Response of Soil Biological Properties and Bacterial Diversity to Different Levels of Nitrogen Application in Sugarcane Fields

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Abstract

Field experiments were performed in early March 2019 at the farm of the Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences. Four concentrations of nitrogen application were employed as follows: that is, urea applications 964, 482, 96, 0 kg ha^{-1}, respectively. And 300 kg ha^{-1} calcium, magnesium, and phosphorus were likewise applied in 4 different treatments. The results showed that the soil microbial biomass carbon and phosphorus were altered significantly by non- and low-nitrogen input. Moreover, the indexes of soil bacterial richness and diversity in the sugarcane field could be significantly improved, even by low nitrogen input. At the genus level, *norank_f__SC-I-84*, *Mycobacterium*, *norank_f__Micropepsaceae*, *norank_f__norank_o__Saccharimonadales*, *norank_f__norank_o__Subgroup_2* and *norank_f__Acetobacteraceae* were the unique dominant bacteria in the soil with the high nitrogen input treatment. *norank_f__JG30-KF-CM45* and *Jatrophihabitans* were the unique dominant genera in the moderate nitrogen input treatment. *norank_f__norank_o__norank_c__Subgroup_6*, *HSB_OF53-F07*, *Streptomyces*, *norank_f__67 − 14*, *norank_f__norank_o__SBR1031* and *norank_f__norank_o__norank_c__KD4-96* were the unique dominant genera in the low nitrogen input treatment. In contrast, *FCPS473*, *Actinospica*, *1921-2*, *Sinomonas*, and *norank_f__Ktedonobacteraceae* were the unique dominant genera in CK (no nitrogen application treatment). It suggested that low nitrogen input was the most significant effect on the soil microbial biomass carbon and phosphorus in the sugarcane field. Moreover, low nitrogen input also can improve the diversity and richness of sugarcane soil bacteria. The dominant bacterial genera of low nitrogen input and the other treatments were different for the compositions of dominant bacteria, and the largest abundance difference of dominant bacterial genera was *norank_f__norank_o__norank_c__Subgroup_6*. However, whether low nitrogen stress can improve the yield and quality of sugarcane warrants further research.

1. Introduction

Sugarcane (*Saccharum officinarum* L.), an important sugar crop, is utilized as a source of biofuel and renewable bioenergy around the world (Chandel et al., 2011). China is the third-largest sugar-producing country in the world followed by Brazil and India. In China, approximately 90% of the sugarcane crops are planted in southern and southwest regions, including Guangxi, Guangdong, and Yunnan provinces. In particularly, Guangxi Province is the top sugarcane and sugar producer of China, accounting for more than 65% of the sugar production of the nation since 1993 (Li, 2004). However, low sugarcane yield is still a major problem in China. To improve cane yield, chemical fertilizers were overused by farmers in Guangxi, China. The nitrogen (N) fertilizer is applied at 600–800 kg ha^{-1} annually for sugarcane in China, but only 60 kg ha^{-1} for newly planted canes and 80–120 kg ha^{-1} for ratoon canes are applied in Brazil (Li & Yang, 2015). Moreover, overuse of chemical fertilizer not only negatively influences microbial systems but also disrupts terrestrial and aquatic ecosystem functions (Robertson & Vitousek, 2009). A method to reduce chemical fertilizer inputs and enhance crop production in an ecofriendly manner is an urgent need for sugarcane production, particularly in Guangxi, China.

Nitrogen (N) is one of the important essential nutrients affecting the growth of crops and is also a key pillar of global food security (Mueller et al., 2012). N is a limited resource; even so, the yield and quality of crops are largely determined by plant demand for N (Hawkesford, 2014). The N surplus, can cause massive losses through denitrification, leaching, volatilization, and runoff, making the soil unable to meet human demands for clean water, clean air, and abundant healthy food (Matson et al., 1998; Erisman et al., 2013). In contrast, N input consistently below crop N requirements (*i.e.*, N lacks) leads to soil nitrogen extraction and soil quality degradation (Sanchez, 2002; Sanchez & Swaminathan, 2005). Currently, face a challenge in finding an effective balance between N input and crop N requirements to achieve high crop yields while maintaining soil quality and reducing the environmental footprint (Lassaletta et al., 2014; Zhang et al., 2015a). Niu et al. (2021) found that water soluble fertilizer affected the enrichment of microorganisms by improving the nutrient content of the soil, thereby affecting the growth and yield of sugarcane.

Soil quality depends on numerous physical, chemical, biological, biochemical and microbiological parameters (Chaer et al., 2009). In particular, the latter two parameters are the most sensitive indicators that respond rapidly to changes in soil quality (Bastida et al., 2008). Soil enzyme activity is not only a sensitive biochemical indicator of quality (Raiesi & Beheshti, 2014) but is also capable of reflecting ecosystem processes (Doran & Zeiss, 2000). However, enzymatic activity is presented only in absolute terms, and soil microbial biomass carbon (MBC), microbial biomass N (MBN) and microbial biomass phosphorus (MBP) are also used as tools for monitoring soil quality (Pandey et al., 2014). In addition, soil microorganisms play an important role in soil biogeochemical processes such as N, phosphorus and other element cycles (Urbanová et al., 2015). Soil microbial community composition and diversity are imperative to maintain soil health and crop productivity (Mangan et al., 2010).

Therefore, in this study, objectify to (1) compare soil fertility and (2) analyze the response of the soil bacterial community structure to different nitrogen applications.

2. Method

2.1 Field site description and experimental designs

The samples were collected on May 12, 2020, from the Experimental Base of Sugarcane Research Institute, Guangxi Academy of Agricultural Sciences, where is located on Longan County (107°75'E and 23°17'N), Guangxi Zhuang Autonomous Region, China. The sugarcane variety Guitang49 was planted in early March 2019.

Four treatments were designed as follows: application of urea 964 kg ha^{-1}, with pure N 450 kg input (H); application of 482 kg ha^{-1}, with pure N 225 kg input (M); and application of urea 96 kg ha^{-1}, with pure N 45 kg input (L) and no urea application (CK). And 300 kg ha^{-1} calcium, magnesium, and phosphorus were likewise applied in 4 different treatments. The conventional field management measures were carried out identically except for the differences in nitrogen application levels. Each nitrogen application

pattern was randomly treated with three replications. A total of 12 plots, each plot area was 63 m^2. In every plot, there were five rows, and the row length and space were 7 m and 1.8 m, respectively.

