Nitrogen Application Increases Soil Organic Carbon And Crop Productivity By Altering Soil Autotrophic Bacterial Community On the Semiarid Loess Plateau

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Research Article

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Abstract

Background and aims Soil autotrophic microorganisms play crucial roles in carbon fixation and crop productivity. However, the information is remains limited to whether and how soil autotrophic microbe mediate SOC pool dynamics and crop productivity.

Methods Here, we investigated the effects of the structure and co-occurrence network of soil autotrophic bacterial community on SOC storage and maize yield. A long-term field experiment was conducted with four chemical nitrogen (N) application rates on the semi-arid Loess Plateau, including N application at 0 kg ha\(^{-1}\) (N0), 100 kg ha\(^{-1}\) (N1), 200 kg ha\(^{-1}\) (N2), and 300 kg ha\(^{-1}\) (N3), respectively.

Results Our results showed that the N application significantly impacted the structure and co-occurrence network of soil \textit{cbbL}-carrying bacterial community via changing soil pH, nitrate nitrogen (NO\(_3\)-N), and soil water content (SWC). The diversity of soil autotrophic bacterial community decreased with the increasing rate of N application. We detected a high abundance of the autotrophic bacterial dominant genera \textit{Xanthobacter}, \textit{Bradyrhizobium}, \textit{Aminobacter}, and \textit{Nitrosospira}. The co-occurrence network of autotrophic bacteria contained four distinct modules that consisted of closely interconnected microorganisms. Structural equation modeling further indicated that the diversity, composition, and network of autotrophic bacterial community had significant relationships with SOC storage and maize yield.

Conclusion Taken together, our results highlight that the soil autotrophic bacterial community may drive carbon fixation SOC accumulation and contribute positively to crop productivity.

Introduction

Soil organic carbon (SOC) is a key predictor for soil quality and crop production (Wiesmeier et al. 2019). Given that soil organic carbon is closely related to crop yield, agricultural system productivity can be enhanced by increasing soil organic carbon (Yuan et al. 2021a). Agricultural management regimes can drive soil microbial community structure to improve SOC storage through a serious of biochemical reactions, including adding soil organic matter to soils (Jiang et al. 2018; Berhane et al. 2020), reducing the decomposition rate of organic matter (Zang et al. 2017), and the combination of these measures. Hitherto, the question remains how the biological mechanisms of soil microbial community regulating SOC dynamics and crop productivity.

Soil autotrophic bacterial community plays crucial roles in mediating SOC dynamics through catabolism and anabolism (Wang et al. 2021b; Zheng et al. 2021), and substantially influences soil sustainability and plant productivity (Qaswar et al. 2020). Soil autotrophic bacteria have developed six pathways of carbon dioxide (CO\(_2\)) fixation in different terrestrial ecosystems (Berg 2011), the Calvin-Benson-Bassham (CBB) cycle is the dominant pathway (Yuan et al. 2012a; Qin et al. 2021). In the Calvin cycle, Ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) is responsible for catalyzing the first rate-limiting step of autotrophic CO\(_2\) fixation (Selesi et al. 2005; Yuan et al. 2012a). To date, four RubisCO forms (forms I–
IV) have been found to be different in structure, catalytic property, and O$_2$ sensitivity, and form I of RubisCO is the most abundant among the four forms. The $cbbL$ gene, which encodes a large subunit of RubisCO I, has been often used as a phylogenetic marker to investigate the autotrophic bacterial community (Kovaleva et al. 2011). A growing body of evidence has supported that fertilization regimes, tillage, and mulching practices have strong impacts on the soil autotrophic bacterial community and enzyme activities through altering SOC, pH, bulk density, and available phosphorus (Yuan et al. 2012b; Lu et al. 2019; Liao et al. 2020; Wang et al. 2020). However, relatively few studies have sought to explore how agronomic practices alter the structure and network of the soil autotrophic bacterial community.

