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Ureolytic microbial community is modulated by fertilization regimes and particle-size fractions in a Black soil of Northeastern China

Li Wang[a,b], Xuesong Luo[a,b], Hao Liao[a], Wenli Chen[a,*], Dan Wei[c], Peng Cai[a,b], Qiaoyun Huang[a,b,**]

[a] State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China
[b] Key Laboratory of Arable Land Conservation (Middle and Lower Reaches of Yangtze River), Ministry of Agriculture, College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, China
[c] Institute of Soil Fertilizer and Environment Resources, Heilongjiang Academy of Agricultural Sciences, Harbin 150086, China

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ABSTRACT

Ureolytic microbes are important contributors to N cycling in soil. The distribution of the ureolytic microbial community in different particle-size fractions (2000-250, 250-53, < 53 μm) was determined by high-throughput sequencing and q-PCR analyses in 35-year-old fertilization experiment on Black soil. Fertilizer treatments included an unfertilized control, chemical NPK fertilizer, horse manure and NPK fertilizer plus horse manure. Each fertilizer treatment harbored a structurally distinct ureolytic microbial community. The dominant ureolytic microbes were putatively associated with *Rhizobiales*, *Burkholderiales*, *Rhodocyclales*, *Pasteurellales*, *Pseudomonadales*, *Oceanospirillales*, *Myxococcales*, *Micrococcales* and *Corynebacteriale* species. The OTUs associated with the same fertilizer treatment or particle-size fraction were scattered across the phylogenetic tree, and only sequences related to *Rhizobiales* showed a similar increase in response to manure application. The abundances of *ureC* genes ranged from $1.5 \pm 0.38 \times 10^7$ to $9.1 \pm 0.91 \times 10^7$ g^{-1} dry soil and accounted for 0.4% to 2.9% of the total bacteria (represented by the copy numbers of the 16SrRNA genes). Significantly lower *ureC* gene abundance was observed in manure-amended soil, whereas the macro- and microaggregates had higher *ureC* gene abundances than the silt + clay fraction. The abundance of ureolytic microorganisms was significantly correlated with soil NH_4, total nitrogen (TN) and carbon (TC) concentrations. This indicates that variation in ureolytic microbial communities was associated with soil nutrient levels. Urease activities in the particle-size fractions were as follows: microaggregate > macroaggregate > silt + clay. Additionally, urease activities were correlated with the TN, TC and soil organic carbon (SOC) concentrations, but not with the abundances of the *ureC* genes. In summary, our study first revealed the heterogeneity in the ureolytic microbial communities and activities across different particle-size fractions under long-term fertilization. Microaggregates seems to be a "hotspot" for nutrients, ureolytic microorganisms and urease activity.

1. Introduction

Urea is a nitrogen compound that is ubiquitous and naturally occurring in soils, which is excreted by organisms as detoxification product of the biodegradation of nitrogenous compounds such as purines and amino acid (Vogels and van der Drift, 1976). More importantly, as a common fertilizer urea makes up almost 60% of the consumption of nitrogen fertilizers around the world (Glibert et al., 2014). Urea applied to soils usually results in rapid hydrolysis to NH_3/NH_4^+ by urease (urea amidohydrolase, EC 3.5.1.5) and provides available nitrogen sources for soil microbes and plants (Mobley and Hausinger, 1989). Therefore, urease activity plays an essential role in soil fertility and has been widely used as an important indicator of soil quality (Jr et al., 1985; García-Ruiz et al., 2008; Cetin et al., 2009).

Most organisms (except yeasts and some chlorophytes) use the nickel requiring metalloenzyme urease to catalyse the hydrolysis of urea (Bekheet and Syrett, 1977). The primary amino acid sequence of urease is approximately 800 amino acids in length and is highly conserved. It can be encoded by a single gene (i.e. in one protein subunit) or divided into two or three genes (two or three protein subunits; Mobley et al., 1995). The C-terminal ~600 amino acids are undivided in all known ureases, forming the alpha subunit, and the gene encoding the alpha subunit is usually called *ureC*. In addition to the structural subunits, urease synthesis requires several accessory genes (*ureD*, *ureE*,

* Corresponding author.
** Corresponding author. State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China.
E-mail addresses: wlchen@mail.hzau.edu.cn (W. Chen), qyhuang@mail.hzau.edu.cn (Q. Huang).

