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Long-Term Organic Manure Application Alters Urease Activity and Ureolytic Microflora Structure in Agricultural Soils

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Abstract: Ureolytic microbes in soil produce urease to catalyze the hydrolysis of urea to NH_3/NH_4^+. Manure is widely applied in agriculture and has the potential to influence soil urease activity. In this study, we examined the responses of the ureolytic microbial community to manure application in two agricultural soils from north (N) and south (S) China using high-throughput sequencing of the *ureC* genes. We found that N soil and S soil harbored significantly distinct ureolytic communities, as no OTU was shared between two locations. The slight variation of the ureolytic community (32.2%, Adonis) was observed in N soil where low rates of manure were applied. However, dramatic alteration of the structure of ureolytic community (83.4%, Adonis) was found, possibly by promoting the growth of *Betaproteobacteria* and *Deltaproteobacteria* and the suppression of the growth of *Actinobacteria* in S soil where high rates of manure were inputted. The total C and C/N ratio were the main environmental factors driving the microbial communities. The relative ratios of *ureC* to *16S rRNA* genes ranged from 1.5 to 3.5% among the two soils. The abundance of *ureC* genes was significantly and positively correlated with total phosphorus (TP, $r = 0.87$, $p < 0.001$). Positive correlations between the urease activity and soil available NH_4^+ ($r = 0.81$, $p = 0.001$), TP ($r = 0.84$, $p = 0.001$), and the abundance of *ureC* ($r = 0.87$, $p < 0.001$) were observed in our study. We speculate that sufficient soil phosphorus promotes the growth of ureolytic microbes, which results in higher urease activity and the greater release of available NH_4^+.

Keywords: urease; ureolytic microflora; *ureC*; manure; high-throughput sequencing

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1. Introduction

Urea is an organic nitrogen compound commonly found in soil. In natural soils, urea is an intermediate product during the degradation of nitrogenous compounds [1]. It is also a widely used fertilizer in agriculture, and makes up approximately 60% of the world's consumption of nitrogen fertilizer [2]. Urea applied to soil is usually rapidly hydrolyzed to NH_3/NH_4^+ by urease (EC 3.5.1.5), and provides an effective nitrogen source for soil microorganisms and plants [3]. Therefore, urease plays a crucial role in determining soil fertility and is widely used as a soil quality indicator [4–6].

Soil urease was mainly produced by microbes. It was shown that 17 to 30% of the cultivable bacterial population in soil was ureolytic [7]. The functional ureolytic microbial community can also be identified by targeting the *ureC* gene and this gene encoding the alpha subunit of urease [8]. A number of studies employed clone library technology by targeting the *ureC* gene and revealed the ureolytic microbial community composition in groundwater [9], grassland [10], and open-ocean and estuarine planktonic communities [11]. In addition, these low-throughput sequencing techniques may underestimate the diversity of ureolytic microorganisms. High-throughput sequencing technologies offer the means to explore the environmental microbiota with higher resolution, coverage, and flux. Therefore, this study used high-throughput sequencing technology to reveal the diversity of ureolytic microorganisms in two typical agricultural soils in China.

The black soil area of northeast China (Udic Mollisol, USDA classification) is about 15 Mha (Institute of Soil Research, Chinese Academy of Sciences, 1978), which is one of the three major black soil areas in the world. Known for its soil fertility and productivity, the region provides 20 percent of China's grain production. Red soil is widely distributed in southern China, accounting for about 56.9 Mha land area and 6.5% of the total cultivated land area in China (National Soil Survey, 1998). Although rich in hydrothermal resources, red soils are usually less productive because of their acidity and sterility. Many studies have addressed the promotive effects of organic fertilization on soil urease activity [12–15]. However, the response of the ureolytic microbial community to organic manure fertilization in soils is still unclear. Therefore, we intend to investigate the difference of the ureolytic microbial communities in Black and Red soils as influenced by long-term organic manure fertilization. The high-throughput sequencing technology of the ureolytic microorganism marker genes (*ureC*) was employed to examine the community of ureolytic microorganisms in the two soils. Urease activity, *ureC* and *16S rRNA* gene abundances were also quantified.