Nitrogen (N) is the important limiting element for plant growth in farmland ecosystem (LeBauer and Treseder 2008). The long-term excess chemical fertilizer leads to decline soil quality, nutrient balance and crop productivity (Shen et al. 2010; Miao et al. 2011; Agegnehu et al. 2016). Appropriate N application can not only improve N utilization efficiency and reduce the waste of fertilizer resources (Zhang et al. 2015; Wang et al. 2019), but also promote SOC and crop productivity via regulating soil autotrophic bacterial community (Liao et al. 2020; Wang et al. 2021a). However, the information remains limited regarding how the soil autotrophic bacterial community influence SOC storage and crop productivity under long-term chemical N fertilization.

Here, we evaluated the importance of soil autotrophic bacterial community on SOC and crop productivity under field conditions. We performed an 8-year field experiment with four N application rate on the semiarid Loess Plateau. We asked the following two questions: (1) How do the composition and co-occurrence network of soil autotrophic bacterial community response to the N treatments? (2) How does the soil autotrophic bacterial community drive SOC dynamics and crop productivity? We hypothesized that the N treatments significantly changed the composition and co-occurrence network of soil autotrophic bacterial community. Furthermore, we expected that soil properties and the autotrophic bacterial community jointly mediated SOC dynamics and crop productivity.

**Materials And Methods**

Field experiment description

The long-term fertilization experiment was conducted at the Rainfed Agricultural Experimental Station of Gansu Agricultural University (35°28’N, 104°44’E) in Gansu province, China. The experiment site located in a warm temperate zone with a continental monsoon climate, with mean annual temperature of 6.4°C and mean annual precipitation of 390 mm. The soil is classified as Calcaric Cambisol according to the Food and Agricultural Organization (FAO) classification system. The long-term field experiment followed a completely randomized design with four nitrogen application rates (three replicates) at 0 kg ha$^{-1}$ (N0), 100 kg ha$^{-1}$ (N1), 200 kg ha$^{-1}$ (N2), and 300 kg ha$^{-1}$ (N3), respectively. The experiment was started in 2012, which consisted of 12 plots. Nitrogen fertilizer was applied as urea (46% N) in two splits: one-third was broadcast on the soil surface and incorporated by moldboard plowing to soil and the remaining two-
thirds was applied at jointing stage of maize. Triple superphosphate (P$_2$O$_5$ 16%) was applied at 150 kg P$_2$O$_5$ ha$^{-1}$, and was evenly broadcast on the soil surface of all plots.

The experimental plots were 18.7 m$^2$ (4.25 m length and 4.4 m width) and consisted of narrow ridges (15 cm height and 40 cm width) alternated with wide ridges (10 cm height and 70 cm width). All ridges were covered by the plastic film (0.01 mm thickness and 140 cm width). Maize monoculture (cv. Pioneer 335) was planted annually from April to October, with a density of 52,500 plants ha$^{-1}$. No management regimes were adopted, expect for weeding by hand.

Soil sampling and soil properties assays

Soil samples from each plot were collected at the flowering stage in early August 2019. In each plot, 10 soil cores were collected from the surface layer (0–20 cm) using a Dutch auger (5 cm diameter), and mixed to form a composite sample. After field collection, fresh samples were placed on ice and immediately transported to the laboratory, and then were sieved (2 mm) to remove visible residues. Soil samples were homogenized to analyze soil chemical properties, microbial biomass carbon, soil autotrophic bacterial community, and RubisCO activity.

Soil pH was measured by a pH meter (Mettler Toledo FE20, Shanghai, China) with water: soil ratio of 2.5:1 (v/w). SOC was determined by a modified Walkley–Black wet oxidation method (Nelson and Sommers 1983). TN was determined using the micro–Kjeldahl method (Sparks et al. 1996). NO$_3$–N and NH$_4$–N were extracted with 2 M KCl and measured using a continuous flow analyzer (Skalar, Breda, Netherlands). Available phosphorus (AP) was extracted with sodium bicarbonate and measured using the molybdenum–blue method (Olsen 1954). Dissolved organic carbon (DOC) was determined by the method of Jones and Willett (2006). Microbial biomass carbon (MBC) was determined by the CHCl$_3$ fumigation extraction (Vance et al. 1987). Soil water content (SWC) was measured using the oven-dry method (O’Kelly 2004).

