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Aggregational differentiation of ureolytic microbes in an Ultisol under long-term organic and chemical fertilizations

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Abstract

Ureolytic microorganisms play a crucial role in soil nitrogen transformation. Soil aggregates and associated microbes are reported to modify the impact of agricultural management on soil nutrient cycling. However, the responses of ureolytic microbial communities in various soil aggregates to long-term fertilization regimes are still unclear in acid soils. In this study, we characterized the ureolytic microflora as well as urease activity in three soil aggregate fractions (2-0.25, 0.25-0.053, <0.053 mm) from an Ultisol with 26-year fertilization experiment. The results showed that long-term chemical fertilization (NPK) significantly decreased the abundance, richness and activity of ureolytic microbial community across soil aggregates ($P < 0.05$) due to strong soil acidification. While manure application (M and MNPK) could mitigate these negative impacts and markedly ($P < 0.05$) improved the abundance, α -diversity and activity of soil ureolytic microflora. Long-term fertilization regimes also drove the differentiation of ureolytic microbial compositions in soil aggregates (Adonis, $F = 17.4$, $P = 0.001$, $R^2 = 33.6\%$), and manure application appeared to be the most important driver. This variation partly contributed to the aberrance of soil urease activity (structure equation model, path coefficient: 0.45, $P = 0.008$). No significant differences were found for ureolytic microbial community among soil aggregates, which was in accordance with the distribution patterns of soil nutrients, indicating the dominant role of resources availability in determining ureolytic microbiota in micro-environment. The ureolytic microbial community among different soil aggregates responded uniformly to long-term fertilizations. Our study revealed that manure application was a sustainable fertilization regime to alleviate the loss of soil ureolytic microbial diversity and activity in acid soils.

Keywords

Ultisol, Fertilization, Soil aggregates, Urease, Ureolytic microbiota

1. Introduction

Chemical fertilization is one of the most widely employed management practices in conventional agricultural systems to improve soil fertility and crop yield (Erisman et al., 2008). Nevertheless, there are growing concerns about the ecosystem degradation and productivity loss caused by long term chemical fertilization which may induce soil acidification and other deterioration processes (Tilman et al., 2001; Foley et al., 2005). Organic fertilization is presumed to be a promising practice to mitigate these negative impacts and support sustainable agriculture (Gomiero et al., 2011). However, we still have an incomplete understanding of the benefits and limitations of organic and chemical fertilizations on soil microbiome which play a crucial role in virtually all soil processes such as soil nutrient cycling, crop productivity and atmospheric greenhouse gas (GHG) emissions (Barrios, 2007, van der Heijden et al., 2008, Singh et al., 2010).

Ureolytic microorganisms produce urease (EC 3.5.1.5) to catalyze the hydrolyzation of urea to bioavailable ammonium. The released ammonium is further assimilated by plants and microbes (Mobley and Hausinger, 1989). Ureolytic microbes are important contributors for soil fertility (Hasan, 2000). It is reported that urea transformation in sediment (with a mass of ureolytic microbes) was 100 times higher than that in water column without ureolytic microbes in ambient temperatures (Thorén, 2007). Ammonium oxidation is believed to be the rate-limiting step for soil nitrogen cycling and is also assumed to be the principal source of N_2O in soil ecosystems (Mathieu et al. 2006; Hu et al. 2015). It is likely that the urea hydrolysis

may be a rate-determining step for the other nitrogen cycling processes in soils. The positive correlations of N₂O efflux with the abundance of ammonium-oxidizing gene *amoA* and urease gene *ureC* in Tibetan grasslands indicated that the rate of NH₃ released from urea hydrolysis was the limiting factor for the subsequent ammonia oxidation (Yue et al. 2015). Therefore, exploring the responses of soil ureolytic microbes to different fertilizations is needed for a better understanding of soil management on soil fertility and nitrogen dynamics.

Based on the quantitative real-time PCR (qPCR) and high throughput sequencing technology, Wang et al. (2018) reported that the abundance and composition of soil ureolytic microbes were dramatically distinct between different long-term fertilization regimes in a Mollisol (near neutral pH) in Northeastern China. Sun et al. (2019) revealed that long-term urea fertilization could increase the abundance, decrease the richness, and alter the composition of soil ureolytic microbial community in a fluvo-aquic soil (alkaline pH ~8.2) from North China Plain. These results imply that fertilization may be an important factor in driving the niche differentiation of ureolytic microbial communities in neutral and alkaline soils. However, there is still a knowledge gap concerning the response of ureolytic microflora to long-term organic and chemical fertilizations and their linkage with urease activity in acid soils.