2.2 Soil sampling and soil biological properties analysis

Soil samples were collected in July 2020 from 12 plots that represented all the treatments in different nitrogen application experiments. To collect soil samples, the auger was sprayed with 75% ethanol for disinfection firstly, and then soil samples were collected by sterilized auger with the same depth of 40 cm in each treatment plot. From each plot, soil samples were collected from 12 random sites and mixed well. These soil samples were collected in sterile plastic bags and placed on ice in an ice box. The samples were immediately transferred to the laboratory, where they were sieved through a 2-mm mesh stainless steel sieve, and then stored in a refrigerator at 4°C for immediate analysis or were stored at -80°C for later use. Meanwhile, portions of the soil samples were air dried for soil chemical analyses (Yang et al., 2021). The chemical properties of the soil were as follows: pH 4.5–5.6, and the contents of organic matter, total nitrogen, phosphorus and potassium were 17.6 g kg^{-1}, 0.92 g kg^{-1}, 0.92 g kg^{-1} and 0.56 g kg^{-1}, respectively. The contents of available nitrogen, phosphorus and potassium were 85 mg kg^{-1}, 35.3 mg kg^{-1} and 125 mg kg^{-1}, respectively.

2.2.1 Soil enzyme activities

Soil microbial biomass carbon, nitrogen and phosphorus and the activity of enzymes such as *β*-glucosidase, phosphatase and protease were analyzed using the following methods:

β-Glucosidase (EC.3.2.1.21) and exocellulase (EC.3.2.1.91) assays were based on *ρ*-nitrophenol (*ρ*NP) release after cleavage of a synthetic substrate (*ρ*-nitrophenyl-*β*-D-glucoside and *ρ*-nitrophenyl-*β*-D-cellobioside, respectively). The color of released *ρ*-nitrophenol was measured at 400 nm in a spectrophotometer (UV-1700, Shimadzu, Japan). A standard curve was plotted using 0–80 μg mL^{-1} *ρ*-nitrophenol. The enzyme activities were expressed as *n* moles *ρ*NP released per g dry soil per minute (*n* mol *ρ*NP g^{-1} min^{-1}) (Deng & Tabatabai, 1994).

Phosphatase (phosphodiesterase and phosphomonoesterase) activity in soils was estimated by measuring the amount of *ρ*-nitrophenol released after incubating the samples with *ρ*-nitrophenyl phosphate (Alef et al., 1995). In a reaction tube, 0.25 mL toluene, 4.0 mL modified universal buffer (pH 6.0; made by dissolving 12.1 g tris, 11.6 g maleic acid, 14.0 g citric acid and 6.3 g boric acid in 500 mL 1 M NaOH and making the volume 1 L) and 1.0 mL *ρ*-nitrophenyl phosphate (15 mmol L^{-1}) were added to 1.0 g soil sample and the mixture was incubated at 37°C for 1 h. The reaction was terminated by adding 1.0 mL of 0.5 mol L^{-1} $CaCl_2$ and 4.0 mL of 0.5 mol L^{-1} NaOH to the mixture prior to filtration. The absorbance of released *ρ*NP was measured at 400 nm in a spectrophotometer (UV-1700, Shimadzu, Japan), and the phosphatase activity was expressed in mg *ρ*-NP g^{-1} h^{-1}.

Aminopeptidase activity was measured by the method described by Pansombat et al. (1997) using 0.002 M *N*-benzoyl-Lxycarbonyl glycyl L-phenylalanine (ZGP). The absorbance was measured in a spectrophotometer at 570 nm wavelength. All the analyses were conducted in 5 replicates.

2.2.2 Soil microbial biomass

Soil microbial biomass N (MBN) and soil microbial biomass C (MBC) were determined using the chloroform fumigation-extraction method as described by Brookes et al. (1985) and Vance et al. (1987). Soil microbial biomass P (MBP) was determined by the phosphorus molybdenum blue colorimetric method (Powlson et al., 1987).

2.3 Analysis of soil microbial diversity

Microbial community genomic DNA was extracted from samples using the E.Z.N.A.® soil DNA Kit (Omega Biotek, Norcross, GA, U.S.) according to manufacturer's instructions. The DNA extract was checked on a 1% agarose gel, and DNA concentration and purity were determined with a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA). PCR amplification and sequencing of total DNA extraction from rhizosphere soil samples were performed by Shanghai Majorbio Biopharm Technology Co., Ltd. PCR amplification was performed by ABI GeneAmp 9700 (ABI, USA), and the PCR products were recovered by 2% agar-gel electrophoresis. The products were purified by an AxyPrep DNA Gel Extraction Kit (Axygen, USA) and quantified by a Quantus Fluorometer (Promega, USA). Purified amplicons were pooled in equimolar amounts and paired-end sequenced (2×300) on an Illumina MiSeq platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw reads were deposited in the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP302628).

2.4 Statistical analyses

All treatments were performed in triplicate by a completely random design. Statistical analyses were carried out by SPSS software using a multiple range test at a 0.95 level of probability to determine significant differences ($p < 0.05$) between the treatments. The results are shown as the standard deviation of the mean (mean ± SD). The experimental data were analyzed using Excel 2019 and Statistical Product and Service Solutions (SPSS) Statistics 21, and online data analysis was conducted by using the free online platform of the Majorbio Cloud Platform (www.majorbio.com) of Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

3. Results

3.1 Soil enzyme activities

The trends in soil enzyme activity under different N applications are shown in Table 1. The activities of *β*-glucosidase and acid phosphatase in soil under high N application (H) were all significantly higher than the activities in CK. However, the activity of *β*-glucosidase in the M or L treatments was not significantly

different between CKs. The activity of acid phosphatase in the L treatment was significantly higher than the activity of acid phosphatase in the CK, but there was no significant difference between the M treatment and the CK. In addition, the activities of aminopeptidase in N applications were all significantly lower than those in CK, and there were no significant differences between each of the N applications. This result suggested that the activities of soil enzymes related to carbon, N and phosphorus cycles in soil were all affected by N application. The activities of *β*-glucosidase and acid phosphatase were significantly increased by higher N application, but the activity of aminopeptidase was significantly decreased by N application.

Table 1

Soil enzyme activities under four different N application treatments (nmol g^{-1} min^{-1})

Treatments	*β*-Glucosidase	Aminopeptidase	Acid phosphatase
H	0.25 ± 0.08a	14.00 ± 1.57b	0.42 ± 0.14a
M	0.17 ± 0.02ab	14.09 ± 0.20b	0.25 ± 0.06b
L	0.06 ± 0.01c	11.68 ± 1.87b	0.43 ± 0.04a
CK	0.12 ± 0.01bc	18.55 ± 1.22a	0.12 ± 0.03b

Notes: All data are presented as the mean ± SD (standard deviation). Different letters in the same column indicate significant differences between treatments at $P < 0.05$ among the means of the four treatments. H: high N application in the sugarcane soil (964 kg ha^{-1}), M: moderate N application in the sugarcane soil (482 kg ha^{-1}), L: low N application in the sugarcane soil (96 kg ha^{-1}), CK: no N application in the sugarcane soil (0 kg ha^{-1}).