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Table 1
Soil chemical properties of aggregate fractions as influenced by fertilization.

Main test[a]	TC	TN	C/N	SOC	TP	AP	NH_4^+	pH
	(F/P)	(F/P)	(F/P)	(F/P)	(F/P)	(F/P)	(F/P)	
Fraction	**112.9/ < 0.001**	**160.3/ < 0.001**	**59.4/ < 0.001**	**108.1/ < 0.001**	**66.6/ < 0.001**	**194.4/ < 0.001**	**50.1/ < 0.001**	ND[c]
Fertilization	0.6/0.652	2.5/0.088	**13.6/ < 0.001**	**3.7/ < 0.001**	**175.6/ < 0.001**	**13.5/ < 0.001**	**20.9/ < 0.001**	**43.2/ < 0.001**
Fertilization*Fraction	0.4/0.901	0.2/0.972	3.1/0.021	0.3/0.921	**3.3/0.017**	**2.8/0.032**	0.8/0.604	ND
Pairwise test[b]	(mean ± s.e.)	(mean ± s.e.)	(mean ± s.e.)	(mean ± s.e.)	(mean ± s.e.)	(mean ± s.e.)	(mean ± s.e.)	
Fraction	(%)	(%)		(g/kg)	(mg/kg)	(mg/kg)	(mg/kg)	
L	1.76 ± 0.21[b]	0.122 ± 0.011[b]	14.4 ± 0.9[c]	24.9 ± 4.8[b]	391 ± 141[b]	36.5 ± 29.9[a]	13.4 ± 4.1[b]	ND
M	3.14 ± 0.58[a]	0.173 ± 0.026[a]	18.1 ± 1.9[a]	45.6 ± 7.5[a]	426 ± 157[a]	33.3 ± 26.1[a]	22.9 ± 7.1[a]	ND
S	0.76 ± 0.09[c]	0.050 ± 0.008[c]	15.3 ± 1.1[b]	13.2 ± 4.6[c]	281 ± 96[c]	24.4 ± 20.1[b]	10.9 ± 3.6[b]	ND
Fertilization								
CK	1.83 ± 1.15[a]	0.102 ± 0.052[a]	17.3 ± 2.8[a]	22.7 ± 15.5[b]	207 ± 38[c]	4.2 ± 1.2[d]	11.5 ± 5.6[b]	6.37 ± 0.05[a]
NPK	1.82 ± 1.04[a]	0.117 ± 0.053[a]	15.1 ± 1.9[c]	29.5 ± 13.8[a]	471 ± 93[a]	51.8 ± 11.9[b]	19.5 ± 7.2[a]	5.42 ± 0.15[c]
M	2.03 ± 1.13[a]	0.120 ± 0.058[a]	16.4 ± 1.4[b]	29.0 ± 16.2[a]	287 ± 56[b]	11.6 ± 2.9[c]	11.7 ± 4.3[b]	6.50 ± 0.07[a]
MNPK	1.87 ± 1.04[a]	0.121 ± 0.059[a]	15.1 ± 1.3[c]	30.4 ± 14.6[a]	500 ± 99[a]	58.1 ± 12.0[a]	20.1 ± 7.3[a]	5.78 ± 0.21[b]

Abbreviations: L, macroaggregates; M, microaggregates; S, silt + clay; CK, without fertilization; M, horse manure; NPK, chemical fertilizers included nitrogen, phosphorus, and potassium fertilizers; MNPK, chemical fertilizers plus horse manure.

[a] Effects of main factors and their interactions were assessed by ANOVA. Values at $P < 0.05$ are shown in bold.

[b] Different letters represent significant differences at $P < 0.05$ for pairwise test.

[c] ND: not determined.