Soils are composed of various sizes of aggregates. It is supposed that the distinct biotic and abiotic factors among diverse soil aggregate fractions may support the distribution of microorganisms with different functions, which further regulates soil biogeochemical cycling (Vos et al., 2013; Wilpiseski et al., 2019). For example, the

larger soil aggregates are favourable for copiotrophic communities and are linked to C turnover and productivity, while smaller soil aggregates are associated with oligotrophic microorganisms and contribute to C storage (Trivedi et al., 2018). The grazing of nematodes at aggregate level may significantly affect the dynamics of specific microbial community such as ammonia oxidizers and alkaline phosphomonoesterase producing bacteria, and then influences the nitrification and organic phosphate cycling in soils (Jiang et al. 2014; 2017). In addition, the microbes associated with different soil aggregates are reported to respond differently to long-term fertilizations and can modify the impact of agricultural management on soil nutrient cycling (Trivedi et al., 2015 and 2018). However, how soil aggregation affects ureolytic microbial communities and urease activity under long-term fertilizations in acid soils remains unclear.

Red soils are typical acidic soils are widely distributed throughout south China and account for 6.5% of the total arable land in the country (National Soil Survey, 1998). Although rich in hydrothermal resources, the productivity of Red soils (Ultisols) is usually low since they are acidic and infertile. In this study, we sampled soils with different fertilization treatments from Qiyang, south China and three soil aggregates were obtained to study the effects of long-term fertilization and aggregate size on ureolytic microbial communities. We hypothesized that (1) various long-term fertilization regimes may significantly differentiate the ureolytic microbial communities and alter soil urease activity; (2) there is a hierarchy of the abundance, richness, composition, and activity for ureolytic microbial community among soil

aggregate fractions.

2. Materials and methods

2.1 Experimental site description, soil sampling and aggregate separation

The soil was collected from Qiyang Red Soil Experimental Station (26° 45' N, 111° 52' E, 120 m altitude), Hunan Province, China. The average annual temperature and precipitation is 17.8 °C and 1255 mm, respectively. The soil is classified as Ultisol (USDA soil taxonomy) with a silty clay texture of 45.0% clay, 46.3% silt and 8.7% sand. The long-term fertilization experiment was started in 1990 with a wheat (*Triticum aestivum* L.) -maize (*Zea mays* L.) rotation system and including four different fertilization treatments: control without fertilization (CK); chemical fertilization of nitrogen, phosphate, and potassium (NPK); pig manure (M); and chemical NPK fertilization plus pig manure (MNPK). The nitrogen fertilizer was applied at a rate of 300 kg N ha⁻¹ each year as pig manure or urea, phosphate fertilizer at 53 kg P ha⁻¹ as superphosphate [Ca(H₂PO₄)₂], and potassium fertilizer at 100 kg K ha⁻¹ as potassium chloride (KCl). The total nitrogen content of pig manure was 1.67% based on dry matter basis. In MNPK, the ratio of organic N to inorganic N was 2.3:1. The fertilizers were applied before crop planting, while 30% were used for wheat and 70% were for maize.

Soil samples were collected from three replicates of each treatment during wheat growing season in November, 2016. Six soil cores (5 cm in diameter) were randomly collected from the 0-20 cm depth in each plot (10×20 m), and mixed to one composite sample. The field moist soils were gently broken up along the natural breakpoints, and

the visible organic debris was removed. Then soil samples were immediately delivered to the laboratory within 24h.

As soon as soil samples were transported to the laboratory, three water-stable aggregate fractions including macroaggregates (2-0.25 mm; L), microaggregates (0.25-0.053 mm; M), and silt + clay (< 0.053 mm; S) were separated by wet-sieving method following Elliott (1986). The soil aggregate fractions were then freeze-dried and stored at -80°C for further analysis.