3.2 Soil microbial biomass

The soil microbial biomass N (MBN) in the different nitrogen application treatments was significantly higher than the MBN in the CK treatment. In contrast, soil microbial biomass carbon (MBC), except in the low N application treatment, was significantly lower than that in the CK. The soil microbial biomass phosphorus trend was similar with MBC except in the M treatment, which was not significant between the M treatment and CK. These results indicated that soil microbial biomass C, N and P were also significantly affected by N application. However, the trends of soil microbial biomass were dependent and affected by N application (Table 2).

Table 2

Effect of different N applications on soil microbial biomass C, N and P in sugarcane fields (mg kg^{-1})

Treatments	Microbial biomass C	Microbial biomass N	Microbial biomass P
H	112.42 ± 3.83c	29.23 ± 2.34b	44.50 ± 1.05c
M	12.91 ± 1.00d	35.85 ± 0.62a	202.41 ± 7.78b
L	146.65 ± 5.33a	20.20 ± 0.62c	242.16 ± 19.81a
CK	130.33 ± 9.26b	8.50 ± 0.81d	198.81 ± 8.97b

Notes: All data are presented as the mean ± SD (standard deviation). Different letters in the same column indicate significant differences between treatments at $P < 0.05$ among the means of the four treatments. H: high N application in the sugarcane soil (964 kg ha^{-1}), M: moderate N application in the sugarcane soil (482 kg ha^{-1}), L: low N application in the sugarcane soil (96 kg ha^{-1}), CK: no N application in the sugarcane soil (0 kg ha^{-1}).

3.3 Soil bacterial diversity and richness

In Table 3, the Shannon index was significantly higher only in the low nitrogen application treatment than in the other treatments. The Simpson index showed the opposite trend to the Shannon index, which was significantly lower than those of the other treatments. In addition, the Ace and Chao1 indexes, which were used as indicators of bacterial richness, in the N application treatments were all significantly higher than those in the CK. Moreover, the highest Ace and Chao1 indexes were all shown in the low N application treatment, which was significantly higher than all the other treatments. This result suggested that soil bacterial diversity and richness in sugarcane fields could all be improved by N application. In particular, the greatest effect was shown by the low N application.

Table 3
Indexes of soil bacterial diversity and richness in sugarcane fields under four N application treatments

Treatments	Shannon index	Simpson index	Ace index	Chao1 index	Coverage
H	6.19 ± 0.06b	0.0053 ± 0.0001a	2726.32 ± 276.81b	2697.06 ± 257.66b	0.98
M	6.19 ± 0.15b	0.0052 ± 0.0011a	2902.96 ± 279.16b	2856.06 ± 258.19b	0.98
L	6.64 ± 0.12a	0.0035 ± 0.0007b	3600.80 ± 36.77a	3621.92 ± 28.72a	0.98
CK	6.04 ± 0.09b	0.0059 ± 0.0007a	2133.27 ± 155.38c	2138.07 ± 159.11c	0.99

Notes: All data are presented as the mean ± SD (standard deviation). Different letters in the same column indicate significant differences between treatments at $P < 0.05$ among the means of the four treatments. H: high N application in the sugarcane soil (964 kg ha^{-1}), M: moderate N application in the sugarcane soil (482 kg ha^{-1}), L: low N application in the sugarcane soil (96 kg ha^{-1}), CK: no N application in the sugarcane soil (0 kg ha^{-1}).

3.4 Bacterial community structure and composition

As shown in Fig. 1, the bacterial community structure at the phylum level in the four N applications consisted of 12 bacterial phyla with relative abundances greater than 1%., *i.e.*, Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria, Gemmatimonadetes, Bacteroidetes, Planctomycetes, WPS-2, Patescibacteria, Firmicutes, Verrucomicrobia and others. However, their proportions in different N application treatments were quite different. Table 4 lists the compositions and relative proportions of the dominant soil bacterial at phylum level which were identified in this study. The numbers of identified bacterial phyla in the H, M, L and CK treatments were 11, 10, 9 and 9, respectively. All these results showed that the N applications not only changed the proportions of dominant soil bacterial phyla, but also altered the compositions of soil bacterial communities (Table 4).

All the above results indicate that Proteobacteria, Actinobacteria, Chloroflexi and Acidobacteria are the four most abundant soil bacterial phyla in sugarcane fields. Proteobacteria are easily enriched under high N application conditions, and Actinobacteria and Acidobacteria sensitively responded to low or moderate N applications. By contrast, Chloroflexi could be enriched in the soil of sugarcane fields without N application.

Notes: H: high N application in the sugarcane soil (964 kg ha^{-1}), M: moderate N application in the sugarcane soil (482 kg ha^{-1}), L: low N application in the sugarcane soil (96 kg ha^{-1}), CK: no N application in the sugarcane soil (0 kg ha^{-1}).

Table 4
The proportion of dominant bacterial communities at phylum level under four N application treatments (%)

Phylum	H	M	L	CK
Actinobacteria	28.54	32.57	25.15	22.65
Proteobacteria	29.44	25.52	23.33	25.10
Chloroflexi	15.31	21.48	24.26	26.16
Acidobacteria	13.62	10.56	15.98	12.71
WPS-2	1.59	1.93	-	4.58
Gemmatimonadetes	2.35	1.48	2.67	-
Planctomycetes	1.87	1.37	1.77	1.68
Bacteroidetes	1.95	1.01	1.20	1.91
Patescibacteria	1.41	-	-	1.21
Firmicutes	1.11	1.25	-	-
Verrucomicrobia	-	-	1.03	-
others	2.01	1.79	2.81	1.96

Notes: H: high N application in the sugarcane soil (964 kg ha^{-1}), M: moderate N application in the sugarcane soil (482 kg ha^{-1}), L: low N application in the sugarcane soil (96 kg ha^{-1}), CK: no N application in the sugarcane soil (0 kg ha^{-1}).

As shown in Fig. 2, at the genus level, there were 28, 22, 26 and 25 dominant bacterial genera with relative abundances greater than 1% in the high, moderate, low and no N application treatments, respectively. Compared to the CK, the dominant bacterial genera all increased in the high- or low-nitrogen treatments, but they decreased in the moderate-nitrogen application treatments. Meanwhile, there were special dominant bacterial genera in every N application treatment, *Mycobacterium*, *norank_f__SC-I-84*, *norank_f__norank_o__Saccharimonadales*, *norank_f__Micropepsaceae*, *norank_f__norank_o__Subgroup_2*and *norank_f__Acetobacteraceae* were the unique dominant genera in the H treatment. *norank_f__JG30-KF-CM45* and *Jatrophihabitans* were the unique dominant genera in the M treatment; *norank_f__norank_o__norank_c__Subgroup_6*, *HSB_OF53-F07*, *Streptomyces*, *norank_f__67−14*, *norank_f__norank_o__SBR1031* and *norank_f__norank_o__norank_c__KD4-96* were the unique dominant genera in the L treatment. *FCPS473*, *Actinospica*, *1921-2*, *Sinomonas* and *norank_f__Ktedonobacteraceae* were the unique dominant genera in the CK treatment. All the above results indicate that the soil bacterial community structure in sugarcane fields could be significantly affected by N input. In particularly, more sensitive effects are triggered by low or high N application (Table 5).