2. Materials and Methods

2.1. Experimental Site and Sampling

The soils were sampled from the Black Soil Ecological Experimental Station in north China (N) and the Red Soil Experimental Station in south China (S), respectively. The Black Soil Ecological Experimental Station is located at Haerbin (45°40′ N, 126°37′ E), Heilongjiang Province, and a wheat-corn-soybean rotation system has been established. The annual mean temperature and precipitation for this region are approximately 3.5 °C and 569 mm, respectively. The soil is classified as Udic Mollisols with a clay loam texture, derived from loamy loess, containing 18% sand, 48% silt, and 34% clay. The Red Soil Experimental Station is located in Qiyang (26°45′ N, 111°52′ E), Hunan Province, and a wheat-corn rotation system has been adopted. The mean annual temperature and precipitation for this region are ~17.8 °C and ~1255 mm, respectively. The soil is an Agri-udic Ferrosol with a silty clay texture derived from Quaternary Red Clay Earth, bearing 8.7% sand, 46.3% silt, and 45.0% clay. The treatments in both stations were with three replicates in a completely randomized design. For the current study, the control (CK) and organic manure (M) treatments in two stations were chosen with the total soil samples of 12 (2 sites × 2 treatments × 3 replicates). The CK was used as the control which receives the same management as M treatment except for fertilization. In the north site, the horse manure was applied at the rate of 75 kg/hm^2-N once every 3 years at late October after corn harvesting since 1979. The moisture content of horse manure is 70%. The average organic matter content, total nitrogen, total phosphorus and total potassium are 41.8%, 1.52%, 1.85%, and 0.49%, respectively, on a dry matter basis. In the south site, swine manure was applied at the rate of 300 -N kg/hm^2 every year since 1990. The moisture content of horse manure is 73%. The average organic matter content, total nitrogen, total phosphorus and total potassium are 39.8%, 2.07%, 2.95%, and 0.62%, respectively, on a dry matter basis. The amount of manure applied was in accordance with native farmers' customary dosage at both sites.

Soil samples were collected in July 2015 and November 2016 for the N and S sites, respectively. Nine soil cores at 0–20 cm depth were collected from each plot and mixed carefully to provide a single sample. The soil cores were gently broken apart along the natural break points and sieved (<5 mm) to remove plant residues and organic debris. Each soil sample was divided into three parts. One was frozen at −80 °C for DNA extraction, another was stored at 4 °C for urease activity, and the third portion was air-dried for soil physicochemical analysis.

2.2. Soil Physicochemical Properties and Urease Activity

Soil pH was determined in a soil suspension with deionized water (1:2.5, w/v) using a pH meter (UB-7, UltraBASIC, Denver, CO). The concentrations of total carbon (TC) and nitrogen (TN) were measured by dry combustion analysis using a Vario MAX-CN Elemental

Analyzer (Elementar, Hanau, Germany). Total phosphorus (TP) was determined by flow injection analysis [16]. The soil organic carbon (SOC) was determined by the $K_2Cr_2O_7$—H_2SO_4 oxidation method of Walkley–Black [16]. Soil exchangeable ammonium (NH_4^+-N) was determined on a FIAstar 5000 Analyzer (Foss Tecator, Denmark) after extraction of fresh soil with 2 M KCl (1:5; *w*/*v*).

Urease activity was measured following the methods of Hoffmann [17]. Briefly, 20 mL citrate phosphate buffer (pH 6.7) and 10 mL 10% urea solution were added to 5 g air-dried soil as substrates, the mixture was incubated at 38 °C in the dark for 3 h, and then diluted with 38 °C water to 50 mL and filtered. The released ammonium in the soil suspension was determined by the indophenol blue reaction and measured spectrophotometrically at 578 nm using an L6 UV-Visible Spectrophotometer (INESA, Shanghai, China).

2.3. DNA Extraction

The total genomic DNA was extracted from 0.5 g soil as described previously by Griffiths et al. [18]. Humic acid, a PCR inhibitor, was removed from the soil DNA by using DNA-EZ Reagents M Humic acid-Be-Gone B (Sangon Biotech, Shanghai, China). The purification protocols were performed according to the manufacturer's instructions. The quality and concentration of DNA was determined using a Nanodrop spectrophotometer (NanoDrop Technologies, Wilmington, NC, USA).

