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Microbial response to $CaCO_3$ application in an acid soil in southern China

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ABSTRACT

Calcium carbonate ($CaCO_3$) application is widely used to ameliorate soil acidification. To counteract soil and bacterial community response to $CaCO_3$ application in an acidic paddy soil in southern China, a field experiment was conducted with four different dosages of $CaCO_3$ addition, 0, 2.25, 4.5 and 7.5 tons/ha, respectively. After one seasonal growth of rice, soil physicochemical properties, soil respiration and bacterial communities were investigated. Results showed that soil pH increased accordingly with increasing dose of $CaCO_3$ addition, and 7.5 tons/ha addition increased soil pH to neutral condition. Moderate dose of $CaCO_3$ application (4.5 tons/ha) significantly increased soil dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) content, enhanced soil respiration, while the excessive $CaCO_3$ application (7.5 tons/ha) decreased these soil properties. High-throughput sequencing results illustrated that moderate dose of $CaCO_3$ application increased the richness and alpha diversity of soil bacterial community. Compared with control, the relative abundance of Anaerolineaceae family belonging to Chloroflexi phylum increased by 38.7%, 35.4% and 24.5% under 2.25, 4.5 and 7.5 tons/ha treatments, respectively. Redundancy analysis (RDA) showed that soil pH was the most important factor shaping soil bacterial community. The results of this study suggest that proper dose of $CaCO_3$ additions to acid paddy soil in southern China could have positive effects on soil properties and bacterial community.

Introduction

Soil acidification is a widespread problem. In recent years, continuous cropping and high levels of nitrogen (N) fertilization have driven soil acidification both directly and indirectly in southern China (Guo et al., 2010; Zhu et al., 2016). Besides, decades of atmospheric acid depositions also have significantly contributed to soil acidification in these regions. According to study of Zhu et al. (2016), in central subtropical China, paddy soil pH averagely declined at the rate of 0.031 units/year between 1980s and 2014. Soil acidification is known for deteriorating paddy soil physical and chemical properties and causing disastrous effects on soil microbial community structure and biochemical activity of microbes (Wang et al., 2015). Probst et al. (1999) has found that acidification can reduce nutrient recycling and water quality

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in forest ecosystem. Recently, soil acidification has received increasing attention because of its significant impacts on the phyto-availability of heavy metals in soils. It is well known that soil acidification could largely increase the bioavailability of cadmium (Cd) in soils, thus increase the Cd accumulation in plants, and consequently pose health risk due to long term intake of Cd contaminated food (Wang et al., 2015; Zhao et al., 2015).

Considering the hazardous effects of soil acidification, lots of remediation methods have been applied to improve and sustain soil productivity. Liming materials such as burnt lime (CaO) and limestone ($CaCO_3$), are widely used in China and western countries to increase soil pH and the content of base cation, such as Ca^{2+} and Mg^{2+}, reduce possible toxicity of Mn^{2+} and Al^{3+} (Ingerslev, 1997). Studies in Norway (Hindar et al., 2003; Hindar, 2005) have shown that long-term (8–20 years) liming at doses of 2–5 tons/ha can contribute to increasing concentrations of Ca^{2+}, and decreasing concentrations of Al^{3+} in the stream water over the entire basin. Importantly, liming could significantly decrease the bioavailability of heavy metal (Chen et al., 2018). Zhu et al. (2016) showed that burnt lime (about 75% CaO, <0.01 mm) application at a rate of 1500 kg/(ha·year) increased soil pH by 0.50 units, and decreased rice grain Cd by 35.3%. Salomé et al. (2011) showed that 6.7 tons/ha of liming significantly increased the soil pH and reduced the Cu^{2+} activity compared with the unamend control. Tyler and Olsson (2001) also tested 55 elements in limed soil and found that increasing pH led to decrease of available Al, As, B, Ba, Ge, Li, Fe, Zn, K and Mn. In addition, lime addition may also affect soil microbial communities and ecosystem functions through changing environmental conditions, such as soil pH and soil organic carbon. Dong et al. (2010) also showed that liming can significantly increase the soil bacterial functional diversity index and PLFA content measured by Biolog and PLFA methods. Therefore, liming could be an effective and economical method to improve soil acidification (Zhu et al., 2016), and which has been strongly recommend in the central subtropical China where soil acidification and Cd contamination are coexist (Zhao et al., 2015; Zhu et al., 2016).