Notes: H: high N application in the sugarcane soil (964 kg ha^{-1}), M: moderate N application in the sugarcane soil (482 kg ha^{-1}), L: low N application in the sugarcane soil (96 kg ha^{-1}), CK: no N application in the sugarcane soil (0 kg ha^{-1}).

Table 5
The proportion of dominant bacterial communities at genus level under four N application treatments (%)

Genus	H	M	L	CK
Acidothermus	6.02	6.3	1.68	4.66
norank_f__norank_o__Gaiellales	5.25	4.76	4.49	2.73
norank_f__norank_o__norank_c__AD3	1.94	6.03	2.05	6.16
norank_f__Xanthobacteraceae	3.27	2.97	4.20	1.36
norank_f__norank_o__norank_c__TK10	1.89	2.94	4.78	2.12
norank_f__norank_o__Acidobacteriales	2.88	2.56	2.72	3.13
Bradyrhizobium	2.97	2.89	1.7	3.2
Conexibacter	1.33	3.55	1.37	3.35
norank_f__norank_o__norank_c__norank_p__WPS-2	1.59	1.93	-	4.58
norank_f__norank_o__Elsterales	2.38	2.38	1.73	1.83
norank_f__JG30-KF-AS9	3.34	2.25	-	2.1
norank_f__norank_o__norank_c__Subgroup_6	-	-	5.32	-
Candidatus_Solibacter	2.17	2.22	1.89	-
Sphingomonas	1.66	1.63	1.37	2.27
norank_f__norank_o__B12-WMSP1	-	-	1.08	4.58
Bryobacter	1.73	1.93	1.31	1.53
norank_f__norank_o__IMCC26256	1.41	1.56	1.75	-
Burkholderia-Caballeronia-Paraburkholderia	1.18	1.87	-	1.95
unclassified_f__Micromonosporaceae	1.58	1.54	1.47	-
norank_f__Gemmataceae	1.28	-	1.43	1.16
unclassified_f__Acidobacteriaceae_Subgroup_1	1.90	-	-	2.00
norank_f__norank_o__norank_c__JG30-KF-CM66	1.39	1.12	-	1.31
unclassified_f__Ktedonobacteraceae	-	-	1.02	2.16
norank_f__Gemmatimonadaceae	1.62	-	1.93	-

Note. H: high N application in the sugarcane soil (964 kg ha^{-1}), M: moderate N application in the sugarcane soil (482 kg ha^{-1}), L: low N application in the sugarcane soil (96 kg ha^{-1}), CK: no N application in the sugarcane soil (0 kg ha^{-1}).

Genus	H	M	L	CK
Acidibacter	1.18	1.11	-	-
norank_f__Roseiflexaceae	-	-	2.71	1.42
norank_f__JG30-KF-CM45	-	1.26	-	-
norank_f__SC-I-84	1.67	-	-	-
Gaiella	-	1.00	2.00	-
Mycobacterium	1.14	-	-	-
FCPS473	-	-	-	1.16
norank_f__Micropepsaceae	1.34	-	-	-
norank_f__norank_o__Saccharimonadales	1.13	-	-	-
Jatrophihabitans	-	1.28	-	-
HSB_OF53-F07	-	-	1.06	-
Actinospica	-	-	-	1.10
norank_f__norank_o__Subgroup_2	1.13	-	-	-
Streptomyces	-	-	1.08	-
1921-2	-	-	-	1.04
norank_f__67 − 14	-	-	1.13	-
Sinomonas	-	-	-	1.09
norank_f__Acetobacteraceae	1.04	-	-	-
norank_f__Ktedonobacteraceae	-	-	-	1.12
norank_f__norank_o__SBR1031	-	-	1.45	-
norank_f__norank_o__norank_c__KD4-96	-	-	1.32	-
others	33.69	29.41	36.51	30.75

Note. H: high N application in the sugarcane soil (964 kg ha^{-1}), M: moderate N application in the sugarcane soil (482 kg ha^{-1}), L: low N application in the sugarcane soil (96 kg ha^{-1}), CK: no N application in the sugarcane soil (0 kg ha^{-1}).

The number of bacteria obtained at the OTU (Operational Taxonomic Units) level under the H, M, L and CK treatments was 3237, 3318, 3923 and 2576, respectively. The numbers of unique bacteria in the H, M, L and CK treatments at the OTU level were 222, 152, 852 and 254, respectively (Fig. 3A). In addition, the numbers of bacteria in the H, M, L and CK treatments at the genus level were 587, 588, 631 and 508, respectively. Moreover, the numbers of unique bacteria in the H, M, L and CK treatments at the genus level

were 18, 4, 59 and 11, respectively (Fig. 3B). All the above results suggested that the soil bacterial community structure could be significantly altered by nitrogen application. However, higher nitrogen input (964 kg ha^{-1} and 482 kg ha^{-1}) was not helpful for improving the number of unique soil bacteria in sugarcane fields. On the contrary, low nitrogen application (96 kg ha^{-1}) was more efficient for improving soil bacterial diversity and richness in sugarcane fields.

Note H: high N application in the sugarcane soil (964 kg ha^{-1}), M: moderate N application in the sugarcane soil (482 kg ha^{-1}), L: low N application in the sugarcane soil (96 kg ha^{-1}), CK: no N application in the sugarcane soil (0 kg ha^{-1}).

The top 50 most abundant soil bacteria at the genus level in sugarcane fields under different nitrogen applications were selected to form the heat map (Fig. 4). The horizontal level represents the different treatments, and the longitudinal direction shows the abundance of bacterial species. As seen in Fig. 4, the distribution of soil dominant bacteria under low, high and moderate nitrogen applications was different from the distribution under the CK, and there was also a difference between each treatment. However, the distribution of soil dominant bacteria was quite similar between the CK and high or moderate nitrogen application treatments. However, the composition and abundance of dominant soil bacteria under low nitrogen application changed significantly between CK. This phenomenon indicates that the response of the soil bacterial community structure to nitrogen application is more sensitive to low nitrogen input at 96 kg ha^{-1}.