2.4. Quantification of ureC and 16S rRNA Genes

The copy numbers of *ureC* genes and *16S rRNA* genes were quantified in triplicate by real-time PCR on an ABI Prism 7300 cycler (Applied Biosystems, Germany) with the primer pairs *ureC*-F/*ureC*-R (5′− TGG GCC TTA AAA THC AYG ARG AYT GGG −3′/5′− GGT GGT GGC ACA CCA TNA NCA TRT C −3′) [8] and *16S*-338F/*16S*-518R (5′− ACT CCT ACG GGA GGC AGC AG −3′/5′− ATT ACC GCG GCT GCT GG −3′) [19], respectively. Standard curves for the *ureC* and *16S rRNA* genes were obtained using a dilution series (10^2 to 10^{10} copies) of plasmid DNA containing *ureC* and *16S rRNA* genes fragment. Reaction mixtures (25 μL) contained 12.5 μL of 2 × SYBR Premix Ex Taq (TaKaRa, Dalian, China), 0.2 μM of each primer and 1 μL of DNA template (1–10 ng). Negative controls without any template were randomly distributed in the plate.

2.5. PCR Amplification of ureC Gene and Sequencing

Fragments of the *ureC* gene for high throughput sequencing were amplified in a 25 μL PCR system containing 12.5 μL Taq PCR Master Mix (Vazyme, Nanjing, China), 2 μM of *ureC* primers, 1μL DNA templates and 9.5 μL DEPC water with an annealing temperature of 49 °C. To enable samples mixed while sequencing, barcodes were incorporated between the adapter and reverse primer. Triplicate PCRs were performed for each sample, and the amplicons were pooled and purified. Equimolar concentrations were sequenced on an Illumina MiSeq platform according to the standard protocols.

The obtained sequences were extracted, trimmed, and quality screened using Qiime http://qiime.org/ (version 1.7.0, accessed on 12 July 2017). In brief, sequencing reads were assigned to each sample according to their unique barcodes, and low-quality sequences (quality score < 20; length < 150 bp) were removed. The remained reads were spliced using FLASH (version 1.2.7, http://ccb.jhu.edu/software/FLASH/, accessed on 12 July 2017) based on overlap sequence (length > 10 bp), and no ambiguous base was allowed in the overlap region. After splicing, the chimeras were identified using the Mothur command chimera.uchime with default parameters. The remaining high-quality reads were aligned and clustered into operational taxonomic units (OTUs) based on 97% sequence similarity, and singleton OTUs (with only one read) were removed. The longest sequence of each of the remained OTU were selected as the representative for BLASTn searches against the NCBI nonredundant (nr) database, with a minimum E value of 1e-20 to retrieve NCBI sequence identifiers. Subsequently, the OTUs were assigned to the top hit reference sequences with maximum score.

2.6. Statistical Analysis

The soil chemical properties, urease activity, and ureolytic community parameters for each treatment were calculated by averaging the three replicates for each sample. A one-way ANOVA was carried out to examine the differences in soil chemical properties, urease activity, and ureolytic community parameters by the SPSS 19.0 statistical software (SPSS, Chicago, IL, USA), where the between- and within subject variation was determined for the four treatments. The Fisher's least significant difference (LSD) test was used at the 95% probability level for the assessment between pairs of treatment means ($p < 0.05$). The relationships between soil chemical properties, ureolytic community parameters, and urease activity were measured by Pearson's correlation analysis. In order to account for differences in sequencing depth between samples, the read set was normalized to approximately 28,500 sequences for each sample. The richness and diversity indices including Ace, Chao1, Shannon, and Simpson indices were calculated using Mothur (version 1.40.0; http://www.mothur.org/wiki/MainPage, accessed on 30 August 2022). Nonparametric multivariate analysis of variance (Adonis) using distance matrices (method = "bray") were used to examine the community difference among groups [20]. Microbial community structure and soil properties were compared using redundancy discriminate analysis (RDA) with the 'vegan' package in R. Mantel tests (1000 permutations) using the 'mantel' function in the 'vegan' package were performed to determine the effects of selected soil chemical variables on the composition of the ureolytic community.