Currently, in southern China, relatively small doses (0.75–1.5 tons/ha) of CaO is being recommended to apply to topsoil of paddy fields at rice tillering growth stage. However, such dose of CaO application has been shown is not very effective at decreasing grain Cd concentration (Chen et al., 2018; Wang et al., 2018). While large dose of CaO application would harm crops due to rapid reacts in soil. Study showed that large dose of CaO in soil would result in poor permeability and soil compaction. Furthermore, the activity of some enzymatic and soil microbes would be influenced (Dan et al., 2017). Therefore, large dose of calcium carbonate ($CaCO_3$) was recommended to be used in instead of CaO, because $CaCO_3$ reacts slowly in soil and is safe for crops (Chen et al., 2018). However, although large dose of $CaCO_3$ application has been shown to be highly effective at reducing Cd accumulation in rice grains and without effecting rice production (Chen et al., 2018), it unclear that whether large dose of $CaCO_3$ application would be harmful to soil microorganisms.

Soil microorganisms play vital roles in the terrestrial ecosystem functioning such as soil structural dynamics, energy transfer and nutrient cycling (Chu, 2013). Bacterial community composition is a parameter playing a leading role in biogeochemical cycles, any changes in bacterial populations can sensitively affect the nutrient cycling and energy flow in soils (Cruz-Paredes et al., 2017). Therefore, in addition to ameliorating soil acidification and reducing Cd accumulation in rice grains, it is also important to evaluate comprehensively how large dose of $CaCO_3$ application affect soil microbial community structure and function, which has important implications for sustaining soil productivity in agro-ecosystems. Recently, high-throughput sequencing technique provides a useful way to estimate microbial communities' response to environmental factors, and in this study, which was employed to monitor the microbial response to $CaCO_3$ application in an acid paddy soil. Therefore, the main objectives of the present study were: (1) to clarify the variation of soil physicochemical properties and soil respiration due to different dose $CaCO_3$ addition; (2) to determine the effects of $CaCO_3$ application on soil microbial community structure; and (3) to suggest the optimal dose of $CaCO_3$ application for acid soil amelioration in southern China in terms of soil microbial community structure and function.

1. Materials and methods

1.1. Study site and experiment set up

A field trial was conducted in Xiangtan City (112.87°E, 27.77°N), Hunan Province with a subtropical monsoon humid climate. The soil at this site was initially acid with pH at 5.5. The field experiment, consisted of 16 plots with 30 m^2 for each one as described by Chen et al. (2018). Four treatments, (1) Control (No $CaCO_3$), (2) LC (low calcium carbonate, 2.25 tons/ha $CaCO_3$), (3) MC (4.5 tons/ha $CaCO_3$) and (4) HC (7.5 tons/ha $CaCO_3$), were included in this study, and replicated in four plots. According to the RothLime model (www.rothamsted.ac.uk/rothlime), to raise soil pH from 5.5 to the target of 6.5, the dose of $CaCO_3$ was predicted to use 7.5 tons/ha. Calcium carbonate was broadcast onto the soil surface and mixed thoroughly into the soil layer two weeks before rice planting. All the plots were arranged randomly and under the same management.

1.2. Soil sampling and analysis

After a seasonal growth of rice, a total of 16 soil samples from 0 to 20 cm were collected. In each plot, litter on the topsoil was first stripped, five-spot-sampling method was used to collect soil samples, and then samples were immediately stored at 4°C and transported to the laboratory. For each sample, about 10 g soil was preserved at −80°C after frozen dried to analyze the soil microbial community, another part of 20 g was stored at 4°C to test soil respiration immediately, and the remaining was air-dried to test the physicochemical properties.

Soil pH was measured at a ratio of soil/water of 1:2.5 (*m*/*V*) with a Delta 320 pH meter (Mettlere-Todedo, Shanghai, China). The concentration of soil dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) were determined by TOC analyzer (Vario TOC, Elementar, Germany). Soil

respiration was measured by gas chromatograph (Agilent GC7890A, Agilent Technologies, USA) equipped with Porpak Q capillary column and a solid-state detector after incubation for 24 hr at room temperature.

1.3. Soil DNA extraction and PCR amplification

Microbial DNA was extracted with 0.5 g soil using a FastDNA SPIN Kit for Soil (MP Biomedicals, USA) according to the guidance of manufacturer's instructions. The quality and quantity of extracted DNA were tested by NanoDrop (NanoDrop Technologies, Wilmington, DE, USA). A primer pair 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′), were used for the polymerase chain reaction (PCR) amplification of DNA fragments targeting the V3–V4 hypervariable regions of bacterial 16S rRNA genes (Klindworth et al., 2012). A PCR mixture (50 μL) contained 25 μL NEB Phusion master mix (High-Fidelity PCR Master with GC Buffer), 1.5 μL of each primer, 1 μL template DNA, and 21 μL sterile ultrapure water. The thermal amplification protocol consisted of an initial denaturation for 5 min at 95°C, followed by 30 cycles for 30 sec at 94°C, 30 sec at 94°C, 30 sec at 55°C, and a final extension for 30 sec at 72°C. PCR products were purified from 1.5% agarose gels with a QIAquick Gel Extraction Kit (Qiagen, Germany). Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, USA) was used to measure the concentration of purified PCR amplicons.