Note A color zone represents relative abundance. H: high N application in the sugarcane soil (964 kg ha^{-1}), M: moderate N application in the sugarcane soil (482 kg ha^{-1}), L: low N application in the sugarcane soil (96 kg ha^{-1}), CK: no N application in the sugarcane soil (0 kg ha^{-1}).

3.5 Principal Component Analysis

As seen in Fig. 5, the contribution rates of the first and second principal components (PC1 and PC2) were 40.75% and 19.04%, respectively. In addition, low and moderate nitrogen applications were distributed mainly in the positive direction of PC1, but high and nonnitrogen applications were found primarily in the negative direction of PC1. Meanwhile, low, moderate and nonnitrogen applications were distributed mainly in the positive direction of PC2, and only high nitrogen application was found in the negative direction of PC2. Moreover, only the low nitrogen application treatment was located on the first quadrant, which suggested that low nitrogen application was positively correlated with the first and second principal components.

Note H: high N application in the sugarcane soil (964 kg ha^{-1}), M: moderate N application in the sugarcane soil (482 kg ha^{-1}), L: low N application in the sugarcane soil (96 kg ha^{-1}), CK: no N application in the sugarcane soil (0 kg ha^{-1}).

4. Discussion

Guangxi is the main producing area for sugarcane in China, and more than 60% of sugar is produced in Guangxi (Li & Yang, 2015). However, overuse of chemical fertilizer and a low utilization rate of fertilizer remain the principal problems in Guangxi sugarcane production (Deng et al., 2017).

Soil is a habitable zone for numerous microorganisms, which play a dominant role in promoting terrestrial biogeochemical cycles such as the N cycle (Madsen, 2011). Microorganisms play a prominent role in agricultural ecosystems, and with the gradual recognition of people, the effect of N fertilizer on soil microorganisms has received increasing attention (Zhou et al., 2017b; Wang et al., 2018). Numerous studies have found that long-term application of N fertilizer could significantly change soil microbial composition and decrease microbial biomass, resulting in loss of microbial diversity (Wang et al., 2018). A study also found that the addition of N to black soil could enrich Proteobacteria and dilute Acidobacteria and Nitrospirae in northeast China (Zhou et al., 2017a). Pan et al. (2014) found that the application of N fertilizer to the soil in grasslands would lead to excessive Actinobacteria. Many studies have shown that soil acidification is an important way for N fertilizer to change the soil microbial community (Sun et al., 2015b; Zhang et al., 2015; Zhou et al., 2017a), although other soil changes such as soil organic carbon (SOC) and available N also affect the soil microbial community (Luo et al., 2014; Zhou et al., 2017b; Guo et al., 2019). Liu et al. (2020) found that N fertilizers decreased the population of microbial N fixers (Sun et al., 2015), thus weakening the soil biological N fixation capacity (Xie et al., 2015; Liu et al., 2020) and decreasing the ecological integrity of agroecosystems (Crews & Peoples, 2004).

First, soil microbial diversity, soil enzyme activity and crop yield may be affected by land management measures (Carney et al., 2004; Kaye et al., 2005; Acosta-Martínez et al., 2010). Soil enzymes play key biochemical functions throughout the decomposition of organic matter in the soil system (Ellert et al., 1997), not only catalyzing microbial life processes in soil and stabilizing soil structure, decomposing organic waste, forming organic matter and cycling nutrients (Dick et al., 1994) but also maintaining soil ecological physicochemical properties and soil health.

In our study, the activities of β-glucosidase and phosphatase in the sugarcane field under high N application (H) were all significantly higher than the activities of β-glucosidase and phosphatase in CK. However, the activity of aminopeptidase in all N application treatments was significantly lower than the activity of aminopeptidase in CK. In contrast, the activities of β-glucosidase and phosphatase in the sugarcane field under moderate N application were not significantly different between CKs. However, except for the activities of β-glucosidase and aminopeptidase, the activity of acid phosphatase in the sugarcane field under low N stress (L) was significantly higher than the activity of acid phosphatase of CK. These results suggest that the activities of soil enzymes were sensitively affected by N application, but the activities of soil enzymes were not higher with higher N input. In contrast, the activity of some enzymes such as acid phosphatase was also significantly improved by low N application.

Soil microbial biomass is an important indicator of soil quality to maintain soil fertility and crop productivity (Powlson et al., 1987). The greater the microbial biomass in the soil, the greater is the

capacity of the soil to provide nutrients to plants through mineralization of organic nutrients (Dwivedi & Soni, 2011). Among these organic nutrients, soil microbial biomass carbon can not only promote the formation of new humus with high activity in soil but also reflect the slight change in the soil before the change in soil total carbon content (Doran et al., 1996). Soil microbial biomass N can also reflect the availability of soil N and play an important role in the supply and circulation of soil N (Doran et al., 1996). Soil microbial biomass phosphorus can reflect the supply level of soil phosphorus (Kwabiah et al., 2003). In addition, although soil microbial biomass phosphorus cannot be directly absorbed and utilized by plants, it can be slowly released as inorganic phosphorus, so it has always been considered the source of available phosphorus in the soil, which is very important for plant growth (Khan & Joergensen, 2009).

The soil microbial biomass C and P in the sugarcane field under high N application (H) were significantly decreased, but the microbial biomass N was only significantly increased compared with CK. In the moderate N application treatment, only the microbial biomass N increased, but the microbial biomass C was significantly decreased, and there was no significant difference in soil microbial biomass P between CK and the moderate N application. However, in contrast to CK, the soil microbial biomass C, N and P in the sugarcane field under low N application (L) were all significantly increased. This result indicated that low N application (96 kg ha^{-1}) is more effective in improving soil fertility than other N applications (964 kg ha^{-1} and 482 kg ha^{-1}) in sugarcane fields.

Soil bacterial diversity and richness were also triggered by N application, but only low N application (96 kg ha^{-1}) showed a significant effect on the soil bacterial diversity and richness. In addition, *FCPS473*, *Actinospica*, *1921-2*, *Sinomonas* and *norank_f__Ktedonobacteraceae* were the unique dominant bacterial genera in CK. In contrast to CK, *norank_f__SC-I-84*, *Mycobacterium*, *norank_f__Micropepsaceae*, *norank_f__norank_o__Saccharimonadales*, *norank_f__norank_o__Subgroup_2* and *norank_f__Acetobacteraceae* were the unique dominant soil bacterial genera in the sugarcane field under high N application. *norank_f__JG30-KF-CM45* and *Jatrophihabitan; norank_f__norank_o__norank_c__Subgroup_6*, *HSB_OF53-F07*, *Streptomyces*, *norank_f__67 − 14*, *norank_f__norank_o__SBR1031* and *norank_f__norank_o__norank_c__KD4-96* were the unique dominant soil bacterial genera under moderate and low N applications, respectively.