Crop yield and RubisCO activity

All maize plants were manually harvested at the maturity. After harvest, the air-dried grain in each plot was weighted to calculate grain yield. The aboveground biomass was determined on a dry-weight basis by oven drying the crop samples at 105°C for 45 min and subsequently to constant weight at 85°C (Xie et al. 2020).

Soil RubisCO activity was determined by the method described by Yuan et al. (2012b). Briefly, 2 g of freeze-dried soil sample was placed in centrifuge tubes and suspended in protein extractant containing Tris–HCl buffer and dithiothreitol. The soil suspension was disrupted by ultrasonication in ice bath. After centrifuged, the supernatant was amended with solid ammonium sulfate to reach 80% saturation, and then stirred for 30 min and centrifuged at 4°C. The precipitate was dissolved to determine RubisCO activity. The absorbance of reaction mix was measured at 340 nm wavelength using a
spectrophotometer (UV−2450, Shimadzu, Japan). All reactions were carried out in triplicate, and negative controls were set up. The RubisCO activity was expressed as nmol CO$_2$ g$^{-1}$ soil min$^{-1}$.

The $cbbL$ gene copy number and Illumina sequencing

Total genomic DNA was extracted from soil samples using the EZNA. Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to manufacturer's protocols. The quality of DNA was determined by 1% agarose gel electrophoresis, and the concentration and purity were detected using a NanoDrop−2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). DNA samples were stored at −80°C for subsequent analysis.

The copy number of $cbbL$ gene was determined by quantitative PCR using the primers K2f (5’-ACCAYCAAGCCSAAGCTSGG-3’) and V2r (5’-GCCTTCSAGCTTGCCSACCRC-3’) (Tolli and King 2005). The reactions were performed in triplicate 20 µL mixture containing 4 µL of 5 × FastPfu Buffer, 2 µL of 2.5 mM dNTPs, 0.8 µL of each primer (5 µM), 0.4 µL of FastPfu Polymerase, and 10 ng of template DNA. The PCR parameters were as follows: a pre-denaturation 95°C for 2 min, followed by 25 cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 5 min. The standard curve was obtained using a 102 to 108 dilution of plasmid DNA carrying the $cbbL$ gene fragment. Melting curve analysis was performed at the end of the PCR amplification to check the specificity of amplification. The $cbbL$ copy number was calculated according to the standard curve.

Purified PCR products were quantified by Qubit3.0 (Life Invitrogen) and mixed equally. The amplicon library was paired-end sequenced (2 × 300) on an Illumina platform. Raw data were extracted, trimmed, and quality screened using the Quantitative Insights Into Microbial Ecology (QIIME, v1.8.0) (Caporaso et al. 2010). Briefly, the low-quality sequencing reads with length <150, with an average Phred scores <20, with ambiguous bases in barcodes and with mononucleotide repeats greater than 8 bp were filtered out. The paired reads from the original bacterial DNA fragments were merged using FLASH (version 1.2.7). After chimera detection, sequence analyses were performed with the UPARSE (version 7.1) software package, and the operational taxonomic unit (OTU) was clustered at 97% sequence identity by UCLUST (Edgar 2013). Alpha-diversity (Chao1 richness and Shannon index) of the autotrophic bacterial community was calculated using MOTHUR software (Schloss et al. 2009), after rarefying all samples to an equal number of 24,138 reads. We have deposited sequencing reads in the Sequence Read Archive at National Center for Biotechnology Information (NCBI) under the accession number PRJNA773586.

