence library. The amplification product was first repaired at the end and the self-connecting fragments of the joint were then removed by magnetic bead screening. Following the library system after the addition of the joint, the sequencing library template was enriched and purified. First, an Agilent High Sensitivity DNA kit (Axygen) was used for quality inspection of the library, followed by quantitative analysis of the library using a Quant-iT™ PicoGreen™ dsDNA Assay Kit (Axygen), with a concentration of 2.5 nmol/L. The qualified sequencing libraries were diluted in gradient, mixed in proportion according to the quantities required for sequencing, denatured by NaOH to a single strand, and then sequenced.

2.4 Statistical analyses

Microsoft Excel was used for statistical collation of the test data, and IBM SPSS Statistics 20 analysis software was used for analysis of variance (ANOVA) and correlation analysis. The least significant difference (LSD) method was used for comparison and the significant difference level was 5%.

3 Results

3.1 Effects of Si on N and P nutrient mineralization in paddy soil

The ratio of soil available nutrients to total nutrients is often used to evaluate soil nutrient status. Organic nutrients are transformed into available nutrients for plants to absorb and utilize through the mineralization process. Using the most representative N-P nutrients as examples, Table 2 shows the effects of different Si dosages on nutrient mineralization in paddy soil, and that added Si significantly increased the ratio of available nutrients to total nutrients in soil compared with the control group. At a Si application rate of 15.6 μg/kg, the ratios of available N/total amount of N (AN/TN) and available P/total amount of P (AP/TP) in soil reached the maximum values. AN/TN and AP/TP increased by 19.78% and 105.09%, respectively, compared with the control group, indicating that the appropriate Si dosage (about 15.6 μg/kg) could increase the rate of mineralization of soil nutrients, significantly increase the content of mineralized nutrients in paddy soil, reduce the fixation of soil nutrient, and thus increase the content of nutrients that could be absorbed and utilized by rice in paddy soil. Appropriate supply of Si not only affects the Si cycle in soil–plant ecosystems, but also affects the N and P cycles in these ecosystems, so that Si can be used as a soil conditioner in agricultural soil to improve nutrient utilization rate and nutrient content of paddy soil.

Table 2 Effects of different dosages of Si on mineralization of N and P nutrients in paddy soil

Level of Si treatment	AN/TN (%)	AP/TP (%)
CK	9.81±0.14^{b}	8.25±0.47^{d}
S1	10.33±0.44^{b}	12.32±1.17^{c}
S2	11.34±0.66^{a}	14.97±0.58^{b}
S3	11.75±0.40^{a}	16.92±1.20^{a}
S4	10.95±0.35^{b}	13.45±3.21^{bc}

Data are expressed as mean±standard deviation (SD), n=6. Different letters in column indicate significant differences between treatments at P<0.05. AN/TN: available nitrogen/total nitrogen; AP/TP: available phosphorus/total phosphorus; CK: control check; S1: Si, 5.2 μg/kg; S2: Si, 10.4 μg/kg; S3: Si, 15.6 μg/kg; S4: Si, 20.8 μg/kg.

3.2 Influence of Si content on microbial activity in paddy soil

About 95% of the energy required in the process of organic matter turnover in the soil environment comes from biodegradation by soil microorganisms. Therefore, soil microbial activity reflects microbial biochemical processes in soil nutrient cycles in a manner, and can be used as an index to evaluate the transformation of organic matter in soil crop ecosystem. Because FDA hydrolase is a good indicator of microbial activity and transformation of organic matter in soil ecosystems, it is considered as a useful biological index of soil health.

An increase in FDA hydrolase activity could indicate that soil microorganisms are more active in nutrient transformation. Table 3 shows the effects of different Si concentrations on microbial activity in paddy soil; as increasing amounts of Si are added, microbial activity in soil initially increases and then decreases. At a Si dosage of 15.6 μg/kg, activity of FDA hydrolase reached its maximum value (102.53 μg/g), about 7.96% higher than that of the control group. In conclusion, appropriate application (about 15.6 μg/kg) of Si fertilizer can substantially improve soil microbial activity to some extent and enhance recycling and utilization of nutrients in soil ecosystem.