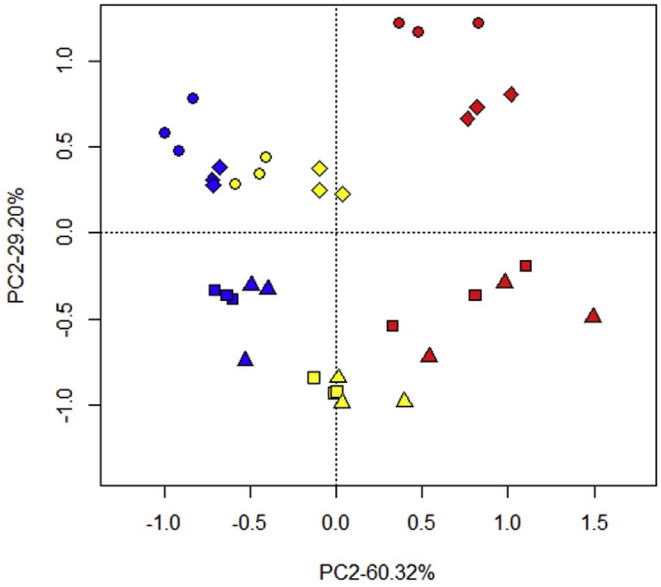

Fig. 1. PCA ordinations of Euclidean distances calculated based on soil chemical parameters (TC, TN, SOC, C/N, TP, AP and NH_4^+). L (yellow), M (red), S (blue); CK (circle), NPK (square), M (diamond), MNPK (triangle). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ureF and *ureG*), which are also contiguous with the structural subunits in most organisms (Mobley et al., 1995). The functional ureolytic microbial community is commonly identified by targeting the *ureC* gene (Reed, 2001). Fisher et al. (2017) quantified the abundance of ureolytic microbes in an incubation soil using q-PCR technology targeting the *ureC* gene and found that high pH encouraged the growth of ureolytic microorganisms and contributed to a higher urea hydrolysis rates. A few studies that employed clone library technology have revealed the ureolytic microbial community composition in groundwater (Greshan et al., 2007), grassland (Singh et al., 2009), and open-ocean and estuarine planktonic communities (Collier et al., 2009). However, these low throughput sequencing technologies might underrate the diversity of environmental ureolytic microbes. High throughput sequencing technologies offer ways to explore the environmental microbiota at higher resolution, coverage and throughput, and have the potential to exhaust their diversity and community. Therefore, we employed high throughput sequencing technology to uncover the diversity of ureolytic microbes in an agricultural soil in Northeast China.

The cultivated Black soil (Udic Mollisol, USDA taxonomy) region in Northeast China covers approximately 15 million ha (Institute of Soil Science of the Chinese Academy of sciences 1978), and is one of the three famous Black soil regions in the world. Owning to the fertile and productive soils, this region is well known as one of the most important crop production bases and provides 20% of the grain yield in China. Chemical fertilizers, organic manures and their combinations are widely used fertilization practices in this region. Many studies have addressed that fertilization enhanced soil urease activity and organic manures usually bring higher increases than chemical fertilizers on urease activity (Kandeler et al., 1999; Nayak et al., 2007; Liang et al., 2014). However the effect of fertilization on urease activity as well as ureolytic microbial community in Black soils is still unclear.

Soils have a complex hierarchical structure including pore distribution and aggregates. Soil aggregates provide spatially heterogeneous habitats for microorganisms, which vary in nutrient availability, water potential and oxygen concentration as well as predation pressure (Ranjard and Richaume, 2001; Jiang et al., 2013). The heterogeneity may substantially affect the microbial community composition (Smith et al., 2014; Chen et al., 2015) and further affect their function. The characteristics of urease activity across various particle-size fractions have been reported by Li et al. (2016). Nevertheless, the effects of aggregate size on the abundance and community composition of ureolytic microorganisms are still unknown.

In the present study, we employed high-throughput sequencing technology of the ureolytic microorganism marker genes (*ureC*) to examine the community diversity and distribution patterns of ureolytic microorganisms across different particle-size fractions of Black soils under 35-years of fertilization. Urease activity, *ureC* and *16S rRNA* gene abundances were also quantified. The objectives of the work were to answer the following questions: (i) how do the abundance and structure of the ureolytic microbial community respond to long-term fertilization and soil aggregation in the Black soil, and (ii) what are the key factors controlling the ureolytic microbial community and urease activity?