Statistical analyses

The analysis of variance was analyzed by Tukey's honest significance test at $P \leq 0.05$ using SPSS 22.0 (IBM SPSS, USA). Pearson's correlation coefficients were used to assess linear relationships among soil properties, the abundance and composition of soil autotrophic bacterial community, RubisCO activity, DOC, MBC, and crop yield across N treatments. Principal coordinate analysis (PCoA) was used to evaluate the Bray-Curtis distances of soil autotrophic bacterial community compositions using the 'vegan' package in R software.
To describe the potential co-occurrence patterns, the autotrophic bacterial network was constructed using the Spearman's correlation and Kullback-Leibler dissimilarity (Faust et al. 2012). The OTUs more than five-sixths of soil samples were kept for network construction. A valid co-occurrence was considered a statistically robust correlation between taxa when the correlation coefficient ($r$) was $> 0.6$ or $< -0.6$ and the $P$ value was $< 0.05$. The co-occurrence network visualization was conducted using Gephi software (Bastian et al. 2009), and the modules were defined as clusters of closely associated nodes (i.e., groups of co-occurring microbes) (Layeghifard et al. 2017).

Random forest tool was used to assess the important predictors of RubisCO activity and maize yield. Random Forest modeling was conducted using the ‘RandomForest’ package (Bento et al. 2002), and the ‘rfPermute’ package was used to determine the model significance and predictor importance (Archer, 2016). The significant predictors in the random forest analyses were further chosen to perform structural equation modeling (SEM) analysis. SEM analysis was performed to estimate the direct and indirect contributions of soil properties and the autotrophic bacterial community to RubisCO activity and maize yield. SEM analysis performed by the robust maximum likelihood evaluation using AMOS 22.0 (SPSS, Chicago, IL, USA). The model fitness was determined according to chi-square test ($P > 0.05$), goodness of fit value, and root mean square error of approximation (Hooper et al. 2008).

Results

Soil properties, MBC, grain yield, and Rubisco activity

One-way analysis of variance showed that the N treatments have significant effects on soil properties ($P < 0.05$, Table S1). TN and NO$_3$–N were significantly ($P < 0.05$) increased with the increasing rates of N application, while soil pH and SWC exhibited an opposite trend ($P < 0.05$). However, no significant difference was found in SOC ($P > 0.05$), NH$_4$–N ($P > 0.05$), and AP ($P > 0.05$) among three N treatments (N1, N2, and N3). DOC and MBC were enhanced by the N application (Fig. S1), with the significantly ($P < 0.05$) higher values under the N3 treatment than under the N1 and N0 treatments. The N2 and N3 treatments were characterized by significantly ($P < 0.05$) higher grain yield and aboveground biomass than the N1 and N0 treatments (Fig. S1), as well as RubisCO activity ($P < 0.05$, Fig. 1).

Abundance and structure of soil autotrophic bacterial community

The abundance of autotrophic bacteria indicated by the copy number of $cbbL$ gene ranged from $0.82 \times 10^6$ to $2.78 \times 10^6$ copies g$^{-1}$ soil. The clear differences were observed among treatments ($P < 0.05$), with the highest abundance of $cbbL$ gene under the N3 treatment (Fig. 1). A total of 289,656 sequences of soil autotrophic bacterial community were obtained using Illumina sequencing after quality control. The diversity of autotrophic bacteria indicated by Chao 1 richness was significantly higher under the N0 and N1 treatments than under the N2 treatment, with intermediary value under the N3 treatment ($P < 0.05$, Fig. 1). However, there was no significant difference in Shannon index among the four treatments ($P = 0.10$, Fig. 1).
Across all samples, the autotrophic bacterial community was dominated by Alphaproteobacteria (32.4%), Betaproteobacteria (10.7%), and Actinobacteria (10.4%) (Fig. 2). The dominant communities were mainly affiliated with facultative autotrophic bacteria within the genera *Xanthobacter* (10.8%), *Bradyrhizobium* (10.1%), *Aminobacter* (5.2%), *Nitrosospira* (6.1%) and, *Mycobacterium* (2.9%), followed by the rare genera *Nocardia* (1.9%), *Oscillochloris* (1.8%), *Sphingomonas* (1.5%), and *Saccharomonospora* (1.3%), (Fig. 2). Principal coordinates analysis indicated that the composition of autotrophic bacterial community under the N2 and N3 treatments exhibited a significant ($P < 0.05$) separation from that under the N1 and N0 treatments (Fig. S2). The relative abundance of *Xanthobacter* under the N3 treatment was significantly ($P < 0.05$) higher than those under the N0, N1, and N2 treatments, while *Nocardia* and *Saccharomonospora* under the N1, N2, and N3 treatments were significantly ($P < 0.05$) lower than that under the N0 treatment (Fig. 2).