2.2 Soil physicochemical characteristics and urease activity

Soil physicochemical characteristics, including soil organic carbon (SOC), total nitrogen (TN), ammonium ($\text{NH}_4^{+}\text{-N}$), available phosphorus (AP) and soil pH, were measured at the aggregate level by the same methods used in our previous study (Wang et al., 2018).

Soil urease activity was measured using urea as the substrate according to Hu et al. (2014). Briefly, 5 g soil aggregate samples were weighted into 50-ml volumetric flask and 2 mL methylbenzene was added to inhibit the soil microbial activity. Then the soil aggregate samples were incubated with 10 mL urea solution (10%) and 20 mL citrate phosphate buffer (pH 6.7) at 38°C for 3 h. Finally, the mixture was diluted to 50 mL and filtered. The NH_4^{+} in the soil suspension was detected by the indophenol blue reaction at 578 nm on a spectrophotometer (L6, Shanghai, China).

2.3 Soil nucleic acid extraction, quantitative PCR (qPCR) and high throughput sequencing

Soil nucleic acid (DNA) was extracted from 0.5 g soil aggregates following the

method of Griffiths et al. (2000). Then the extracted solutions were purified using DNA-EZ Reagents M Humic acid-Be-Gone B (Sangon Biotech, Shanghai, China) according to the manufacturer's protocol. The quality and quantity of DNA were determined by Nanodrop-2000 spectrophotometer (Nanodrop Technologies, PeqLab, Germany).

The abundances of soil bacteria and ureolytic subgroups were quantified on an ABI 7300 FAST Real-time PCR system (Applied Biosystems, U. S. A.). The primers used for total bacteria were *16S*-338F/*16S*-518R (Fierer et al., 2005) and for ureolytic microbes were *ureC*-F/*ureC*-R (Reed, 2001). Reaction mixtures (20 μ L) contained 10 μ L SYBR Premix Ex Taq II (2 $\times$) (Takara Bio, Otsu, Shiga, Japan), 0.5 μ L forward primer (10 μ M) and 0.5 μ L reverse primer (10 μ M), 1 μ L DNA extracts (1-10 ng) or 1 μ L standard plasmid, and 8 μ L ddH₂O. Standard curves were generated using a 10-fold dilution series of plasmid containing the *16S rRNA* and *ureC* amplified fragments, respectively. The annealing temperature of the qPCR assay was 60°C for *16S rDNA* and 54 °C for *ureC*. The amplification efficiencies of different samples were between 91% and 109%.

The compositions of ureolytic microbial communities were determined by barcode sequencing using Illumina HiSeq 2500 platform. DNA libraries were prepared using the PCR products amplified with the same primers in qPCR assay except that a 7 bp oligonucleotides was added at the 5' end of the primers to distinguish the samples. Downstream processing and bioinformatics analysis was performed following Wang et al. (2018 and 2019). Operational taxonomic units

(OTUs) were clustered based on 97% sequence similarity and annotated in accordance with Jin et al. (2016).

2.4 Statistical analysis

In this study, two-way ANOVA was carried out on SPSS 19.0 with Student–Newman–Keuls (SNK) test at the 95% probability level ($P < 0.05$). Pearson's correlation analysis was performed to assess the correlations. Shannon and Chao1 indexes were estimated based on the normalized read set using Mothur software. Redundancy analysis (RDA) was carried out with vegan package of R 3.4.2 using the Bray-Curtis distance matrices. The nonparametric multivariate analysis of variance (Adonis), similarities analysis (ANOSIM), and partial Mantel tests (999 permutations) were executed with the vegan package, using the “anosim”, “adonis” and “mantel.partial” function, respectively. Samples were clustered using unweighted pair group method with arithmetic averages (UPGMA) with 100 jackknife resamplings, based on the weighted UniFrac distance constructed in QIIME. The figure was displayed using MEGA 7.0. Significant taxonomic differences between soil aggregate fractions and fertilization regimes were tested using linear discriminate analysis (LDA) effect size (LEfSe) (Segata et al., 2011). Structural equation modelling (SEM) was performed to explore how soil chemical properties altered the abundance, diversity, population composition and activity of ureolytic microorganisms. The analysis was performed with AMOS 7.0 using the maximum likelihood estimation method. We adjusted the model by stepwise eliminated the least non-significant relationship from the fully specified model. The model fit to the data was judged by values of the χ^2 and

root mean square error of approximation (RMSEA) (Byrne, 2010).