1.4. High-throughput sequencing of bacterial 16S rRNA gene

The PCR products were combined in equimolar ratio into a single tube, and then sequenced using Hiseq 2500 (Illumina, USA) at Illumina sequencing platform. Raw reads were processed by NGS Tookit (version 2.3) to filter the low-quality reads, then the sequences were processed with a common pipeline based on Mothur (Kozich et al., 2013). Effective bacterial sequences were clustered into operational taxonomic units (OTUs) at a 97% sequence similarity level in the UPARSE software (version 7.1) (Edgar, 2010) and the dominant sequence was chosen as the representative sequence for each cluster. Ace, Chao, Simpson and Shannon index was used to estimate the alpha diversity in each sample. Bacterial community composition in each treatment was counted by QIIME 1.9.1 (Caporaso et al., 2010).

The original sequence data have been submitted to The Sequence Read Archive (SRA), numbered SRR8163560-8163575.

1.5. Statistical analysis

One-way ANOVA analysis was used to test the significant difference among each treatment by SPSS 22.0 (SPSS Inc. Chicago, USA). Data preparation and analysis of soil respiration were carried out by Microsoft Office Excel 2010. Data expressed in Venn and heatmap were standardized and analyzed by R software (version 3.3.3) to identify the differences and similarity in microbial community among diverse treatments. The alpha diversity of soil bacterial community was calculated by Mothur microbial software v.1.30.1. Redundancy analysis (RDA) was used to clarify the relationships between environmental factors and soil bacterial community structure by Canoco for Windows version 4.5 (Wageningen, Netherlands).

2. Results

2.1. Characterization of soil properties

Significant difference of soil chemical properties was found between treatments (Table 1). With increasing dose of $CaCO_3$ addition, soil pH increased accordingly. The $CaCO_3$ addition at 7.5 tons/ha increased soil pH to neutral condition (average pH 7.01), and which was little higher than the prediction of RothLime model (prediction pH at 6.5). Soil DOC content in the treatment of moderate $CaCO_3$ application was significantly higher than that of control, average DOC increased by 14%, while low and high $CaCO_3$ applications did not statistically significantly affect soil DOC content. Soil DON contents exhibited the same variation pattern with that of soil DOC content, e.g. soil DON content of MC was significantly higher than that of control (average DON increased by about 20%), while those of LC and HC were not significant different with that of control.

2.2. Soil respiration

The application of $CaCO_3$ resulted in pronounced changes in soil respiration (Fig. 1). Soil respiration rate of MC treatment was the highest among all these four treatments, comparing with control, the average soil respiration rate of MC treatment increased by about 7%. However, soil respiration rate of both LC and HC treatments were significantly lower than that of control, and HC treatment exhibited the lowest soil respiration rate. The average soil respiration rates of LC and HC treatments decreased by about 4% and 8%, respectively, comparing with control.

2.3. Bacterial community structure

A total of 41 phyla were found in all the treatments. The dominant phyla in the control were Proteobacteria, Chloroflexi and Actinobacteria, accounting for 39.98%, 17.10%, 12.70% of total bacterial sequences, respectively (Fig. 2). After application of $CaCO_3$, the dominant phyla in soil were similar to those of

Table 1 – Chemistry properties of soils in different treatments.

Plot	pH	DOC (mg/kg)	DON (mg/kg)
Control	5.54 ± 0.15 d	762.10 ± 7.53 b	125.93 ± 8.43 b
LC	6.00 ± 0.09 c	786.05 ± 26.56 b	125.82 ± 3.59 b
MC	6.45 ± 0.16 b	868.58 ± 15.33 a	150.33 ± 1.03 a
HC	7.01 ± 0.15 a	757.12 ± 17.88 b	131.12 ± 4.23 b

Results are expressed as mean value ± standard error ($n = 4$). Different letters denote significant differences among treatments ($p < 0.05$). LC: 2.25 tons/ha $CaCO_3$; MC: 4.5 tons/ha $CaCO_3$; HC: 7.5 tons/ha $CaCO_3$; DOC: dissolved organic carbon; DON: dissolved organic nitrogen.