Table 3 Effects of different Si dosages on soil microbial activity in paddy soil

Level of Si treatment	FDA hydrolase activity (μg/g)	Amplitude (%)
CK	94.97±0.51^{d}	
S1	97.97±1.08^{bc}	3.16
S2	99.55±1.42^{b}	4.82
S3	102.53±0.84^{a}	7.96
S4	96.60±0.60^{cd}	1.72

The values for FDA hydrolase activity are expressed as mean±standard deviation (SD), n=6. Different letters in column indicate significant differences between treatments at P<0.05. FDA: fluorescein diacetate; CK: control check; S1: Si, 5.2 μg/kg; S2: Si, 10.4 μg/kg; S3: Si, 15.6 μg/kg; S4: Si, 20.8 μg/kg.

3.3 Influence of Si dosage on the numbers of functional microorganisms of N and P nutrient metabolism in paddy soil

As the driving force of soil nutrient cycling, the change in soil microbe numbers can sensitively reflect changes in nutrient content of its environment. Phosphate-solubilizing bacteria can dissociate the soluble and insoluble P in soil through their own metabolic activities, and form a P-supply region in the crop rhizosphere, thus promoting the absorption and utilization of P by crop plants. Ammonia-oxidizing bacteria are important players in the decomposition of N-containing organic matter, and they play a decisive role in ammonization. An increase in numbers of these two types of bacteria helps to enhance mineralization of organic nutrients in soil, thus providing greater concentrations of small molecular nutrients for plants to absorb and use. Table 4 shows the effects of different Si dosages on the number of functional microorganisms involved in N and P nutrient metabolism in paddy soil, from which we learn that with an increase of added Si, the numbers of P-solubilizing bacteria and ammoniated bacteria in soil initially increase and then decrease. At a dosage of Si of 15.6 μg/kg, the numbers of P-solubilizing bacteria and ammoniated bacteria reached a maximum (8.13×10^{2} colony forming units (CFU)/g and 15.00×10^{2} CFU/g, respectively), representing an increase of 125.94% and 92.31%, respectively, compared with the control group. In conclusion, appropriate application of Si fertilizer can significantly increase the number of functional microorganisms in soil, especially with respect to P-solubilizing bacteria and ammoniated bacteria. Thus, Si fertilizer can promote the activation of soil nutrients and improve the availability of nutrients available for crop use in soil.

3.4 Influence of Si dosage on the activity of important N and P nutrient metabolic enzymes in paddy soil

Soil enzymes come mainly from soil microorganisms, and their biological activity represents the exuberant degree of potential nutrient components activating metabolism in soil, and to some extent reflects the absorption and utilization of nutrients by crops; they are also important indicators of soil fertility (Frankenberger and Dick, 1983; Nannipieri, 1994).

Table 4 Effects of different Si dosages on the numbers of functional microorganisms of N and P nutrient metabolism in paddy soil

Level of Si treatment	P-solubilizing bacteria		Ammoniated bacteria	
	Number ($\times10^{2}$ CFU/g)	Amplitude (%)	Number ($\times10^{2}$ CFU/g)	Amplitude (%)
CK	3.60±0.87^{c}		7.80±1.22^{d}	
S1	4.67±0.81^{bc}	29.61	10.60±2.20^{c}	35.90
S2	5.87±0.64^{b}	62.94	12.40±1.11^{c}	58.97
S3	8.13±0.50^{a}	125.94	15.00±1.06^{a}	92.31
S4	5.47±0.95^{b}	51.83	13.07±1.10^{bc}	67.51

The numbers of P-solubilizing bacteria and ammoniated bacteria are expressed as mean±standard deviation (SD), n=6. Different letters in column indicate significant differences between treatments at P<0.05. CFU: colony forming units; CK: control check; S1: Si, 5.2 μg/kg; S2: Si, 10.4 μg/kg; S3: Si, 15.6 μg/kg; S4: Si, 20.8 μg/kg.