3. Results

3.1 Soil physiochemical properties

As shown in Fig S1 and Table S1, long-term fertilizations significantly ($P < 0.05$) increased soil nutrition status compared to the control across various aggregates, only except for NH_4^+ ($P > 0.05$). Considerably larger improvements of SOC, TN and AP were found in manure-based treatments (M and MNPK) than in chemical-alone treatment (NPK). The soil pH among fertilization treatments followed a sequence of M (6.62) > MNPK (6.13) > CK (5.21) > NPK (4.70). At soil aggregate level, similar pH values and NH_4^+ concentration ($P > 0.05$) were observed among different fractions, while the other nutrients contents, including SOC, TN and AP, usually presented a slightly decreased trend with decreasing soil aggregate size ($P < 0.05$).

3.2 Soil urease activity and the abundance of ureolytic microbes

In this study, soil urease activity ranged from 135.3 ± 20.9 to $900.7 \pm 173.7 \mu\text{g NH}_4^+ \text{g}^{-1} \text{dry soil d}^{-1}$ among all samples (Fig. 1A). Manure-based treatments (M and MNPK) significantly enhanced, while chemical fertilization alone (NPK) notably decreased soil urease activity compared with the control (CK) across different aggregates ($P < 0.05$). The urease activity was significantly different ($P < 0.05$, Table S2) among various soil aggregates and showed a slightly increased mean value with decreasing soil aggregate size (Fig. 1A).

The copy numbers of ureolytic microbes were between $3.1 \pm 0.48 \times 10^7$ and $15.8 \pm 1.8 \times 10^7 \text{ g}^{-1} \text{dry soil}$ (Fig. 1B). Higher heterogeneity of ureolytic microbial

abundance was observed among fertilization treatments than among soil aggregate size fractions (Table S2). Particularly, manure application (M and MNPK) prominently improved the abundance of ureolytic microorganisms in all soil aggregate fractions compared with chemical-alone treatment (NPK) and no fertilizer control (Fig. 1B). The copy numbers of total bacteria were between $1.8 \pm 0.45 \times 10^9$ and $11.3 \pm 6.5 \times 10^9 \text{ g}^{-1}$ dry soil (Fig. 1C). The ratios of ureolytic microbes to total bacteria ranged from 0.6% to 7.1%.

3.3 α -diversity, composition, and community structure of ureolytic microbiota

As listed in Table S3, 29763–97180 high-quality reads were obtained by Illumina HiSeq sequencing per sample and these sequences were clustered to 752–2684 OTUs at a 97% cutoff based on the normalized read set. Significant lower Chao1 and Shannon indexes were found in NPK than in other samples ($P < 0.05$, Fig. 2 and Table S2), implying an impairment of α -diversity for ureolytic microorganisms by long-term chemical alone fertilization. No significant difference of α -diversity was found between various soil aggregates ($P > 0.05$, Fig. 2 and Table S2).

Taxonomic assignment revealed that ureolytic microorganisms were dominated by Alphaproteobacteria (21.6%), Betaproteobacteria (18.3%), Gammaproteobacteria (10.2%), Deltaproteobacteria (4.2%) and Actinobacteria (11.0%). While Firmicutes, Ascomycota, Planctomycetes, Verrucomicrobia, Bacteroidetes, Cyanobacteria, Deinococcus-Thermus, Basidiomycota, Chloroflexi, Streptophyta, Thaumarchaeota, Chlorophyta, Crenarchaeota and the others appear a lower relative abundances, and totally make up 34.7% of the sequences.

Multivariate analyses showed that long-term fertilization managements significantly drove the shift of the ureolytic community structure (ANOSIM, $R = 0.79$, $P = 0.001$), and explained 33.6% of the ureolytic microflora variations (Adonis, $F = 17.4$, $P = 0.001$). Soil aggregate size had no significant influence on the ureolytic community structure (ANOSIM, $R = -0.03$, $P = 0.844$) and merely explained 8.0% the ureolytic microflora variations (Adonis, $F = 2.9$, $P = 0.436$). RDA visually confirmed these results (Fig. 3A) and UPGMA cluster analysis uncovered that organic manure was the most important factor differentiating the ureolytic microbiota (Fig. 3B). Pure-partial Mantel tests showed that the variability of ureolytic communities was notably correlated with soil pH ($r = 0.71$, $P = 0.001$), and then TN ($r = 0.30$, $P = 0.001$), NH_4^+ ($r = 0.09$, $P = 0.011$) and AP ($r = 0.16$, $P = 0.001$) (Table S4).