The abundance and community composition of autotrophic bacteria were positively correlated with NO$_3$–N ($r = 0.57$, $P < 0.05$ and $r = 0.79$, $P < 0.01$), but negatively correlated with pH ($r = -0.57$, $P < 0.05$ and $r = -0.75$, $P < 0.01$) and SWC ($r = -0.62$, $P < 0.05$ and $r = -0.83$, $P < 0.001$) (Fig. 5). The abundance and community composition of autotrophic bacteria had positive correlations with RubisCO activity ($r = 0.58$, $P < 0.05$ and $r = 0.91$, $P < 0.001$), SOC ($r = 0.63$, $P < 0.05$ and $r = 0.75$, $P < 0.01$), DOC ($r = 0.90$, $P < 0.001$ and $r = 0.73$, $P < 0.01$), as well as maize yield ($r = 0.65$, $P < 0.05$ and $r = 0.94$, $P < 0.001$), and aboveground biomass ($r = 0.61$, $P < 0.05$ and $r = 0.91$, $P < 0.001$) (Fig. 5). In contrast, the autotrophic bacterial diversity showed negative correlations with TN ($r = -0.74$, $P < 0.01$), NO$_3$–N ($r = -0.70$, $P < 0.05$), RubisCO activity ($r = -0.57$, $P < 0.05$), DOC ($r = -0.69$, $P < 0.05$), maize yield ($r = -0.71$, $P < 0.05$), and aboveground biomass ($r = -0.73$, $P < 0.01$).

The autotrophic bacterial co-occurrence networks

Co-occurrence networks were constructed to examine the different co-occurrence patterns of the soil autotrophic bacterial community under the four treatments (Fig. 3). In total, there were 367 nodes, 1956 links, and four distinct modules (modules I, II, III, and VI) in the autotrophic bacterial network. In the autotrophic bacterial network, the number of positive correlations (1953 edges) were greater than that of the negative correlations (3 edges). The modules I, II, III, and VI in the bacterial networks consisted of 117, 72, 99, and 79 nodes, respectively. At the phylum level, the relative abundance of Alphaproteobacteria was significantly ($P < 0.05$) higher in modules II and VI than in modules I and III, while those of Betaproteobacteria, Actinobacteria, and Chloroflexi followed the opposite trend (Fig. 4). At the genus level, modules I and II showed the significantly ($P < 0.05$) greater abundances of *Aminobacter* and *Bradyrhizobium*, but lower abundances of *Mycobacterium*, *Nitrosospira*, and *Saccharomonospora* than module III. In contrast, *Xanthobacter* was significantly greater in module VI than in modules I and III. Module VI was positively correlated with TN ($r = 0.77$, $P < 0.01$) and NO$_3$–N ($r = 0.89$, $P < 0.001$), but negatively correlated with pH ($r = -0.71$, $P < 0.01$) (Fig. 5). Furthermore, module VI had positive correlations with RubisCO activity ($r = 0.77$, $P < 0.01$), SOC ($r = 0.63$, $P < 0.05$), DOC ($r = 0.59$, $P < 0.05$), MBC ($r = 0.85$, $P < 0.001$), grain yield ($r = 0.72$, $P < 0.01$), and aboveground biomass ($r = 0.71$, $P < 0.05$) (Fig. 5).
Soil properties and autotrophic bacterial community affected RubisCO activity and maize yield