3. Results

3.1. Soil Physicochemical Properties

The physicochemical properties of the soil samples are shown in Table 1. As compared to the north site, the control soil of the south site shows significantly lower pH, TC, TN, C/N ratio, TP, and SOC (Table 1, pairwise test, $p < 0.05$). The manure application changed the soil physicochemical properties significantly except for a slight improvement of TC and TN in the north soils, while it considerably enhanced the pH, TC, TN, C/N, TP and SOC in the south soils (Table 1).

Table 1. Soil physicochemical properties of the studied soil samples.

Treatment	pH	TC (g/kg)	TN (g/kg)	C/N	NH_4^+-N (mg/kg)	SOC (g/kg)	TP (mg/kg)
NCK	6.33 ± 0.01^{a}	14.49 ± 0.04^{b}	1.12 ± 0.02^{b}	12.93 ± 0.22^{a}	5.11 ± 0.58^{b}	8.19 ± 0.53^{a}	385.43 ± 0.67^{ab}
NM	6.23 ± 0.12^{a}	16.73 ± 0.31^{a}	1.37 ± 0.06^{a}	12.23 ± 0.8^{a}	4.87 ± 0.15^{b}	9.56 ± 1.02^{a}	465.59 ± 25.91^{ab}
SCK	5.06 ± 0.05^{b}	4.04 ± 0.28^{d}	0.73 ± 0.01^{c}	5.53 ± 0.43^{c}	9.44 ± 2.40^{a}	3.91 ± 0.42^{b}	300.90 ± 9.07^{c}
SM	6.35 ± 0.06^{a}	10.67 ± 0.14^{c}	1.34 ± 0.01^{a}	7.78 ± 0.13^{b}	10.54 ± 1.18^{a}	8.27 ± 0.09^{a}	1138.61 ± 95.11^{a}

Abbreviations: TC, total carbon; TN, total nitrogen; C/N, carbon nitrogen ratio; NH_4^+, ammonium nitrogen; SOC, soil organic carbon; TP, total phosphorus; NCK, no fertilization in North site; NM, organic manure treatment in North site; SCK, no fertilization in South site; SM, organic manure in South site; Data are expressed as the mean ±SE values; $n = 3$; Different letters represent significant differences at $p < 0.05$ given from Fisher's least significant difference (LSD) between four treatments.

3.2. The Urease Activity and Abundance of the Ureolytic Microflora

The urease activities for the control soils from the two sites were not significantly different (Figure 1a). Manure application enhanced soil urease activities for both sites. The average copy numbers for the *16S rRNA* and *ureC* genes in CK ($1.16 \times 10^9 \pm 1.88 \times 10^8$, $3.85 \times 10^7 \pm 5.14 \times 10^6$ copies g^{-1} dry soil, respectively) and M ($1.04 \times 10^9 \pm 9.66 \times 10^7$, $3.06 \times 10^7 \pm 4.81 \times 10^6$ copies g^{-1} dry soil, respectively) of the North were not significantly different either (Figure 1b,c). However, the mean abundances of the *16S rRNA* and *ureC* gene in M ($6.42 \times 10^9 \pm 6.29 \times 10^8$, $1.28 \times 10^8 \pm 3.22 \times 10^7$ copies g^{-1} dry soil, respectively) were prominently higher than those in CK ($1.85 \times 10^9 \pm 3.20 \times 10^8$, $4.26 \times 10^7 \pm 2.31 \times 10^6$ copies g^{-1} dry soil, respectively) of the South (Figure 1b,c). A significant correlation was found between the *ureC* gene copy number and that of *16S rRNA* gene ($r = 0.926$, $p < 0.001$).

The relative ratios of *ureC* gene to *16S rRNA* gene ranged from 1.5% to 3.5%. The urease activity was also significantly correlated with the copy numbers of *ureC* ($r = 0.727$, $p = 0.007$).