The LEfSe analysis verified the significant associations between microbial phyla and fertilization regimes, with Ascomycota, Betaproteobacteria, Deltaproteobacteria, Acidimicrobiia, Streptomycetales and Pseudonocardiales being more abundant in M treatments; Thermoleophilia, Rhodospirillales, Rhizobiaceae, and several groups from Myxococcales including Polyangiaceae, Myxococcaceae and a unidentified cluster, *Bradyrhizobium*, *Geodermatophilus* and *Alkalilimnicola* in MNPK; and Oceanospirillales, Pseudomonadales, Sphingomonadales, Frankiales, Mycobacteriaceae, Thermomonosporaceae and Phyllobacteriaceae in NPK; Firmicutes, Micrococcales, Geodermatophilales, Micromonosporales, Streptosporangiaceae, Comamonadaceae, Nocardaceae, Brucellaceae, Xanthobacteraceae, *Methylibium* and *Burkholderia* in CK (Fig. 3D, Fig. S2A and

Table S5). The small impact of soil aggregate fractions on ureolytic community was confirmed by LEfSe analysis, which revealed no predilection for the overwhelming majority of ureolytic taxonomy across soil aggregates. The only exceptions were that *Ochrobactrum* was more abundant in silt + clay fractions, while *Actinoplanes*, *Actinosynnema*, *Amycolatopsis*, *Methylobacterium* and *Leptothrix* were richer in macroaggregates (Fig 3C, Fig S2B and Table S6).

3.4 Controlling factors for ureolytic community and soil urease activity

The structural equation model was used to evaluate the effects of soil physiochemical properties on ureolytic community parameters and urease activity. This model explains 59% of the variance in ureolytic microbial abundance, 66% in ureolytic microbial composition and urease activity, and 73% in ureolytic microbial diversity. Soil total N (TN) produced the strongest effects on ureolytic microbial abundance, while ureolytic microbial diversity and composition, and urease activity were primarily determined by soil pH. Moreover, soil pH also indirectly influenced soil urease activity through regulating ureolytic microbial composition (Fig. 4).

4. Discussion

Long-term manure applications were usually reported to improve soil microbial biomass as they introduce large amounts of nutrients to support the growth of soil microorganisms (Gong et al., 2009; Chakraborty et al., 2011; Ma et al., 2016). In this study, we observed a remarkable improvement for the abundances of soil ureolytic microorganisms by organic manure, yet (Fig. 1B). However, long-term chemical fertilization was found to have slightly decreased ureolytic microbial abundance in

this acid Red soil, especially in microaggregates (Fig. 1B), which was in contrary to that observed in a fluvo-aquic soil from North China Plain (pH~8.2) (Sun et al., 2019), indicating that the effect of long-term chemical fertilizations on ureolytic microbial abundance may be soil type dependent. Based on a microcosm experiment, Fisher et al. (2017) obtained a positive correlation between soil ureolytic microbial abundance and soil pH in the range of 3.1 to 7.1. The negative effect of long-term chemical fertilization on soil ureolytic microbial abundance may be owing to the strong soil acidification caused by chemical fertilizations in this acidic soil (Fig. S1F). Moreover, there was a significant impairment of α -diversity for soil ureolytic microorganisms by long-term chemical fertilization in this acid soil (Fig. 2), which was similar to the result from alkaline fluvo-aquic soils (Sun et al., 2019). Fortunately, our results suggested that manure application could alleviate this negative effect and improve the α -diversity to the similar level of control soils (Fig. 2). Actually, organic manure could provide more stable and balanced nutrients for soil microorganisms that might support different ureolytic species (Fig. 3B) and enhance their α -diversity.