According to the heat map, we also found that the distribution of soil dominant bacteria under low, high and moderate N application were all different from the CK, and the distribution was also different between each treatment. All the above results indicate that soil bacteria can sensitively respond to changes in N contents in soil.

5. Conclusion

In conclusion, in contrast to the moderate (482 kg ha^{-1}) or high N (964 kg ha^{-1}) applications, low N application (96 kg ha^{-1}) had the most significant effect on soil fertility by improving the activity of soil enzymes and soil biomass C, N and P. Moreover, soil health, which is represented by soil biological indicators such as bacterial diversity and richness were improved by low N input under 96 kg ha^{-1}

application in sugarcane fields. This result suggested that soil fertility and health in sugarcane fields could be improved or maintained by the application of 96 kg ha^{-1} of N compared to the applications of 964 kg ha^{-1} and 482 kg ha^{-1} of N.

Declarations

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Author Contribution Conceptualization: Shangdong Yang, Hongwei Tan; Methodology: Jian Xiao, Shangdong Yang; Formal analysis and investigation: Tian Liang, Weizhong He; Writing - original draft preparation: Jian Xiao, Shangdong Yang; Writing - review and editing: Shangdong Yang, Hongwei Tan; Funding acquisition: Hongwei Tan.

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Data Availability Raw reads during the current study are deposited in the NCBI Sequence Read Archive (SRA) database (Accession Number: SRP302628).

Ethics Approval Not applicable.

Conflict of Interest The authors declare no competing interests.

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Figures

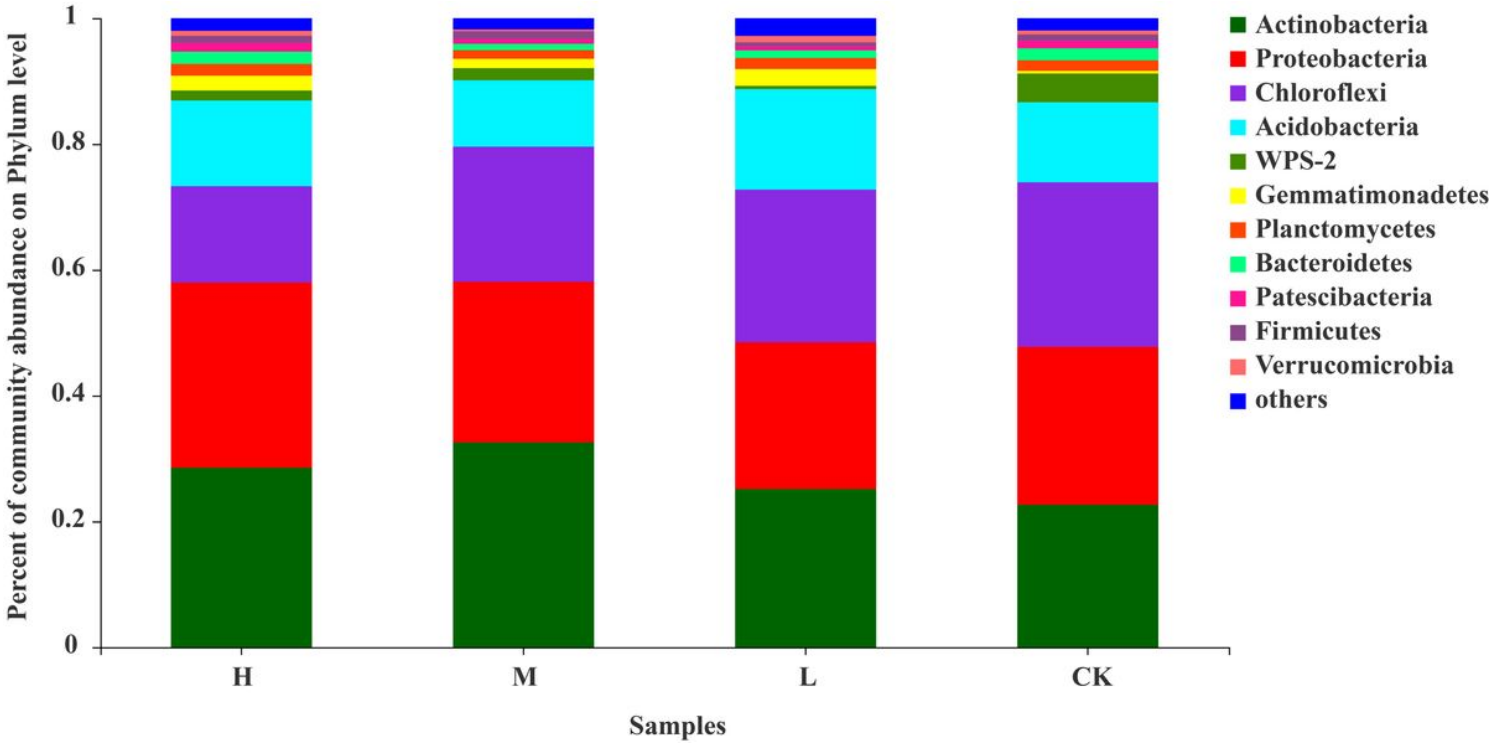

Figure 1

Compositions of soil bacterial communities at phylum level under four N application treatments. Notes: H: high N application in the sugarcane soil (964 kg ha-1), M: moderate N application in the sugarcane soil

(482 kg ha-1), L: low N application in the sugarcane soil (96 kg ha-1), CK: no N application in the sugarcane soil (0 kg ha-1).