Random forest modeling revealed that pH (7.0% and 6.3%, \( P < 0.05 \)), \( \text{NO}_3^- \)N (5.8% and 5.8%, \( P < 0.05 \)), and SWC (10.2% and 9.8%, \( P < 0.01 \)) were the primary predictors among soil properties for RubisCO activity and maize yield (Fig. 6). As for the autotrophic bacteria, RubisCO activity and maize yield were significantly affected by the composition (11.5% and 11.7%, \( P < 0.01 \)), diversity (6.5% and 7.6%, \( P < 0.05 \)), and module VI in the network (5.4% and 6.0%, \( P < 0.05 \)) of the autotrophic bacterial community. Structural equation modeling (SEM) further suggested that soil properties were significantly \( (P < 0.01) \) correlated with the autotrophic bacterial community (Fig. 6). Importantly, soil autotrophic bacterial community might significantly \( (P < 0.05) \) affect RubisCO activity through composition, diversity, and module VI, and profoundly impact SOC storage and maize yield.

Discussion

Nitrogen application affected soil autotrophic bacterial community

Our results showed that N fertilizer application led to great changes in soil properties, and consequently strongly impacted the abundance, diversity, composition community, and co-occurrence network of soil autotrophic bacterial community. The abundance of \( cbbL \) gene was significantly increased with the increasing application rate of N fertilization, with the highest value under the N3 treatment. Increasing \( cbbL \)-containing bacterial abundance has been found to be positively correlated with microbial CO\(_2\) fixation rate (Yuan et al. 2012b; Wu et al. 2015). Observations of the large abundance were probably derived from the high level of TN and available N (\( \text{NO}_3^- \)N and \( \text{NH}_4^- \)N), and pH closer to neutral under the N3 treatments. Soil pH has been evidenced as the main factor influenced the microbial community structure (Jiang et al. 2016; Ramírez et al. 2020). Soil pH closer to neutral is significantly related to high nutrients availability and increased microbial abundance (Rousk et al. 2010; Liu et al. 2021). The nutrients availability may provide spatially adaptive living micro-habitats for the facultative autotrophic bacterial community, and thus favor the growth and colonization of facultative autotrophs. The N application significantly reduced Chao 1 richness, suggesting an overall decline of soil autotrophic bacterial diversity. The decrease in Chao 1 richness under the high rate of N application may be responsible for the disappearance of locally rare species (Wang et al. 2018). In N limited conditions, some specific microorganisms still maintain a high level of the functional diversity and carbon source utilization capacity (Shen et al. 2010; Lupwayi et al. 2012). In contrast, N-enriched conditions may cause positive correlations between species in co-occurrence network and change their ecological interactions (i.e., cooperative symbiosis), thereby reduce the soil autotrophic bacterial diversity (Yang et al. 2020; Yuan et al. 2021b).

In the present study, the impact of N application on soil autotrophic bacterial community composition was significantly associated with soil properties. The majority of the autotrophic bacterial community belonged to the facultative autotrophic bacteria. These populations exhibit diverse eco-physiological traits and advantageous ecological strategies for carbon fixation and the degradation of various carbon-
containing organic compounds (Yuan et al. 2012b; Guo et al. 2015; Ge et al. 2016; Liao et al. 2020). We further revealed that the co-occurrence network of autotrophic bacteria contained distinct microbial modules consist of highly interconnected taxa. The higher positive links in the network imply that significant commensalism and mutualism relationships may exist among bacterial taxa, and thus alleviate competitive interactions (Banerjee et al. 2016; Zhang et al. 2018). In addition, the intimate connections among species in the microbial network may represent their niche sharing (Berry and Widder 2014). The modules have been reported as ecological niche overlap, habitat heterogeneity, and phylogenetic relatedness (Freilich et al. 2010), which have different functions in nutrients exchange and resource availability in agricultural ecosystems (Jiang et al. 2017; Li et al. 2021). We should be cautious about the bacterial relationships from co-occurrence network because statistical correlations do not necessarily represent causal relationships.