For example, phosphatase can hydrolyze organic P compounds in the soil into available P, which can be absorbed and utilized by crops. Urease can catalyze the hydrolysis of urea in soil into ammonia, water, and CO_2, which can be absorbed and utilized by crops. Table 5 shows the effects of different amounts of Si on the activity of important N and P nutrient-metabolizing enzymes in soil, showing that with increased Si, acid phosphatase activity (AcPA) and urease activity in soil initially increase and then decrease. At Si dosage of 15.6 μg/kg, AcPA and urease activity reached maximum values of 7.29 mg/g and 1.46 mg/g, respectively, 14.80% and 28.07% higher than those of the control group. These results indicate that appropriate applications (about 15.6 μg/kg) of Si fertilizer can significantly improve the activity of metabolic enzymes related to N and P metabolism in paddy soil, thereby promoting the metabolism of N and P nutrients in soil and improving the ability of soil to supply available nutrients to crops like rice.

Table 5 Effects of different dosages of Si on important N and P nutrient metabolic enzyme activity in paddy soil

Level of Si treatment	AcPA		Urease activity	
	Activity (mg/g)	Amplitude (%)	Activity (mg/g)	Amplitude (%)
CK	6.35±0.32^{c}		1.14±0.03^{d}	
S1	6.67±0.09^{bc}	5.04	1.24±0.01^{bc}	8.77
S2	6.90±0.12^{b}	8.66	1.27±0.02^{b}	11.40
S3	7.29±0.21^{a}	14.80	1.46±0.03^{a}	28.07
S4	6.86±0.26^{b}	8.03	1.21±0.02^{c}	6.14

The data of AcPA and Urease activity are expressed as mean± standard deviation (SD), n=6. Different letters in column indicate significant differences between treatments at P<0.05. AcPA: acid phosphatase activity; CK: control check; S1: Si, 5.2 μg/kg; S2: Si, 10.4 μg/kg; S3: Si, 15.6 μg/kg; S4: Si, 20.8 μg/kg.

3.5 Effect of Si dosage on microbial abundance in paddy soil

An operational taxonomic unit (OTU) usually refers to the merging of sequences from one or more samples according to a certain artificial sequence similarity threshold; those sequences whose similarity is higher than the threshold will be merged into one OTU (Blaxter et al., 2005). At present, most 16S rRNA gene-based bacterial structural diversity studies take 97% sequence similarity as the OTU threshold. OTU numbers can serve as a useful estimate of microbial diversity. An increase in OTU may indicate that the community structure of soil microorganism becomes more abundant. Table 6 is a statistical table of sequencing and OTU quantities of soil samples under different applications of Si in paddy soil. From Table 6, it can be seen that within the five gradients set by the experiment, the sequencing quantity and OTU number of soil samples initially increased, and then decreased with the increased Si dosages. At Si dosage of 15.6 μg/kg, the sample sequencing and OTU were 56 720 and 4035, respectively, which represent increases of 14.09% and 9.35% compared with the control group. The above results showed that proper application of Si fertilizer (about 15.6 μg/kg) could significantly improve the species richness of soil microbial community structure.

Table 6 OTU statistics of soil samples under different dosages of Si in paddy soil

Level of Si treatment	Sample sequencing quantity	Number of OTU
CK	44 222	3690
S1	45 590	3782
S2	48 455	3870
S3	50 455	4035
S4	43 789	3655

OUT: operational taxonomic unit; CK: control check; S1: Si, 5.2 μg/kg; S2: Si, 10.4 μg/kg; S3: Si, 15.6 μg/kg; S4: Si, 20.8 μg/kg.