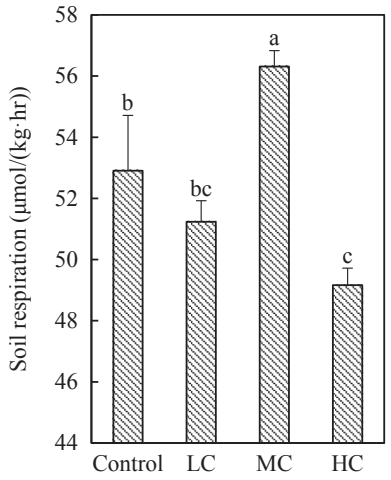

Fig. 1 – Difference in soil respiration among four treatments. Different letters denote significant differences among treatments ($p < 0.05$).

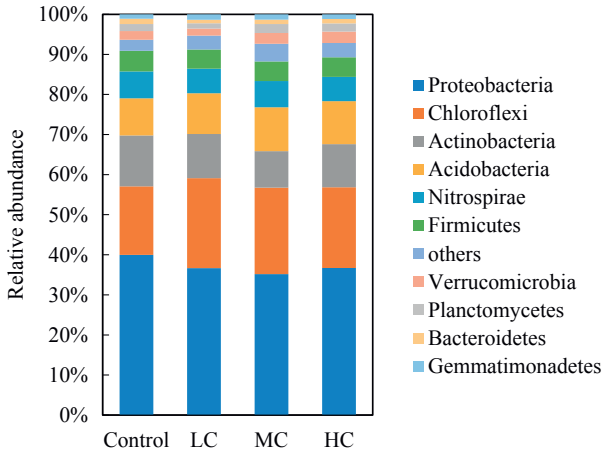

Fig. 2 – Composition of bacteria community in different treatments at phyla level.

control, but the distribution proportion changed among different $CaCO_3$ treatments. For instance, the relative abundances of Acidobacteria, Chloroflexi, Gemmatimonadetes, Planctomycetes and Verrucomicrobia increased, while that of Actinobacteria decreased ($p < 0.05$).

For further understanding the bacterial community composition at family level, the top 17 abundant families in each treatment was selected (Fig. 3). The most dominant family in control was Xanthobacteraceae, accounting for 10.92%, followed by Anaerolineaceae, Rhodospirillaceae, and Nitrospiraceae, accounting for 8.88%, 4.82% and 4.46%, respectively. Under LC, MC and HC treatments, the relative abundance of Xanthobacteraceae reduced to 9.08%, 8.36% and 9.32%, respectively, compared with control, while that of Anaerolineaceae increased to 12.32%, 12.02% and 11.68%, respectively. The results of heatmap showed that $CaCO_3$ application increased the relative abundance of Anaerolineaceae family belonging to Chloroflexi phylum. Under LC, MC and HC treatments, the relative abundance of Anaerolineaceae significantly ($p < 0.05$) increased by 38.7%, 35.4% and 31.5% compared with control, respectively. Meanwhile, $CaCO_3$ application decreased the relative abundance of Xanthobacteraceae and Rhodospirillaceae. Under MC treatment, the relative abundance of Xanthobacteraceae and Rhodospirillaceae significantly ($p < 0.05$) decreased by 23.5% and 34.7%, respectively, comparing with control. Cluster analysis showed that the microbe under MC and HC treatments gathered together firstly, then with LC treatment, while control showed large differences with $CaCO_3$ treatments.

The effects of $CaCO_3$ application on bacterial communities at OTU level were also analyzed (Table 2). Results showed that OTUs number of most phyla were significantly higher under $CaCO_3$ treatments than those of control, indicating that $CaCO_3$ application increased the absolute abundance of microbe in the soil. For example, under LC, MC and HC treatments, the number of OTUs belonging to Chloroflexi increased by 49.7%, 66.8% and 25.2%, respectively, compared with control.

To further characterize the similarity and difference of different treatments, Venn graph of OTU distribution was shown as Fig. 4. The shared OTUs of four treatments were 2709, accounting for 86.85%, 80.05%, 75.04% and 78.73% of all OTUs from control, LC, MC and HC, respectively. Meanwhile, unique OTUs from control, LC, MC and HC were 9, 16, 33 and 11, respectively, making up 0.29%, 0.47%, 0.91% and 0.32% of the total OTUs.

2.4. *Alpha diversity of soil bacterial communities*

There were substantial differences in bacterial alpha diversity among the four treatments (Table 3). According to the Ace and Chao indexes, $CaCO_3$ application significantly increased the richness and diversity of soil microbe. Bacterial community of MC treatment was much richer than that of the other treatments ($p < 0.05$), and control showed the lowest richness. Regarding to Simpson index, control showed significantly higher Simpson index than $CaCO_3$ treatments ($p < 0.05$), and there was no significant difference among the three $CaCO_3$ treatments. For Shannon index, control showed the lowest Shannon index, which was significantly lower than $CaCO_3$ treatments ($p < 0.05$), and there was no significant difference among the three $CaCO_3$ treatments.

