Shifts in the bacterial community along with root-associated compartments of maize as affected by goethite

Peduru Hewa, Jeewani; Chen, Lin ; Van Zwieten, Lukas; Shen, Congcong; Guggenberger, Georg; Luo, Yu; Xu, Jianming

Biology and Fertility of Soils

DOI:
10.1007/s00374-020-01458-9

Published: 01/11/2020

Peer reviewed version

Shifts in the bacterial community along with root-associated compartments of maize as affected by goethite

Peduruhewa H Jeewani[ab], Lin Chen[c], Lukas Van Zwieten[d], Congcong Shen[e], Georg Guggenberger[f], Yu Luo[a*], Jianming Xu[a]

[a]Institute of Soil and Water Resources and Environmental Science, Zhejiang Provincial Key Laboratory of Agricultural Resources and Environment, Zhejiang University, Hangzhou 310058, China

[b]Department of Agriculture, Southern Province, Galle 80000, Sri Lanka

[c]State Key Laboratory of Soil and Sustainable Agriculture Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China

[d]NSW Department of Primary Industries, Wollongbar Primary Industries, Institute Wollongbar, NSW 2477, Australia

[e]Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

[f]Institute of Soil Science, Leibniz Universität, Hannover, 30419 Hannover, Germany

All correspondence to Dr. Yu Luo

Email : luoyu@zjueducn

Figures :3 pages

Tables : 2 pages

Text pages : 24 pages

Supplementary information : 1 page

Article type: Research paper

Abstract

Rhizosphere compartments, including rhizosphere soil, rhizoplane soil, and the endosphere, are comprised of specialized bacterial communities. For better understanding, effect of goethite on modification of microbial composition of soil-microbes in rhizo-compartments of maize (*Zea mays* L.), DNA sequencing was used. The objective of our study was to determine the mechanisms by which goethite (α-FeOOH) shapes the bacterial communities in these various rhizosphere compartments. According to Linear Discriminant Effect Size Analysis *Acidimicrobiales* (Actinobacteria) were relatively enriched in the rhizosphere, *Heminiimonas* in the rhizoplane, and *Enterobacteriales* in the endosphere. Differential Abundance Analysis revealed that goethite addition enriched Actinobacteria and depleted Proteobacteria in all rhizosphere compartments. The goethite amendment enlarged the difference of Shannon diversity between rhizosphere compartments, with much lower diversity in endosphere and rhizoplane compared to rhizosphere soil, indicating a higher selection of microbiome assemblage. This was confirmed by the beta Nearest Taxon Index (βNTI) with the βNTI >+2, value indicating that changes in environmental conditions progressively increase the strength of selection leading to variable selection (deterministic processes) was the dominant microbial assembly process in goethite added soil. According to the distance-based linear modeling (distLM), the assemblage of bacterial communities in the rhizosphere compartments was regulated by specific edaphic variables, with the contributions of goethite (62%), total C (52%), soil pH (50%), and FeOM (25%). Stabilization of

rhizosphere carbon at the presence of goethite would be the selective step for its accessibility and consequent microbial community composition. For instance, the keystone microorganisms e.g., *Pseudomonas* (Proteobacteria), had complex interactions within the co-occurrence network, indicating its narrow niche and wide colonization ability with other microbes. Taken together, goethite narrows down the diversity towards the endosphere and modulate the bacterial community composition. Our study also provided further evidence for modulation of the microbial community at rhizosphere compartments by the "gate selection" effects, which linked with the abiotic controls on rhizodeposits via limiting the bioavailability of rhizo-C.

Keywords: Goethite, rhizo-compartments, microbial assembly processes, niche selection, keystone microbes, enriched and depleted microbes

Introduction

The rhizosphere is a complex micro-ecological zone that is enriched in C, energy, and nutrients. It represents a highly dynamic and diverse zone for biochemical interactions between plant roots and soil biota (Hinsinger et al. 2009). It is also characterized by a unique bacterial community, which is impacted by plant-associated factors such as plant trait variation/developmental stage in addition to the soil type and related edaphic factors such as pH (Bulgarelli et al. 2012; Hassani et al. 2018; Pii et al. 2016; Rousk et al. 2010; Schreiter et al. 2014). Among the soil associated factors, soil organic C (SOC) is the most important factor in affecting soil microbial composition and assembly process (Dini-Andreote et al. 2015).