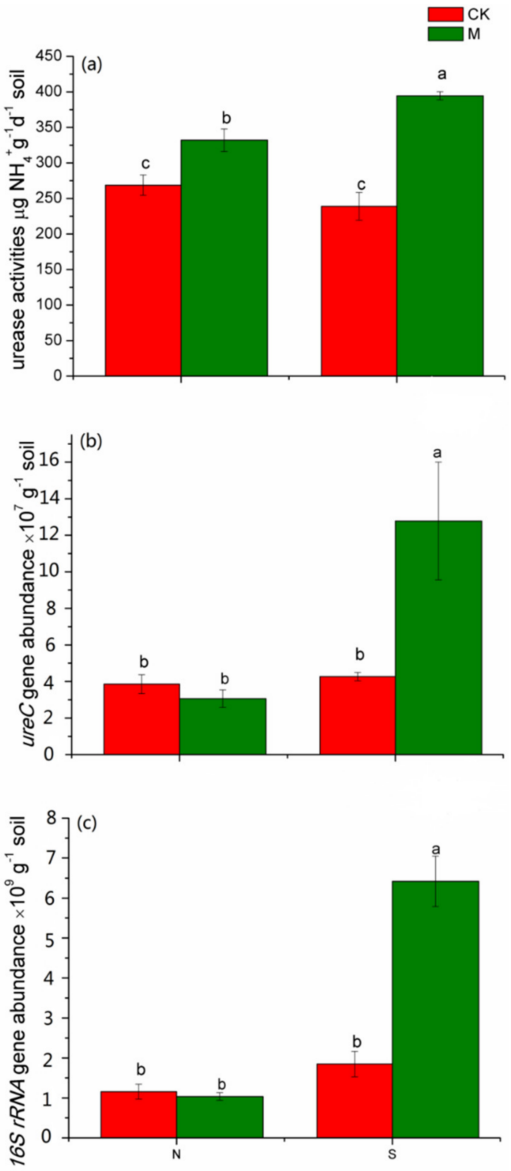

Figure 1. Urease activity (**a**), abundance of *ureC* (**b**) and *16S rRNA* (**c**) genes. Error bars represent standard errors of three replicates. Bars with different letters (shown above each bar) were found to be significantly different ($p < 0.05$) by Fisher's LSD test ($p < 0.05$). Abbreviations: N, north site; S, south site; CK, no fertilization; M, organic manure fertilization.

3.3. The α-Diversity of the Ureolytic Microflora

A total of 667,290 (Table 2) high-quality *ureC* gene sequences with an average read length of 320 bp were obtained from 12 soil samples. Sequence clustering yielded 8738 OTUs, of which 5168 OTUs belong to North samples and 3570 OTUs belong to South samples, and there were no shared OTU between the two sites. The numbers of high quality sequences, even depth high quality sequences and OTUs for each sample, and *α-diversity* indexes, are shown in Table 2. For the control soils, observably lower richness (Ace and Chao1 indexes) and α- diversity (Shannon and Simpson indexes) were found in the south compared to those in the north, indicating the significant effect of location on ureolytic microflora diversity. However, they were not influenced by manure application at either site (Table 2).

Table 2. α-diversity indices of *ureC* gene for the studied soil samples.

Sample	No. of Reads	No. of Even Depth Reads	No. of OTUs	Chao1	ACE	Shannon	Simpson
NCK-1	58,498	28,306	2855				
NCK-2	82,375	28,281	2836				
NCK-3	69,621	28,227	2849	3042 ± 21 [a]	3148 ± 23 [a]	9.54 ± 0.21 [a]	0.994 ± 0.002 [a]
NM-1	62,208	28,275	2432				
NM-2	56,324	28,576	3025				
NM-3	105,332	28,135	2846	2950 ± 353 [a]	3032 ± 371 [a]	9.39 ± 0.41 [a]	0.992 ± 0.004 [ab]
SCK-1	43,249	28,575	2101				
SCK-2	43,883	28,660	1387				
SCK-3	45,248	28,643	1965	1963 ± 418 [b]	1945 ± 422 [b]	8.25 ± 0.68 [b]	0.976 ± 0.010 [b]
SM-1	33,378	28,686	1653				
SM-2	35,007	28,759	1666				
SM-3	32,167	28,777	1684	1670 ± 16 [b]	1670 ± 16 [b]	8.73 ± 0.23 [ab]	0.987 ± 0.007 [ab]