2.5. *Main factors affecting bacterial community*

Redundancy analysis (RDA) was performed to elucidate relationships among environment factors and bacterial composition at phylum level (Fig. 5). The 10 most dominant phyla shown in Fig. 2, which represented on average 97% of the total bacterial community, were used for RDA. The results revealed that the first two axes explained approximately 65.7% of the variation in the bacterial composition, with 60.6% by the first axis and 5.1% by the second axis. Soil pH was the most significant factor regulating the bacterial composition after a Monte Carlo permutation test ($p < 0.05$), followed by soil DOC and DON content. The Proteobacteria, Nitrospirae and

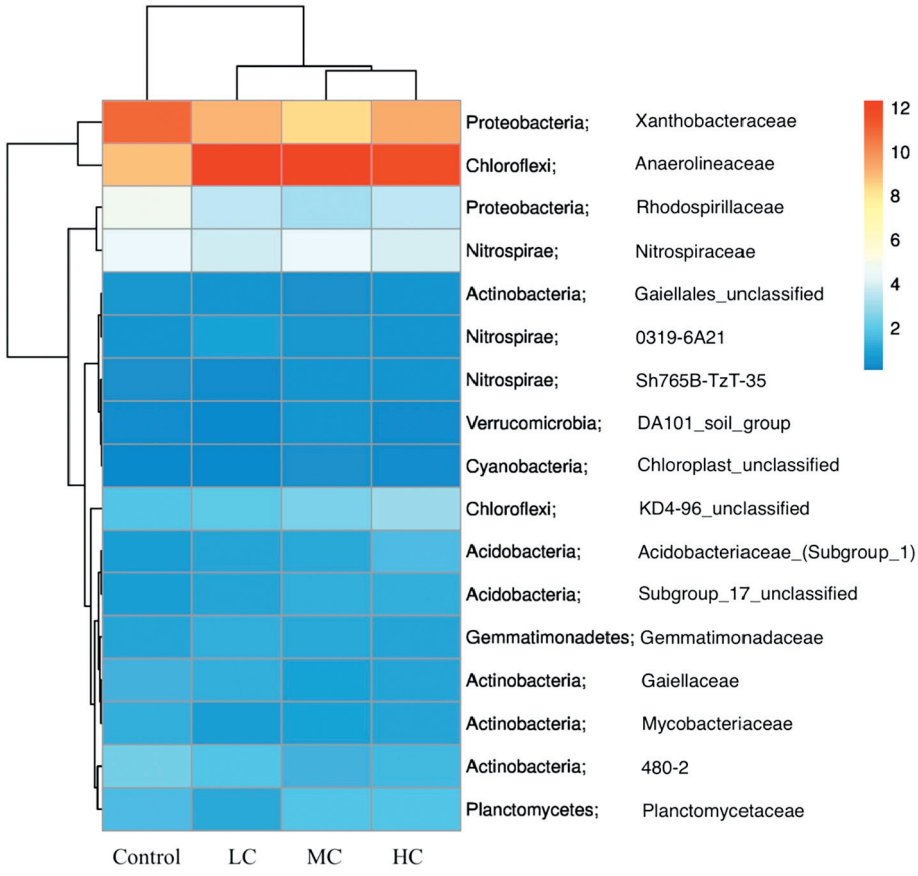

Fig. 3 – Heat map of the top 17 abundant families in each treatment. The color intensity in each cell indicates the transformed relative abundance. The color in the top-right legend represents the relative abundance level. The first column in the legend represents the phylum categories and the second represents the family.

Bacteroidetes showed a strong positive correlation with the DOC contents. A strong negative correlation between pH and Actinobacteria was found. We also found a significant separation among four treatments along the first two axes, which were divided into almost three groups. In addition, long distances between control and $CaCO_3$ treatments further suggested that $CaCO_3$ application significantly affected soil bacterial communities.

3. Discussion

Calcium carbonate, which can gradually neutralize soil acidity as well as release calcium, has been widely used in European and American countries (Zhao et al., 2015). In southern China, there are large areas of acidic paddy soils with moderate levels of Cd contamination. To both mitigate soil acidification

Table 2 – The number of OTUs of dominant bacterial communities at the phyla level.