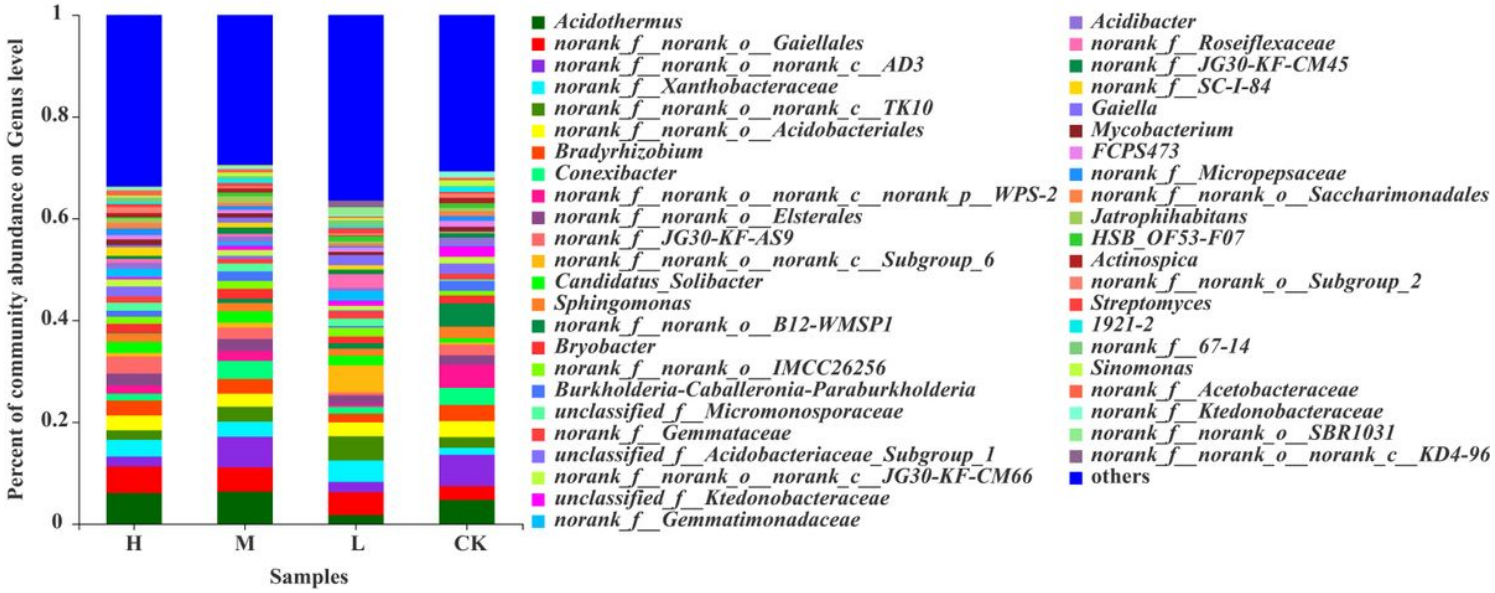

Figure 2

Compositions of soil bacterial communities at genus level under four N application treatments. Notes: H: high N application in the sugarcane soil (964 kg ha-1), M: moderate N application in the sugarcane soil (482 kg ha-1), L: low N application in the sugarcane soil (96 kg ha-1), CK: no N application in the sugarcane soil (0 kg ha-1).

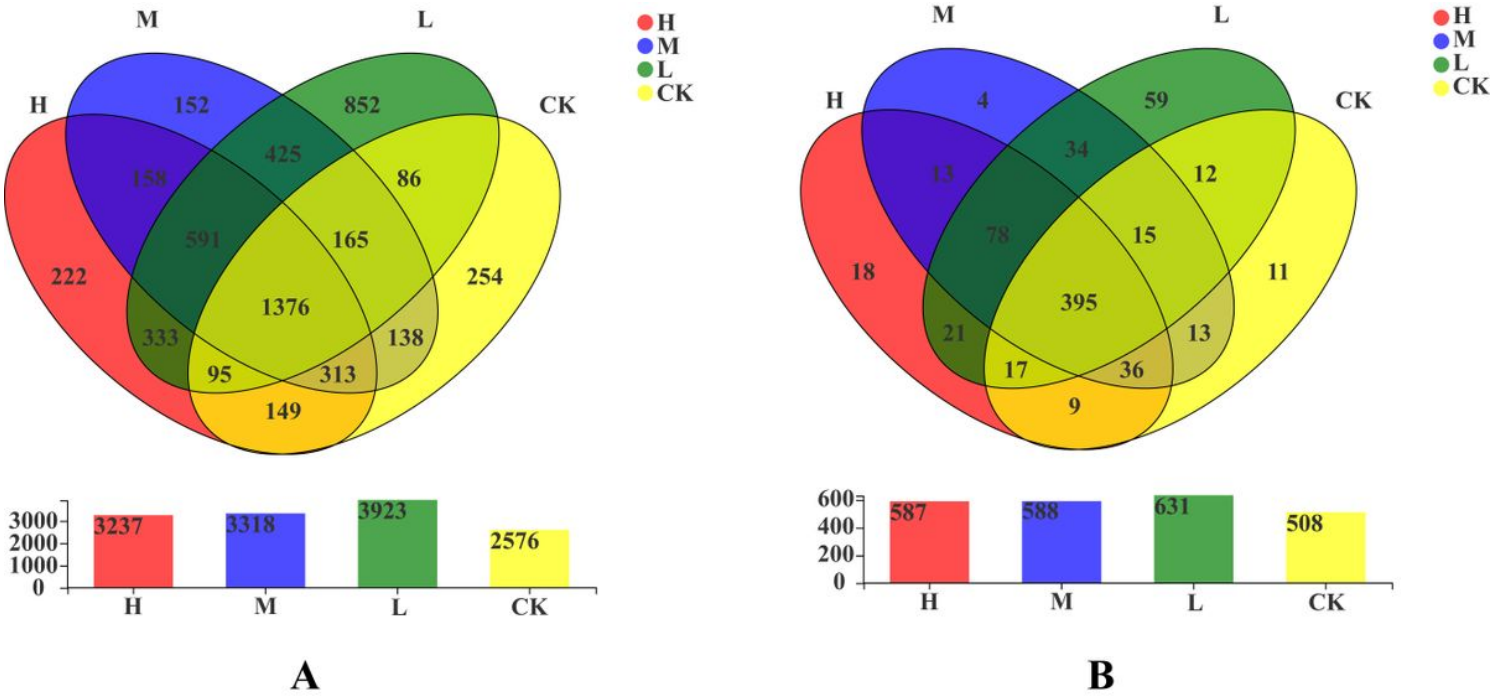

Figure 3

Venn analysis of soil bacteria in sugarcane fields under four N application treatments at the OTU (A) and genus (B) levels