Soil autotrophic bacterial community mediated SOC storage and maize yield

Increasing evidence determines that soil autotrophic bacterial community contributes substantially to carbon fixation and SOC pool dynamics in farmland systems (Yuan et al. 2012a; Ge et al. 2016). A previous study has reported that the microbial carbon fixing rate ranges from 0.84 to 5.57 Mg C km\(^{-2}\) per year in the Qiaozi watershed of Chinese Loess Plateau (Xiao et al. 2018b). We found that the abundance and composition of the autotrophic bacterial community were positively correlated with RubisCO activity and contributed profoundly to maize yield. The closely relationships between RubisCO activity, \(^{14}\)C-SOC, and the abundance of \(cbbL\) gene confirms that soil C fixation is carried out by the soil autotrophic bacterial community by regulating RubisCO activity (Yuan et al. 2012a). Soil microbial diversity is of ecological importance to predict multiple ecosystem functioning, including nutrient cycling and climate regulation (Delgado-Baquerizo et al. 2016; Jiao et al. 2021). However, few studies have explicitly addressed how and to what extent soil autotrophic bacterial diversity drives soil C fixation. Our study showed that the diversity of the autotrophic bacteria had negatively correlations with DOC, MBC, and maize yield. High diversity induced the negative priming of SOC sequestration through the complicate relationship among taxa in microbial networks (Xiao et al. 2018a; Chen et al. 2019). Highly similar species share similar microhabitats and ecological niche may suppress carbon metabolism as species diversity increases (Maynard et al. 2017). We suggest that the reduced autotrophic bacterial diversity was benefit for enhancing RubisCO activity and CO\(_2\) fixation rate of bacterial populations, and improved SOC sequestration and maize productivity. The soil autotrophic bacterial community was dominated by facultative autotrophic bacteria, including \textit{Xanthobacter}, \textit{Bradyrhizobium}, \textit{Aminobacter}, and \textit{Nitrosospira}. In fact, these facultative autotrophs exhibit metabolic flexibility with both heterotrophic and autotrophic metabolic pathways, allowing them to grow on alternative C and energy sources (Esparza et al. 2010; Xiao et al. 2019). Additionally, the genus \textit{Xanthobacter} is the main implementer of fixing CO\(_2\) mainly via the ribulose-biphosphate pathway (Wiegel 2006). Notably, the genus \textit{Xanthobacter} was the dominant taxa in module IV of co-occurrence network, and module IV had positive correlations with SOC storage, which confirmed the potentials of soil autotrophic bacterial community for carbon fixation. Our results
highlight that soil autotrophic bacteria have significant potentials for carbon fixation and contribute to carbon pool and crop productivity.

**Conclusions**

The present study indicated the N treatments strongly impacted the abundance, diversity, composition, and co-occurrence network of soil autotrophic bacterial community via changing pH, NO$_3$-N, and SWC. We provided evidence that the abundance, diversity, and network soil autotrophic bacterial community contributed to RubisCO activity, SOC storage, and maize productivity. Taken together, understanding the biological mechanisms of soil autotrophic bacteria mediating carbon fixation may provide crucial implications for enabling SOC sequestration and crop productivity. Future research by the microcosm incubation and stable isotope-based evidence could help verify the casual relationships between soil autotrophic bacterial community and carbon fixation activity.