Fig. 1 shows a Venn diagram analysis of OTUs in soil samples. It is also clear that the number of unique OTUs among groups in soil samples was highest when the dosage of Si was 15.6 μg/kg, indicating that the soil microbial community was richest in diversity when the application amount of Si was 15.6 μg/kg. However, when treated with S4 (i.e., Si dosage of 20.8 μg/kg), the number of specific OTUs between groups in the samples decreased significantly, which indicated that the more Si applied, the less beneficial it was to the richness of soil microbial community structure. In conclusion, proper application of Si fertilizer (about 15.6 μg/kg) can effectively improve the richness of microbial community structure in paddy soil.

4 Discussion

Many studies have come to the conclusion that proper Si nutrient supply can improve the nutrient utilization efficiency of plants and improve the efficiency of soil nutrient mineralization. Under normal base

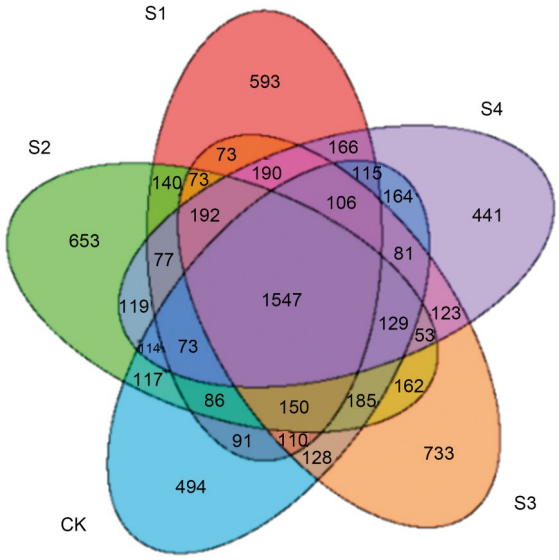

Fig. 1 Shared operational taxonomic unit (OTU) across different soil samples under different applications of Si in paddy soil. CK: control check (without additional Si); S1: Si, 5.2 μg/kg; S2: Si, 10.4 μg/kg; S3: Si, 15.6 μg/kg; S4: Si, 20.8 μg/kg.

fertilizer conditions, the addition of Si nutrient below a certain threshold has a positive effect on the growth of plants and utilization of soil nutrients. When the threshold is exceeded, however, the high levels of Si nutrient may lead to a stress response with negative effects on plant growth and utilization of soil nutrients. However, research on the ecological mechanisms underlying Si promotion of plants growth and nutrient utilization is still scarce. In this project, we studied the ecological mechanism by which Si induces microorganisms, and the main driving force of nutrient cycling in the soil-plant ecosystem, to activate and absorb nutrients in soil. Through microbial metabolism, organic matter in soil can be transformed and recycled so that plants can absorb the required nutrients.

4.1 Effects of different Si dosages on α diversity of soil microorganisms in paddy soil

Alpha diversity refers to diversity in a specific region or ecosystem and is a comprehensive indicator of richness and evenness. It depends mainly on two factors. One is the number of species, that is, species richness. The other is diversity and uniformity of distribution of individuals in the community. Indices of community richness mainly include the Chao1 index and ACE index. The indices of community diversity include the Shannon index and Simpson index (Shannon, 1948a, 1948b; Simpson, 1949; Chao, 1984; Chao and Yang, 1993). The Chao1 index estimates the number of OTUs in the community using the Chao1 algorithm. It is often used in ecology to estimate the total number of species. The larger the value, the more the total number of species. The ACE index is used to estimate the number of OTUs in the community. By default, OTUs with sequence numbers less than 10 are included to estimate the actual number of species in the community. The higher the ACE index, the higher the richness of the community. The Shannon index considers the richness and evenness of the community. The higher the value of the Shannon index, the higher the diversity of community. The Simpson index is one of the indices used to estimate microbial diversity in samples. In ecology, it is often used to quantitatively describe the biodiversity of an area. The higher the Simpson index, the lower the diversity of the community. Table 7 is a statistical table of α diversity, which shows that appropriate Si application in soil can improve the Chao1 index, ACE index, Shannon index, and Simpson index of soil microorganisms in paddy soil in a manner, while excessive Si application can reduce the above index. At a Si application rate of 15.6 μg/kg, the above indices of soil microorganisms reached their maximum values, showing increases of 37.05%, 47.45%, 0.14%, and 2.40%, respectively, compared with the control group, indicating that an appropriate application of Si can effectively improve the Chao1 index and ACE index of soil microorganisms in paddy soil but has no significant effect on the Shannon index or Simpson index. This analysis indicates that application of appropriate Si dosages can significantly increase the richness of soil microbial communities, and also to some extent improve the diversity of soil microbial communities.