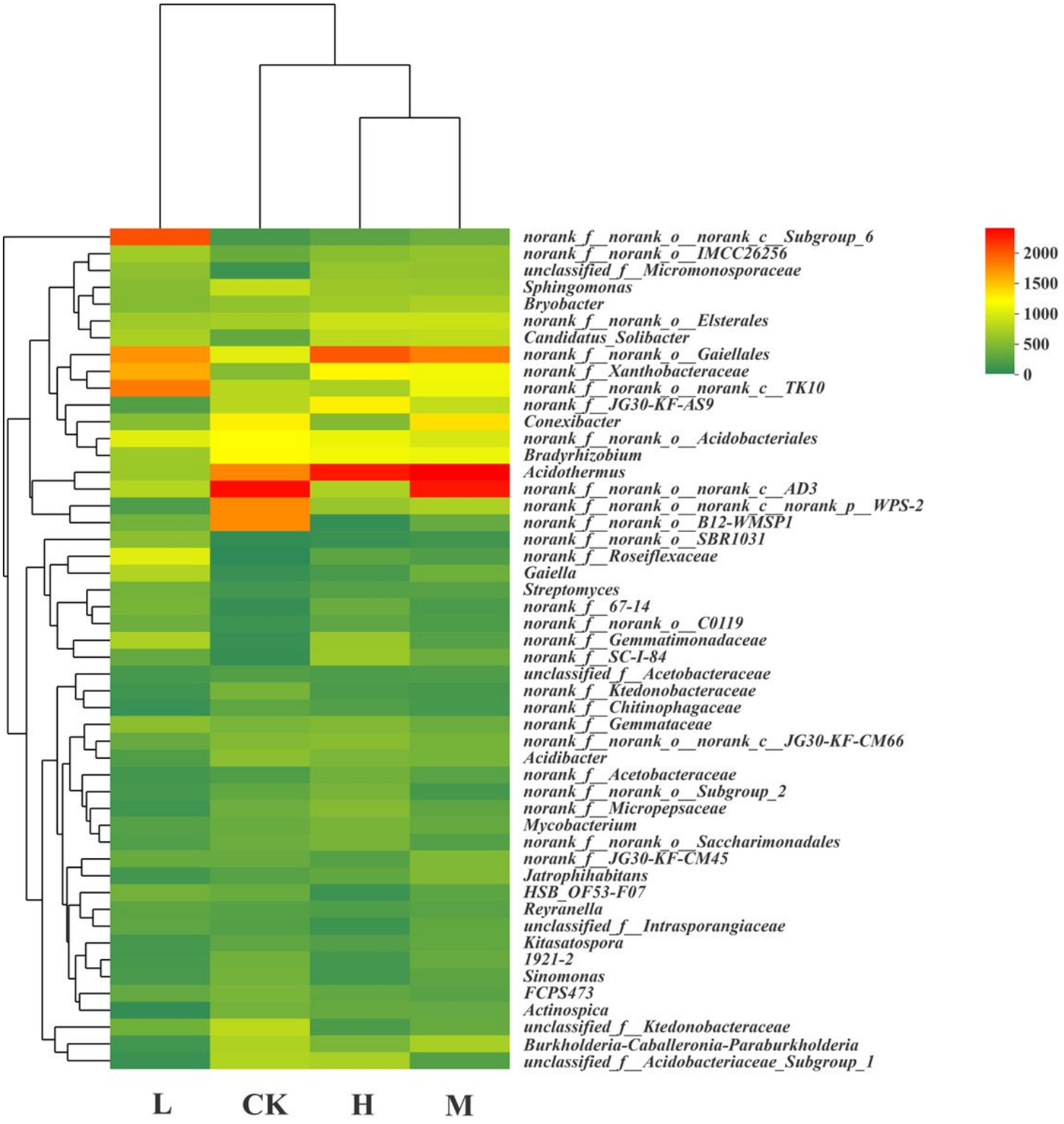

Figure 4

Heatmap of four N application treatments with three replicates based on the relative abundances of the top 50 most abundant genera

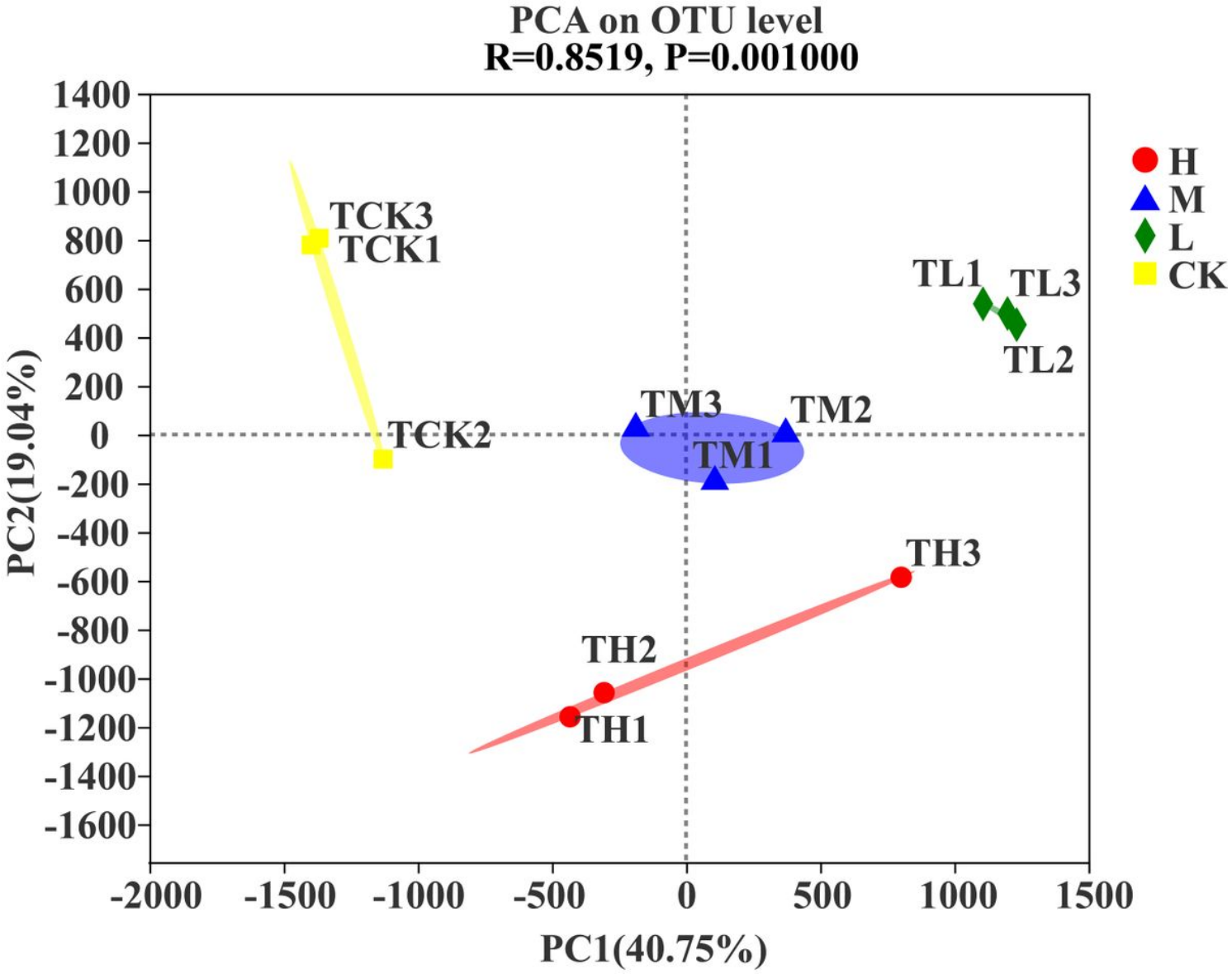

Figure 5

Principal component analysis of the relative abundance of soil bacteria at the OTU level in sugarcane fields under four N application treatments