Bacterial phyla	Control	LC	MC	HC
Acidobacteria	3444.0 ± 10.4 c	4319.7 ± 129.1 b	5389.2 ± 387.7 a	4257.0 ± 120.3 b
Actinobacteria	4731.3 ± 28.3 a	4679.2 ± 78.1 ab	4486.5 ± 200.4 ab	4254.0 ± 187.5 b
Bacteroidetes	500.5 ± 3.5 ab	369.0 ± 13.3 b	543.2 ± 42.4 ab	434.0 ± 6.8 b
Chloroflexi	6373.7 ± 55.2 d	9539.0 ± 315.8 b	10,629.7 ± 294.1 a	7979.7 ± 204.8 c
Firmicutes	1928.7 ± 30.9 b	2044.5 ± 71.6 b	2406.0 ± 108.8 a	1933.7 ± 96.9 b
Gemmatimonadetes	405.7 ± 5.0 d	578.7 ± 14.1 b	647.5 ± 21.2 a	460.2 ± 10.5 c
Nitrospirae	2498.2 ± 33.3 b	2597.2 ± 77.8 b	3218.5 ± 79.6 a	2392.5 ± 95.4 b
Planctomycetes	649.0 ± 3.1 c	566.0 ± 8.3 d	1097.5 ± 29.3 a	814.5 ± 26.4 b
Proteobacteria	14,899.7 ± 196.4 bc	15,572.5 ± 201.9 bc	17,317.5 ± 454.6 a	14,539.0 ± 198.0 c
Verrucomicrobia	814.0 ± 8.7 c	748.0 ± 14.7 c	1329.5 ± 107.4 a	1125.7 ± 30.8 b

Results are expressed as mean ± standard error ($n = 4$). Different letters indicate significant differences among treatments ($p < 0.05$).

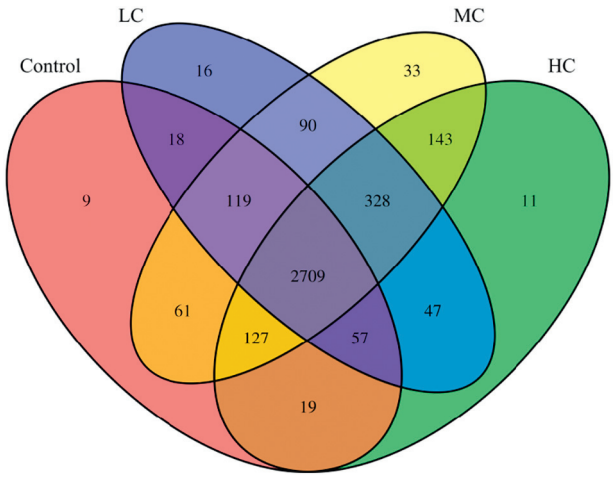

Fig. 4 – Venn diagram showing OTU (operational taxonomic units) distribution of bacteria in different treatments.

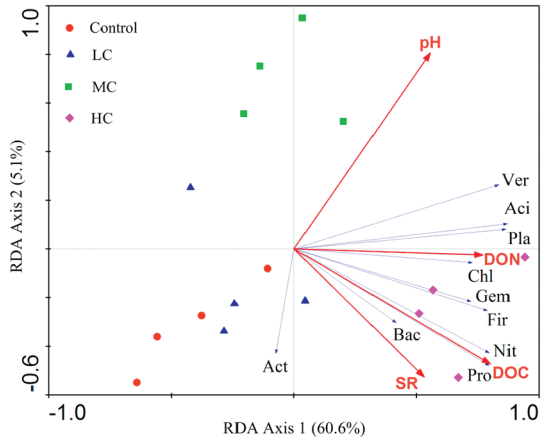

Fig. 5 – Ordination diagram showing the results of RDA (redundancy analysis) of environmental factors and bacterial composition. Aci: Acidobacteria; Act: Actinobacteria; Bac: Bacteroidetes; Chl: Chloroflexi; Fir: Firmicutes; Gem: Gemmatimonadetes; Nit: Nitrospirae; Pla: Planctomycetes; Pro: Proteobacteria; Ver: Verrucomicrobia; SR: soil respiration.

and cadmium accumulation in rice grains, $CaCO_3$ application is recommended as a highly effective method (Chen et al., 2018). However, so far the comprehensive impacts of different dose of $CaCO_3$ application on soil properties and microbial community are still unclear (Huber et al., 2006). Therefore, in the present study, a detailed investigation on how different doses of $CaCO_3$ application affect soil properties, microbial community, richness and diversity was conducted.