Abbreviations: NCK, no fertilization in north site; NM, organic manure treatment in north site; SCK, no fertilization in south site; SM, organic manure in south site; the Ace, Chao1, Shannon, and Simpson index were calculated using Mothur (version 1.40.0) based on the even depth reads table; Data are expressed as the mean ±SE values; $n = 3$; Different letters represent significant differences at $p < 0.05$ given from Fisher's least significant difference (LSD) between four treatments.

3.4. The Structure of Ureolytic Microflora and Drivers

The redundancy analysis (RDA) of the ureolytic microflora and environmental variables with Bray-Curtis dissimilarity matrices were performed in R with the "vegan" package based on the relative abundance of OTU_{ureC}. The most relevant environmental variables combination to ureolytic microflora was selected using the 'bioenv' function (Figure 2). This was supported by the mantel test, which showed that TC ($R = 0.77$, $p = 0.001$) and C/N ($R = 0.86$, $p = 0.001$) were significantly related with ureolytic microflora. The first and second canonical axes explained 27.97% and 13.03%, of the total ureolytic community variation, respectively. The samples were clearly separated on the first canonical axis by location, whereas the influence of manure application became evident on the second axis.

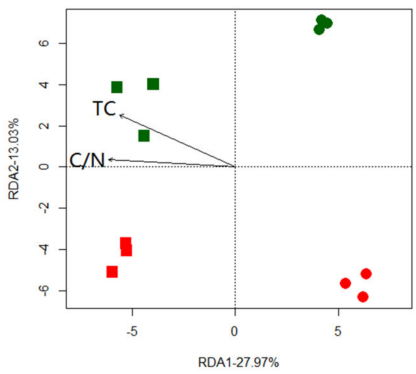

Figure 2. Redundancy analysis (RDA) of the ureolytic microflora and environmental variables with Bray-Curtis dissimilarity matrices based on the relative abundance of OTU_{ureC}. N (square), S (circle); CK (red), M (green). TC and C/N was the most relevant environmental variables to ureolytic microflora selected using the 'bioenv' function in R. Abbreviations: TC, total carbon; C/N, carbon nitrogen ratio. N, north site; S, south site; CK, no fertilization; M, organic manure fertilization.

3.5. The Composition of Ureolytic Microflora

The taxonomic compositions of ureolytic microbial community assemblages at the phylum level in samples are shown in Figure 3, and the relative abundance of abundant (>1%) ureolytic microbiomes at phylum level are listed in Table 3. It is clear that the ureolytic microbial community at both sites are dominated by *Betaproteobacteria* (39.9% and 28.5% for N and S site, respectively), *Alphaproteobacteria* (13.2% and 36.2%, respectively),

Gammaproteobacteria (13.2% and 8.2%, respectively), *Deltaproteobacteria* (10.6% and 9.7%, respectively), *Actinobacteria* (13.9% and 4.7%, respectively) and *Verrucomicrobia* (4.1% and 2.2%, respectively, Figure 3). In addition, *Nitrospirae* (7.0%) and *Bacteroidetes* (3.8%) are also abundant (relative abundance >1%) in the N site but not in the S site. *Planctomycetes*, *Cyanobacteria*, *Firmicutes*, *Thaumarchaeota*, *Ascomycota*, *Deinococcus-Thermus*, *Chloroflexi* are present at lower abundances at both sites, accounting for 2.46% of all sequences (Figure 3). An ANOVA analysis revealed that all abundant phyla are altered by location or manure application except for *Verrucomicrobia,* which are consistent in all samples. *Alphaproteobacteria, Gammaproteobacteria, Actinobacteria* and *Nitrospirae* differ significantly between the two locations ($p < 0.05$) in which *Alphaproteobacteria* and *Actinobacteria* show higher relative abundance in the S site while *Gammaproteobacteria* and *Nitrospirae* are more abundant in the N site ($p < 0.05$). The relative abundance of *Betaproteobacteria* and *Deltaproteobacteria* is the lowest in S-CK than in the other three treatments ($p < 0.05$). *Actinobacteria* was significantly decreased by manure application in the S site ($p < 0.05$).