**Declarations**

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**Author contributions** Ling Li and Yuji Jiang conceived the topic. Jinbin Wang, Junhong Xie, Zhuzhu Luo, and Renzhi Zhang performed the experiments. Jinbin Wang analyzed all statistical data. Jinbin Wang and Yuji Jiang wrote the manuscript. All authors have read and approved the manuscript.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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Figures
Figure 1

Soil RubisCO activity (a) and cbbL gene copy numbers (b), and alpha-diversity (c and d) autotrophic bacteria under different N application rate treatments. Different letters indicate the significant difference among treatments at $P < 0.05$. Bars represent standard errors ($n = 3$). N0, N1, N2, and N3 represent N application at 0 kg ha$^{-1}$, 100 kg ha$^{-1}$, 200 kg ha$^{-1}$, and 300 kg ha$^{-1}$, respectively.
Figure 2

Relative abundance of the autotrophic bacterial phyla under different N application rate treatments (a). The numbers before the treatment name indicate the sampling replications, for example, 1-N0, 2-N0, and 3-N0 means the sampling was taken from replicate 1, 2, and 3 of the field plots, respectively. b, Relative abundance of the autotrophic bacterial genera under different N application rate treatments. Different letters indicate the significant difference among treatments at P < 0.05. Bars represent standard errors (n = 3). N0, N1, N2, and N3 represent N application at 0 kg ha⁻¹, 100 kg ha⁻¹, 200 kg ha⁻¹, and 300 kg ha⁻¹, respectively.
Figure 3

Co-occurrence network of soil autotrophic bacterial communities under four treatments. The network is colored by phyla (a) and modules (b), respectively. Modules I–IV represent four clusters with closely interconnected nodes. Size of each node is proportional to the number of connections (degree), and the thickness of each connection between two nodes (edge) is proportional to the value of correlation coefficients. Blue edges indicate positive connections, red edges negative connections.
Figure 4

Relative abundance of different dominant modules in soil autotrophic bacterial networks at phyla (a) and genera (b)-level. Different small letters indicate the significant difference among modules at $P < 0.05$. Bars represent standard errors ($n = 12$).
Correlation coefficients between soil autotrophic bacterial community, soil properties, RubisCO activity, SOC storage, and yields. NO3−N, nitrate nitrogen; NH4−N, ammonium nitrogen; AP, available phosphorus; SWC, soil water content; RubisCO, RubisCO activity; SOC storage including soil organic carbon (SOC), microbial biomass carbon (MBC), and dissolved organic carbon (DOC). The soil autotrophic bacterial community is represented by abundance of cbbL gene, diversity (Shannon index), composition (first principal coordinates, PCoA1), and the eigengenes of four modules (modules I, II, III, and VI) in trophic co-occurrence network. *** P < 0.001; ** P < 0.01; * P < 0.05.
Random forest modelling was performed based on 12 samples (4 fertilization treatments × 3 replicates). Soil properties include pH, total nitrogen (TN), nitrate nitrogen (NO3−N), ammonia nitrogen (NH4−N), available phosphorus (AP), and soil water content (SWC). The autotrophic bacterial community includes abundance of cbbL gene, diversity (Shannon index), composition (first principal coordinates, PCoA1), and four module eigengenes in trophic co-occurrence network. **c**, Impacts of soil properties and soil autotrophic bacterial community on RubisCO activity, SOC storage, and maize productivity (grain yield) as estimated using structural equation modeling (SEM) analysis. Based on random forest analyses, the significant predictors were chosen to perform the SEM analysis. The latent (soil properties and the autotrophic bacterial community) inside the boxes were used to integrate the effects of multiple multiple conceptually related observed variables into a single-composite effect. Soil properties are represented by pH, nitrate nitrogen (NO3-N), and soil water content (SWC). Soil autotrophic bacterial community (cbbL) is represented by the composition [first principal coordinates (PCoA1)], diversity (Shannon index), and module VI of soil autotrophic bacterial networks. SOC storage is represented by soil organic carbon (SOC), microbial biomass carbon (MBC), and dissolved organic carbon (DOC). *** P < 0.001; ** P < 0.01; * P < 0.05.

**Figure 6**

Mean contribution (% of increased mean square error, MSE) of soil properties and soil autotrophic bacterial community (cbbL) to RubisCO activity (a) and maize yield (b) based on random forest modelling.
Supplementary Files

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