3.1. *Proper dose of $CaCO_3$ improved soil chemical properties*

Soil DOC and DON, as important parts of the nutrition cycling, are vital indices for evaluating soil fertility and characterizing soil productivity, and have essential influence on soil microbial activity and crop growth (Li et al., 2012; Six et al., 2002). Our results showed that the contents of DOC and DON in soil were significantly increased by moderate dose of $CaCO_3$ application (Table 1), indicating that proper application of $CaCO_3$ could significantly improve soil nutrition cycling. This result was well consistent with that of Kreutzer (1995), who found $CaCO_3$ application can modify soil organic matter composition by enhancing DOC contents, C turnover and humus storage, all of which were important processes of soil functions. The increasing of DOC and DON induced by $CaCO_3$ application could be attributed to the mitigation of soil acidification and activation of soil microbes. It has been demonstrated that under neutral pH conditions, the solubility of organic matter could be significantly increased, and activity of microbes involved in decomposition of soil organic matter could be enhanced (Löfgren et al., 2009; Paradelo et al., 2015). Rangelcastro et al. (2005) also demonstrated that turnover of C and N could be more rapid in the limed soil due to higher microbial activities. However, under HC treatment, DOC and DON contents decreased compared to control (Table 1). Excessive $CaCO_3$ application could lead to the inadaptability of microbes, in addition, some DOC can be easily neutralized with other substances such as calcium and magnesium compounds under high pH conditions, resulting in decline of DOC content.

Table 3 – Alpha diversity of soils in different treatments.

Plot	Ace	Chao	Simpson	Shannon
Control	3471.83 ± 30.91 c	3479.09 ± 147.95 b	0.0104 ± 0.0007 a	6.21 ± 0.10 b
LC	3756.17 ± 31.53 b	3738.19 ± 51.70 b	0.0079 ± 0.0006 b	6.39 ± 0.02 a
MC	4059.96 ± 66.17 a	4044.43 ± 58.68 a	0.0065 ± 0.0009 b	6.59 ± 0.06 a
HC	3835.09 ± 31.72 b	3835.05 ± 40.47 ab	0.0076 ± 0.0007 b	6.45 ± 0.11 a

Results are expressed as mean value ± standard error ($n = 4$). Different letters denote significant differences among treatments ($p < 0.05$).

3.2. *Proper dose of $CaCO_3$ application increased soil respiration*

Soil respiration, as the second largest carbon flux of terrene, can significantly influence the atmospheric carbon concentration (Stockmann et al., 2013). Therefore, it has become a key ecological process of global climate change and a pressing issue attracting great attentions (Wei et al., 2013). Soil respiration indicates to the production of carbon dioxide by soil microorganisms and the plants. In our study, due to the same plant and field management conditions in each treatment, the variation of soil respiration could be regarded as a sign of microbial activities response to different $CaCO_3$ additions to the soils. Our data showed that different $CaCO_3$ application resulted in pronounced changes in CO_2 emissions (Fig. 2). Moderate doses of $CaCO_3$ application resulted in a dramatically increase in soil respiration. As discussed above,

under MC treatment, soil DOC content significantly increased (Table 1), which could support the growth of microorganisms and plays an important role in the global carbon cycle. It has been demonstrated that adding organic carbon to soil could enhance the size, diversity and activity of heterotrophic microbial communities, thus increased soil respiration (Giacometti et al., 2013; Poulsen et al., 2013). Previously, several studies also showed that lime application led to increased CO_2 production from acid soils (Kemmitt et al., 2006; Kirkham et al., 2007). Shaaban et al. (2017) demonstrated that the application of dolomite (a liming material), accelerated biological processes and chemical reactions in soils, thus increased CO_2 emission. In LC treatment, soil respiration was not notably changed from control, indicating that soil acidity still remained a limiting factor for microbial activities and metabolism. The significant decreasing in soil respiration in HC treatment mainly because that excessive use of $CaCO_3$ would result in low level of decomposition process. This result is consistent with soil DOC content, which also decreased under HC treatment (Table 1).