4.2 Effects of different Si contents on β diversity of microorganisms in paddy soil

Beta diversity is often used to compare the diversity of different ecosystems, that is, differences between samples collected from different places. This index uses the evolutionary relationship and abundance information of each sample sequence to calculate the distance between samples and reflect the differences in microbial communities among samples. The main methods of β diversity are principal component analysis (PCA), multidimensional scaling (MDS), and clustering analysis to analyze the similarity of community structure among

Table 7 Alpha diversity statistics of soil samples under different applications of Si in paddy soil

Level of Si treatment	Chao1	ACE	Simpson	Shannon
CK	3581.24	3721.55	0.9964	10.43
S1	4915.32	4930.81	0.9974	10.56
S2	5024.16	5239.17	0.9974	10.66
S3	5278.29	5487.54	0.9978	10.68
S4	3536.52	3583.19	0.9978	10.65

CK: control check; S1: Si, 5.2 μg/kg; S2: Si, 10.4 μg/kg; S3: Si, 15.6 μg/kg; S4: Si, 20.8 μg/kg.

different samples. PCA is a visual method to study the similarity or difference of data. With the idea of dimensionality reduction, PCA can find the most important coordinates in the distance matrix. After sorting the complex data with a series of eigenvalues and eigenvectors, the first few eigenvalues are selected to represent the relationship between samples. MDS is a statistical research method that classifies samples or variables according to their similarity or non-similarity. In an MDS map, space and distance are used to reflect the relationship between each point to judge the distribution of each point in the network and the density of the network. Fig. 2 is the PCA of microbial communities under different Si application rates in paddy soil; the principal components of microbial community in soil samples are extracted by PCA, and the differences of soil microbial community structure under different Si treatments are analyzed.

It also shows that there are obvious differences between soil microbial community structure under CK

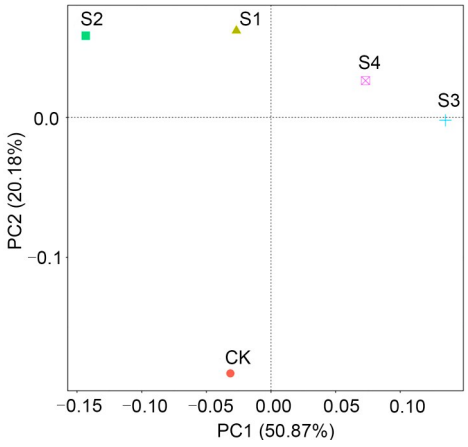

Fig. 2 Principal component analysis (PCA) of microbial communities under different dosages of Si in paddy soil. CK: control check (without additional Si); S1: Si, 5.2 μg/kg; S2: Si, 10.4 μg/kg; S3: Si, 15.6 μg/kg; S4: Si, 20.8 μg/kg.

treatment and soil microbial community structure after Si treatment. The S1, S2, and S4 treatments distribute on the positive axis of principal component 2 (PC2) axis, and CK and S3 distribute on the negative axis of PC2 axis. These results show that the difference in microbial community structure between CK treatment and S1, S2 and S4 treatments is caused mainly by PC2 (20.18%), while the difference of microbial community structure between S1, S2 treatments and S3, S4 treatments is mainly caused by PC1 (50.87%). This result indicates that different Si application rates might cause significant differences in microbial community structure in paddy soil.