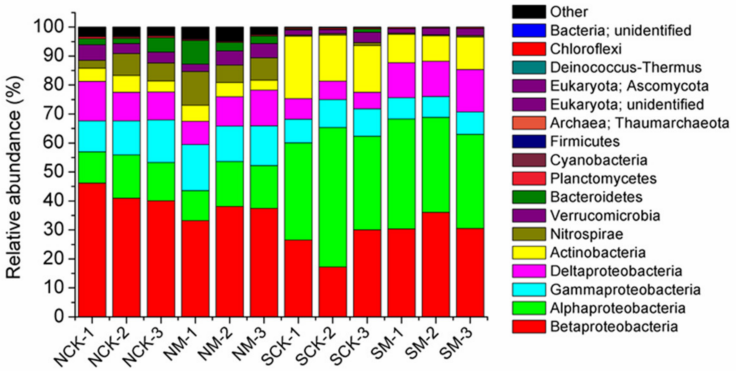

Figure 3. The relative abundance of ureolytic microbes across samples at phylum/class level.

Table 3. Relative abundance of abundant (>1%) ureolytic microbes at phylum level.

Treatment	*Betaproteobacteria*	*Alphaproteobacteria*	*Gammaproteobacteria*	*Deltaproteobacteria*	*Actinobacteria*	*Nitrospirae*	*Verrucomicrobia*	*Bacteroidetes*
NCK	42.3 ± 3.2% [a]	13.0 ± 2.0% [b]	12.7 ± 2.1% [a]	11.3 ± 2.3% [a]	5.0 ± 1.0% [c]	5.7 ± 2.5% [a]	4.0 ± 1.0% [a]	3.0 ± 1.7% [ab]
NM	36.0 ± 2.6% [a]	13.3 ± 2.9% [b]	14.0 ± 2.0% [a]	10.0 ± 2.0% [a]	4.7 ± 1.5% [c]	8.7 ± 3.1% [a]	4.3 ± 1.2% [a]	4.7 ± 2.9% [a]
SCK	24.7 ± 6.8% [b]	38.0 ± 8.7% [a]	9.0 ± 1.0% [b]	6.3 ± 0.6% [b]	18.0 ± 3.5% [a]	0.7 ± 0.6% [b]	2.3 ± 1.5% [a]	0.3 ± 0.6% [b]
SM	32.3 ± 3.2% [ab]	34.3 ± 3.2% [a]	7.3 ± 0.6% [b]	13.0 ± 1.7% [a]	10.0 ± 1.0% [b]	0.0 ± 0.0% [b]	2.0 ± 0.0% [a]	0.0 ± 0.0% [b]

Abbreviations: NCK, no fertilization in north site; NM, organic manure treatment in north site; SCK, no fertilization in south site; SM, organic manure in south site; Data are expressed as the mean ±SE values; $n = 3$; Different letters represent significant differences at $p < 0.05$ given from Fisher's least significant difference (LSD) test between four treatments.

4. Discussion

Ureolytic microbes play a pivotal role in the supply of soil bio-available NH_4^+. In this study, we determined the abundance, structure, and activity of soil ureolytic microflora by high-throughput sequencing and urease activity measurement in two distinct farmlands located in South (S, Red soil) and North (N, Black soil) China, which are both under long-term manure application. The results revealed a strong endemicity of the ureolytic microbial community, as no OTU is shared between the two locations. Their structure and function are significantly influenced by manure application at both sites. However, the strength of the impact was determined by the dosage of manure applied.

Black soil in the Northeast is well known to be fertile and productive for its richness in organic matter. Therefore, the native famers usually applied a small amount of manure to the soil. This low dosage of manure application has a slight but significant influence on soil physicochemical properties, urease activity and ureolytic community. For example, among the test physicochemical properties, only TC and TN were improved by 14.5% and 22.3%, respectively, after manure application in this site. The urease activity was increased by 22.2%. Manure led to a 32.2% (Adonis) variation in the ureolytic community compared

with CK. However, this amount of manure does not significantly change the abundance of ureolytic microorganisms.