3.3. Proper dose of $CaCO_3$ application enriched soil bacterial diversity

In this study, the composition and structure of bacterial populations in an acid soil response to $CaCO_3$ application was investigated by high-throughput sequencing. The top 10 phyla with the highest abundance in soil of different treatments were similar, which were also found in some related studies (Nacke et al., 2011; Rousk et al., 2010; Will et al., 2010), while the abundance of each phyla was varied among treatments (Fig. 2). Proteobacteria and Chloroflexi were the most abundant phyla, represented 35%–40% and 17%–23%, respectively. Proteobacteria is characterized as a high metabolic diversity and were widely known to be involved in biogeochemical cycles in soils (Janssen, 2006). In this study, the relative abundance of Proteobacteria was significantly reduced in the $CaCO_3$ treatments compared with control (Fig. 2). The decrease in relative abundance of Proteobacteria was due to the induced growth of other microbes by $CaCO_3$ application, which could be supported by the improved alpha diversity in the $CaCO_3$ treatments (Table 3). In addition, $CaCO_3$ application might suppress the growth of some members of Proteobacteria, such as Xanthobacteraceae shown in Fig. 3. It has been reported that Xanthobacteraceae family is phenotypically, metabolically, and ecologically diverse, which may be ubiquitous in wet soil and sediments (Oren, 2014). However, some members of Xanthobacteraceae, such as *X. xylophilus* is moderately acidophilic (opt. pH at 5.5, range 4.8–6.8) (Zaichikova et al., 2010), thus increasing soil pH by $CaCO_3$ application could decline the abundance of these acidophilic members. Chloroflexi was the second abundant phyla in our soils (Fig. 2), and the relative abundance of Chloroflexi were significantly increased in the LC and MC treatments (Fig. 2). The Chloroflexi are diverse group, with a phylogenetic range as broad as that of the Proteobacteria (Ley et al., 2006), and usually are linked to carbon cycling and N_2 fixation (Hug et al., 2013; Sorokin et al., 2012; Yamada et al., 2005). In addition, it has been reported that Chloroflexi dominated microbial diversity in some caves which are formed within limestone rock (Banks et al., 2010; Barton et al., 2014). These studies indicate that some members of Chloroflexi are associated with limestone rock environments, and $CaCO_3$ might provide the calcium or carbon for their growth and subsistence (Banks et al., 2010; Barton et al., 2014), and thus, it is reasonable that $CaCO_3$ application could increase the abundance of Chloroflexi. Furthermore, among all the abundant families, Anaerolineaceae belonging to Chloroflexi showed the largest variation in relative abundance, increased by 31.5%–38.7% (Fig. 3), which has been illustrated to play an important role in hydrocarbon degradation (Bo et al., 2016). However, under MC treatment, the absolute abundance of the other soil microorganisms increased much more than that of Anaerolineaceae, such as such as Chloroflexi, Firmicutes (Table 2), thus the relative abundance of Anaerolineaceae decreased compared with LC treatment. Under HC treatment, large dose of $CaCO_3$ application might result in sharp increase in soil pH, which might be harmful to soil microorganisms, including Anaerolineaceae. Therefore, the relative abundance of Anaerolineaceae under LC treatment was higher than that under MC and HC treatments.

The application of $CaCO_3$ also resulted in significant changes in the alpha diversity of bacterial community. In the present study, Shannon and Simpson indices, which was recommended by Haegeman et al. (2013), were used to denote the species diversity (Table 3). Comparison of these indices revealed that $CaCO_3$ additions enriched bacterial diversity, except for HC treatment. It has been suggested that bacterial diversity can reflect the biogeochemical cycles such as denitrification and nitrite oxidation (Wertz et al., 2007). In our study, enhanced alpha-diversity indexes indicated the positive effects of liming on bacterial community in acid paddy field, which was corroborated by previous studies (Fierer and Jackson, 2006; Hartman et al., 2008). However, large dose of $CaCO_3$ application in HC treatment resulted in sharp increase in soil pH, which might be harmful to soil microorganisms.

3.4. Soil pH regulates soil bacterial community

The results of RDA in this study revealed that the bacterial community structure from MC and HC was distinctly separated from control, indicating that $CaCO_3$ applications induced great changes in soil bacterial communities. Furthermore, soil pH, to a large extent, determined the composition of bacterial community (Fig. 5). The close relationship between soil pH and bacterial community was mainly due to the narrow pH optima which most bacteria have, and changes in soil pH altered soil nutrient availability, catabolic activities, soil structure and biomass activities. The vital role of pH in shaping soil bacterial community has been confirmed by several studies (Fierer and Jackson, 2006; Lauber et al., 2009; Qi et al., 2018). Rousk et al. (2010) have demonstrated that soil pH had significant influence on microbial composition and function along the Hoosfield acid strip in UK.

4. Conclusions

Soil pH increased with the increasing dose of $CaCO_3$ application. Low and moderate doses of $CaCO_3$ application (2.25 and 4.5 tons/ha) increased the content of DOC and DON, while

higher doses of $CaCO_3$ application (7.5 tons/ha) decreased the content of DOC and DON. Soil respiration enhanced significantly under MC treatment, while decreased under HC treatment. Furthermore, low and moderate doses of $CaCO_3$ application enhanced soil bacterial diversity and modified bacterial community composition at phyla and family level. RDA analysis showed that soil pH was the most important factor regulating soil bacterial communities. Therefore, proper $CaCO_3$ additions could be beneficial for both ameliorating acid paddy soil and enriching soil microbial community.

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