We found a significant differentiation of ureolytic microbial composition by long-term fertilizations in this Red soil, and manure application acted as the prime motivator. Both pure-partial Mantel tests and structural equation model revealed that soil pH was the paramount soil property that determines the ureolytic microbial community (Fig. 4 and Table S4). It is assumed that the remarkable discrepancy of soil pH between manure (MNPK and M, 6.13 and 6.62) and non-manure (NPK and CK, 4.70 and 5.21) based systems drove the contrasting selection of ureolytic

microbes. The ecological characteristics of these genera might explain their habitat preference. For example, acid resistant *Sphingomonas* was detectable in the extremely acidic mine (Kimura et al., 2011). The genera *Alkaliliminicola*, *Geodermatophilus* and *Thioalkalivibrio* were probably alkaliphilic organisms (Normand & Benson, 2015). Besides, the introduction of various organic C by manure application might also contribute to the differentiation of ureolytic microbial community (Wang et al., 2018). For instance, as copiotrophic microbes whose relative abundances were usually positively correlated with TC or labile C (Fierer et al., 2007; Leff et al., 2015; Trivedi et al., 2018), Betaproteobacteria were found to be significantly enhanced by organic manure application (M) in our study. Rhizobiaceae is a taxon associated with nutritious environment such as plant roots (Jourand et al., 2004). This ureolytic group was reported to be enriched in manure treated plots in both acid Red soil and neutral Black soil (Wang et al., 2018). Different ureolytic species were reported to exhibit various urease producing abilities as well as diverse specific activities. For example, the specific activities of purified urease from different microbes ranged from 14.5 to 7100 $\mu\text{mol urea min}^{-1} \text{mg enzyme}^{-1}$ in laboratory (Krajewska, 2009; Lu and Jia, 2013; Tourna et al., 2011). Therefore, the significant differentiation of ureolytic microbial compositions among various long-term fertilizations might, at least in part, contribute to the variation of urease activity across fertilization regimes (Fig. 4). In addition, the affluent nutrients introduced by manure application might also promote the growth of ureolytic microbes which result in more urease production to satisfy the large requirement of bioavailable NH_4^+ for their rapid growth. This may partially explain

why manure application showed a positive effect on urease activity in our study (Fig. 1A). Finally, but may be most importantly, soil urease is present as both extracellular and intracellular forms. The proportion of extracellular urease was estimated to account for 26.9% to 62.9% of total urease activity in soils (Klose and Tabatabai, 1999). Extracellular soil urease is usually adsorbed by soil colloids, particularly organic matters (Krajewska, 2009), which can resist protease degradation and thermal inactivation (Burns et al., 1972; Pettit et al., 1976). This may be an important reason for the higher urease activity in manure based soil systems. Overall, our study revealed a significant differentiation of ureolytic microbes under long-term organic and chemical fertilizations in the Ultisol which partly contributed to the variation of soil urease activity. These findings supported our first hypothesis.

A broad body of literatures has demonstrated the hierarchical distribution of soil microorganisms across soil aggregate fractions due to the heterogeneity of organic matters, oxygen and water content, and predation pressure among soil aggregates (Tisdall and Oades, 1982; Davinic et al., 2012; Evans et al., 2016; Li et al., 2017; Jiang et al., 2017). In our previous study, we revealed an obvious stratification of the ureolytic microbial communities and urease activity across soil aggregates in Mollisols (Wang et al., 2018), which was regulated by the distribution patterns of nutrients. However, no marked differences for ureolytic microbial community were found in Ultisols, and this disagrees with our second hypothesis. This might be attributed to the small heterogeneity of soil nutrient conditions at soil aggregates in this soil (Fig. S1), which lead to the homogeneous distribution for soil

nitrite-oxidizing bacterial community (Han et al., 2018). Therefore, our results suggested that resources availability might be a dominant factor determining the distribution of soil ureolytic microbes within micro-environment across soil types.

5. Conclusions

In this study we reported the effect of long-term fertilization regimes and soil aggregate size on soil ureolytic microbial communities in an acid Red soil. The ureolytic microflora were shown not to be affected by soil aggregate size, but were differentiated by long-term fertilizations. Chemical fertilization significantly reduced the abundance, diversity and activity of ureolytic microbes while manure application could mitigate these negative impacts. Long-term fertilizations also changed the compositions and structure of ureolytic microbiota. Soil urease activity was mainly determined by soil pH and the compositions of ureolytic microflora. This work revealed how long-term fertilizations influenced soil ureolytic microbes and further regulated soil urease activity in the microenvironments of acid soils. The results obtained would help us better understand the consequence of fertilization practices on soil microbes and their functions in nitrogen cycling of subtropical and tropical regions.