4.3 Effects of different Si dosages on microbial community composition in paddy soil

The heat map presents the data in a two-dimensional matrix or table using color change as an indicator. It can intuitively depict the data value by the defined color depth. According to the needs of species or sample richness similarity clustering, the clustering data are represented on the heat map, and high-abundance and low-abundance species can be clustered in blocks. The similarities and differences in community composition of multiple samples at different classification levels can be reflected by color gradients and degrees of similarity. The community composition heat map at genus level combined with cluster analysis is presented by clustering and sorting the community composition data of each classification level according to abundance distribution of classification units or degrees of similarity between samples, and then reflecting the similarity in community composition between samples by color gradient.

Fig. 3 is a thermal map of genus-level community composition of soil combined with cluster analysis of top 50 genera. Analysis of Fig. 3 shows that different Si dosages lead to changes in the dominant microorganisms in the soil microbial community. The dominant microorganisms in the control group include *Acidibacter* (*Proteus*) and *Pesudolabrys* (*Yellow bacteria*). The dominant microorganisms in soil treated with S1 are *Acidothermus* (*Thermophila*), *Thermoan*, *Aebaculum* (*Thermophilic anaerobic bacterium*), *Syntrophorhabdus*, and others; the dominant soil microorganisms in S2 treatment include *Sphingomonas* (*Sphingosinol monocytes*), *Dyella*, *Fulvimonas* (*Yellow rot bacteria*), *Soil-bacterium-WF55*, and *Bacillus*, among others; the dominant soil microorganisms in S3 treatment included

Nocardia, *Lysobacter* (*Lysobacteria*), *Sigulisphaera*, and *Isospera*. The dominant soil microorganisms in S4 were *Pelomonas*, *Ralstonia*, *Streptacidiphilus*, *Kitasatospora*, and *Minicystis*.

These results show that Si application can promote the transformation of dominant microorganisms in paddy soil, such as *Bacillus*, *Cyclolipidicus*, *Candidatus-soilbacter*, *Candidatus-koribacter*, *Thermophilic* bacteria, *Zooplankton*, and slow-growing Rhizobium (Yu et al., 2016). That is to say, Si application can promote the transformation of soil microorganisms towards the efficient use of soil organic nutrients, thus enhancing soil nutrient recycling and utilization.

Earlier research's data showed that soil microorganisms are abundant, with the number of soil microorganisms approximated at 1029 orders of magnitude. However, because the growth of soil microorganisms is closely related to the soil temperature, nutrient content, soil acidity and alkalinity, and interactions between microorganisms, the simple artificial simulated environment of the laboratory cannot replicate the real growth patterns of microorganisms in natural conditions. Compared with the environment, the amount of soil microorganisms in laboratory culture is less than 1% of the total amount of soil microorganisms (Pace, 1997). For this reason, we studied the effect of Si application on soil microbial