Red soil was barren and lacked organic matter due to intense leaching. The application of large quantities of manure in this region led to a dramatic shift of soil physicochemical properties, urease activity, and the abundance and structure of the ureolytic community. The concentration of SOC, TN, TP, and NH_4^+ in the soil has been improved by 111.5%, 83.6%, 278.4%, and 11.7%, respectively, after manure application. The urease activity was increased by 65.0%. Manure lead to an 83.4% (Adonis) variation of the ureolytic community and an increment of 199.6% for the abundance of ureolytic microorganisms.

Although high throughput sequencing technology provides a sweeping perspective on the composition of soil bacterial communities, it is still hard to interpret the taxonomic survey data in an ecologically meaningful manner. We are unable to determine why certain taxa are more abundant in some soils than in others. At present, one unified concept used to describe the life history and characteristics of microbes is the *r*- to *K*- selection continuum [21], which originates from plant and animal ecology. Microbiologists tend to use the terms copiotroph and oligotroph to describe those microorganisms with ecological attributes typical of r- and Kstrategists, respectively. Copiotrophs preferentially consume labile soil organic C pools and have high nutritional requirements and growth rates at appropriate conditions. In contrast, oligotrophs usually exhibit lower growth rates and are likely to outcompete copiotrophs in conditions of low nutrient availability due to their higher substrate affinities [22,23]. In our study, the Black soil in north China is known as having high nutrient availability, while the Red soil in south China is nutrient deficient (Table 1). However, the high rates of manure application (S-M) made the Red soil become well-nourished (Table 1). Therefore, we speculate that the microbes which are more abundant in Black soil and in manure-treated Red soil are copiotrophic ureolytic microbes. Otherwise, they are oligotrophic ureolytic microbes. Therefore, our results suggest that the *Betaproteobacteria* and *Deltaproteobacteria* are copiotrophs, while *Actinobacteria* are oligotrophs. The Pearson's correlation test also indicated that the *Betaproteobacteria* (r = 0.60, p = 0.041) and *Deltaproteobacteria* (r = 0.70, p = 0.012) are positively correlated with SOC, and *Actinobacteria* are negatively correlated with SOC (r = −0.82, p = 0.001). *Betaproteobacteria* and *Deltaproteobacteria* are commonly considered as copiotrophic bacterial taxa and are associated with substrates rich in organic carbon [24–26]. *Actinobacteria* are known as a copiotrophic group, and a decreased relative abundance in nutrients-rich soils were observed in this study and also in others [27,28]. It seems that *Actinobacteria* are not consistently copiotrophic or oligotrophic [29].

The growth rate hypothesis (GRH) proposes that elevated growth rates are linked to elevated demands for *p* for the synthesis of *p*-rich ribosomal RNA (rRNA) [30–33]. Therefore, high *p* availability was needed to support the fast grow microorganisms [34]. Räsänen et al. [35] suggested that short-term phosphorus addition would benefit bacterial net growth in a fresh water system. We also found a positive correlation between *ureC* abundance and TP (r = 0.87, p < 0.001). The Pearson's test also showed that the urease activity is positively correlated with NH_4^+ (r = 0.81, p = 0.001), TP (r = 0.84, p = 0.001), and *ureC* abundance (r = 0.87, p < 0.001). Therefore, we speculated that a higher concentration of *p* promotes the growth of ureolytic microbiomes, which produces higher urease activity and the release of more available NH_4^+. No significant correlation between urease activity and α-diversity index was found, indicating that α-diversity is not a controlling factor for urease activity.

5. Conclusions

We revealed a strong endemicity of the ureolytic microbial community in the farmlands between south and north China. Long-term manure application significantly altered the ureolytic microflora and improved urease activity in both sites. The relative ratios of *ureC* to *16S rRNA* genes ranged from 1.5 to 3.5%, and the abundance of *ureC* genes was positively correlated with TP. The structure of ureolytic microflora were determined by TC

and C/N. Soil urease activity was positively correlated with soil available NH_4^+, TP, and *ureC* abundance.

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