Conflicts of interest

There are no conflicts to declare.

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Fig. 1 (A) Urease activity, (B) ureolytic microorganism abundance, and (C) total bacterial abundance at aggregate level under long-term fertilizations. Error bars represent standard errors of three replicates. Bars with the different letter are significantly different ($P < 0.05$) by SNK test. Abbreviations: L, macroaggregates; M, microaggregates; S, silt + clay.

Fig. 2 (A) Chao 1, and (B) Shannon indexes for *ureC* gene at aggregate level under long-term fertilizations. Error bars represent standard errors of three replicates. Bars with the different letter (shown above each) are significantly different ($P < 0.05$) by SNK test. Abbreviations: L, macroaggregates; M, microaggregates; S, silt + clay.

Fig. 3 Responses of ureolytic communities to long-term fertilizations and soil aggregate fractions (A) Redundancy analysis (RDA). (B) Weighted UniFrac distance dendrogram. (C) LDA effect size taxonomic cladogram categorized by soil aggregate fractions. Significantly discriminant taxon nodes are colored and branch areas are shaded according to the highest-ranked variety for that taxon. For each taxon detected, the corresponding node in the taxonomic cladogram is colored according to the highest-ranked group for that taxon. If the taxon is not significantly differentiated between sample groups, the corresponding node is marked yellow. Highly abundant and select taxa are indicated: g, *Ochrobactrum*; a, *Actinoplanes*; d, *Actinosynnnerma*; e, *Amicolatopsis*; i, *Methylobacterium*; l, *Leptothrix*. For the complete list of discriminate taxa and ranks used to generate this cladogram, see Fig S1B and Table S6. (D) LDA effect size taxonomic cladogram categorized by long-term fertilization regimes. Highly abundant and select taxa are indicated: f6, *Betaproteobacteria*; h0, *Deltaproteobacteria*; a7, *Streptomycetales*; a4, *Pseudonocardiales*; h, *Thermoleophilia*; d1, *Rhodospirillales*; c5, *Rhizobiaceae*; g6, *Polyangiaceae*; g4, *Myxococcaceae*; b7, *Bradyrhizobium*; q, *Geodermatophilus*; h1, *Alkalilimnicola* h7, *Oceanospirillales*; i0, *Pseudomonadales*; d4, *Sphingomonadales*; p, *Frankiales*; j, *Mycobacteriaceae*; b1, *Thermomonosporaceae*; c3, *Phyllobacteriaceae*; w, *Micrococcales*; t, *Geodermatophilales*; a0, *Micromonosporales*; a9, *Streptosporangiaceae*; e3, *Comamonadaceae*; l, *Nocardiaceae*; c1, *Brucellaceae*; c7, *Xanthobacteraceae*; e5, *Methylibium*; d6, *Burkholderia*. For the complete list of discriminate taxa and ranks used to generate this cladogram, see Fig S1A and Table S5.

Fig. 4 Effects of soil physicochemical properties on ureolytic community parameters and urease activity as estimated using structural equation model ($n = 36$, $\chi^2 = 18.05$, $P = 0.260$, degrees of freedom (df) = 15; root mean square error of approximation (RMSEA) = 0.076, $P = 0.344$). Numbers adjacent to arrows and arrow width indicate the relationship's effect size ($P < 0.05$). Black and grey arrows indicate positive and negative relationships, respectively. The first principal component (PC1, see Figure S3) is used to represent the composition of ureolytic microbial community. Ureolytic microbial diversity was quantified by Shannon diversity index on all samples.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract

Highlights

- Chemical fertilizer reduces ureolytic microbial richness and activity.
- Manure application improves ureolytic microbial abundance and activity.
- Long-term fertilizations change the structure of ureolytic microbiota.
- The ureolytic microbiota among soil aggregates has no significant difference.
- Soil urease activity is determined by soil pH and ureolytic microflora composition.