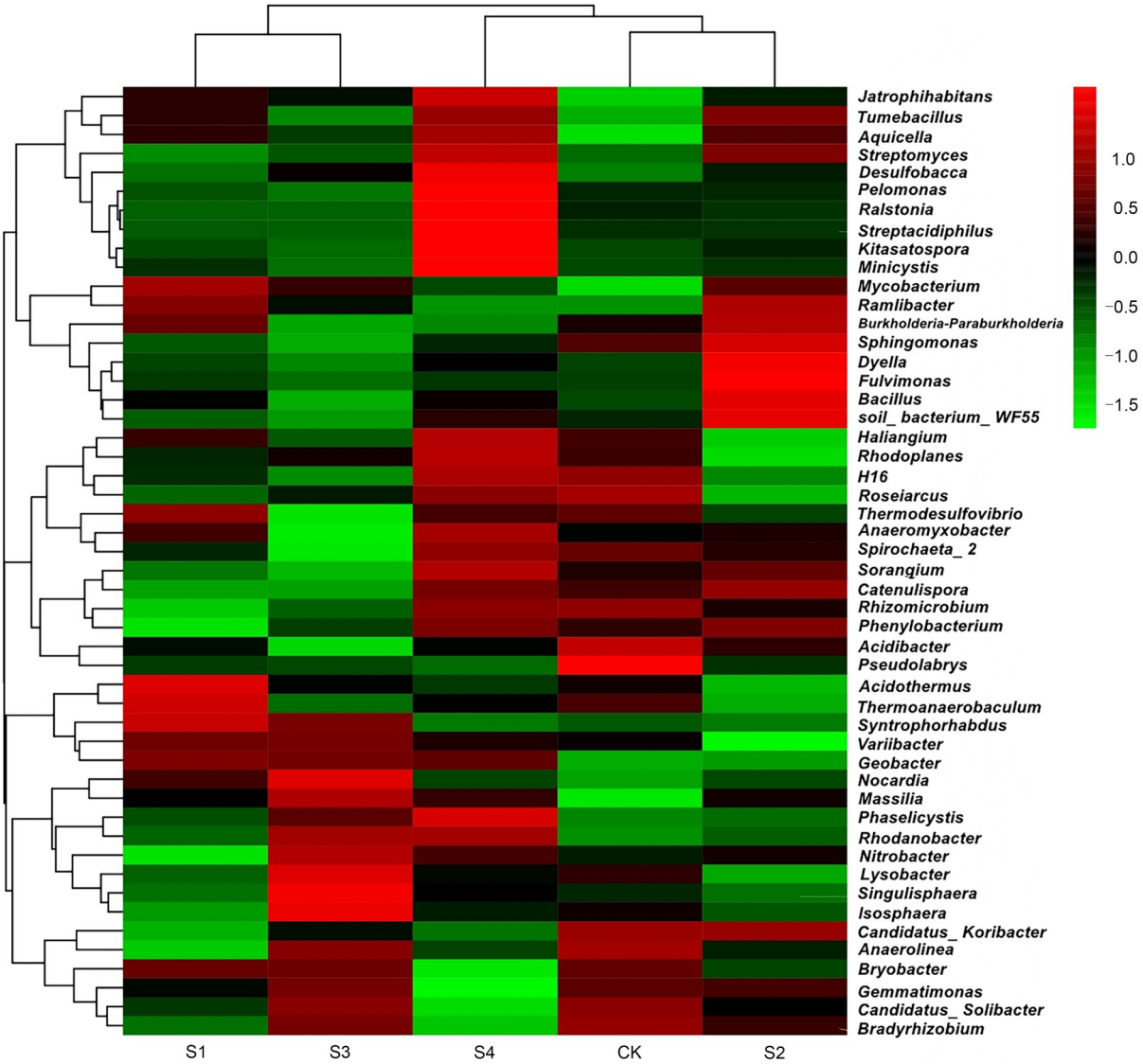

Fig. 3 Heatmap of community composition at genus level of microorganism under different applications of Si in paddy soil. CK: control check (without additional Si); S1: Si, 5.2 μg/kg; S2: Si, 10.4 μg/kg; S3: Si, 15.6 μg/kg; S4: Si, 20.8 μg/kg.

community structure using high-throughput sequencing technology. The results of α diversity analysis showed that appropriate Si dosages could significantly increase the richness of soil microbial communities, and at the same time improve the diversity of soil microbial community to a certain extent. PCA of β diversity showed that there were significant differences in microbial community composition caused by Si fertilizer application in acidic soils. Si can also improve soil pH, increase soil concentrations of basic ions, promote the formation of aggregate structure, and promote the decomposition of organic matter, so as to regulate soil structure and increase the number of soil microorganisms effectively (Du et al., 2016; Cai, 2017). This means that Si fertilizer can effectively improve the microbial community structure of acidic soil both as a basic fertilizer and soil amendment, which is consistent with the results of this study.

4.4 Relationship between the above microbial indicators and N and P nutrient mineralization in paddy soil

Previous experiments have shown that increasing the use of Si fertilize can improve the activity, quantity, and abundance of functional microorganisms in soil and that activity of corresponding enzymes can also be enhanced. Moreover, Si helps to promote the mineralization of soil nutrients, so that the soil can provide more effective nutrients. An analysis of the correlation between some representative indicators in previous experiments and nutrient mineralization indicators is shown in Table 8.

The Shannon index was selected to represent microbial community structure, and AN/TN and AP/TP were used to characterize nutrient mineralization in paddy soil. Table 8 shows that the number of ammoniated bacteria and AcPA are strongly correlated with nutrient mineralization and community structure, indicating that the numbers of functional soil microbes and the activity of related enzymes are related to nutrient mineralization and microbial community structure. The Shannon index also shows good correlation with AN/TN and AP/TP, indicating that microbial community structure is closely related to nutrient mineralization. The ecological mechanism of Si improvement in N and P nutrient availability in paddy soil is due mainly to its role in increase of soil microbial community structure diversity and soil microbial activity, resulting in increased mineralization of soil N and P nutrients, which indicates that appropriate concentration of Si soil supplements (about 15.6 μg/kg) not only affects the Si cycle in soil-plant ecosystem, but also affects the N and P cycles in these systems.

5 Conclusions

Proper application of Si can effectively increase the number of functional microorganisms involved in the N and P cycles in paddy soils. At Si application rate of 15.6 μg/kg, the numbers of ammoniated bacteria and phosphate-solubilizing bacteria, rates of urease activity, and AcPA in paddy soil reached maximum values, showing respective increases of 73.12%, 130.36%, 28.12%, and 20.15% compared with the control group.

The correlation coefficients between soil microbial activity, the number of ammoniated bacteria, urease activity, and AN/TN ratios were 0.764–0.929, and the correlation coefficients of microbial activity, the number of phosphate-solubilizing bacteria, AcPA, and AP/TP were between 0.719–0.937, which further proved that the changes of soil microorganisms and their metabolism-related enzyme activity caused by Si application increased the activity of functional microorganisms and important metabolic enzymes related to N and P cycles in soil, thus promoting the mineralizations of N and P and improving the availability of N and P nutrients.

Table 8 Correlation analysis between microbial indicators and nutrient mineralization indicators in paddy soil

Factor	FDA hydrolase activity	P-solubilizing bacteria	Ammoniated bacteria	AcPA	Urease activity	Shannon
Shannon	0.5730	0.7109	0.9290**	0.8363	0.5088	
AP/TP	0.8290	0.8818*	0.9290**	0.9508**	0.7640	0.9202**
AN/TN	0.7690	0.8982*	0.9140*	0.9366**	0.7197	0.8914*

* indicates a significant correlation at $P<0.05$ and ** indicates a significant correlation at $P<0.01$. AP/TP: available phosphorus/total phosphorus; AN/TN: available nitrogen/total nitrogen; FDA: fluorescein diacetate; AcPA: acid phosphatase activity.

The proper application of Si could effectively improve soil microbial community structure and microbial biodiversity. In particular, appropriate amounts of Si fertilizer could increase the microbial community diversity related to soil C, N, and P cycles, including increases in bacterial taxa such as *Bacillus cyclamatus*, *Thermophilic thermophilus*, *Rhodiola*, *Candidatus-soilbacter*, *Candidatus-koribacter*, *Bradyrhizobium*, and others. At Si dosage of 15.6 μg/kg, sequence number, OTU number, Chao1 index, ACE index, Shannon index, and Simpson index of soil microorganism in paddy fields all reached maximum values, showing increases of 14.46%, 10.01%, 23.80%, 30.54%, 0.18%, and 2.64%, respectively, compared with the control group.

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Author contributions

Yuqi LIANG contributed to data analysis and writing of the manuscript. Min LIAO and Xiaomei XIE contributed to study design, data analysis, and writing and editing of the manuscript. Yuqi LIANG, Zhiping FANG, Jiawen GUO, Xiaomei XIE, and Changxu XU carried out the experimental work. All authors have read and approved the final manuscript and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Yuqi LIANG, Min LIAO, Zhiping FANG, Jiawen GUO, Xiaomei XIE, and Changxu XU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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