Rhizodeposits, such as oxalate, citrate, and secondary compounds, are readily available for microorganisms. Besides, those can be sorbed to the mineral phase, either directly or after microbial metabolization as microbial metabolites (Wan et al. 2019). Biogeochemical

properties of iron (Fe) and SOC are strongly linked through the direct interactions of organic compounds with the mineral matrix and Fe (hydr)oxides are very prominent sorbents for organic substances and well recognized for their ability to stabilize rhizodeposits (Pii et al. 2016; Wan et al. 2019). It was reported that goethite stabilizes organic C through physicochemical interactions (Kaiser and Guggenberger 2007). Dissolved iron can be transported through the soil profile and precipitated on root surfaces and it encounters unique biogeochemical processes closer to root zone via modulating the microbiome community composition (Somenahally et al. 2011). Although the direct effects of goethite on the accumulation/stabilization of SOC have been widely reported, a lack of information exists about the role of goethite in modulation of microbial composition in rhizo-compartments.

Properties of the rhizosphere soil are modified by a range of factors (SOC content, pH) (Philippot et al. 2013; Rousk et al. 2010) and processes that links environmental heterogeneity to shifts in microbial assembly processes such as variable selection and homogenous selection (Caruso et al. 2011; Dini-Andreote et al. 2015). The selective environment may also be spatially heterogeneous, leading to variable selection. In this case, taxa selected for in one place may be selected against in a different place because of spatial variation in the selective environment. Further, it was demonstrated that microbial community selection is occurred at the rhizosphere and that selectivity at the rhizo-compartments might act effectively as a gate for controlling entry of the microbes into the root endosphere via gate filtering effect.

Bacterial community composition can differ substantially amongst different rhizosphere compartments, i.e., the rhizosphere soil, rhizoplane soil, and the endosphere (Chen et al. 2016; Fan et al. 2017). Generally, bacterial diversity decreases from the rhizosphere soil

96 towards the root (Huang et al. 2019). Previous studies have provided new insights into the
97 bacterial composition and organization of microbiomes from different rhizosphere
98 compartments of *Arabidopsis sp*., *Populus sp*., and *Zea mays* (Bulgarelli et al. 2012; Peiffer
99 et al. 2013). Detailed characterization of the root microbiome of *Arabidopsis sp.* showed that
100 the dominant phyla in the endosphere are much less diverse than in the rhizosphere soil
101 (Bulgarelli et al. 2012). A more comprehensive understanding of the microbe-environmental
102 interactions remains challenging due to the complex ecological interactions taking place in
103 separate compartments of the rhizosphere.

104

105 The present study aimed at investigating the role of goethite in controlling the diversity of
106 bacterial communities in the rhizosphere soil, the rhizoplane, and the endosphere of maize.
107 We hypothesized that (i) goethite can modulate the bacterial community composition and
108 their assemblages due to "gate" effects on rhizodeposits (i.e., limiting the bioavailability of
109 rhizosphere C) and (ii) variations in the composition and the diversity of the bacterial
110 community occurs spatially resolved across the rhizosphere soil, the rhizoplane, and the
111 endosphere as affected by goethite.

112

113 **Materials and methods**

114 **Site description and sampling**

115 The soil was collected (0-10-cm) from an experimental plot of the Zhejiang University
116 research station located in Zhejiang province, China. This area experiences a temperate
117 monsoon climate with an average annual rainfall of 1453 mm and a temperature of 23^{o}C. The
118 plant species were dominated by *Osmanthus fragrans* (Sweet osmanthus). After removing
119 visible stones and plant residues, the soil was air-dried and sieved <2mm. The soil was

120 classified as an Alfisol with a sandy clay loam texture and a pH (soil: $H_2O_{deionized}$ 1:2.5) of
121 6.7. Total C content (28.6 g kg^{-1}) and total N content (1.04 g kg^{-1}) were determined by dry
122 combustion (Perkin Elmer EA 2400, Shelton, CT, USA). Total Fe content was 19.1 g kg^{-1}
123 and determined by inductively coupled plasma mass spectrometer (ICP-MS; Perkin Elmer,
124 Shelto, CT, USA).

125

126 **Rhizobox experiment**

127 The experiment consisted of a control and a treatment with goethite applied to the soil at a
128 dose approximately equivalent to 10% w/w of the original Total Fe content of the soil (Hori
129 et al. 2010). Goethite was evenly mixed (applied as finely ground powder form [<0.053mm]
130 (Sigma-Aldrich Chemistry Company, Germany) with soil. The control (n=3) and goethite
131 amended soil (n=3) (1 kg dry weight equivalent) were placed into rhizoboxes (30 x 14 cm)
132 which had a layer of acid-washed quartz sand (250 g) at the bottom to assist drainage and to
133 minimize the potential development of anaerobic conditions. Maize (*Zea mays.* L) seeds were
134 surface disinfected using 30% H_2O_2 for 30 minutes and germinated in a nursery for one week.
135 The germinated seedlings were transferred into the rhizoboxes (1 plant per box), the soil was
136 moistened to 70% of water holding capacity (WHC), and each pot was watered every other
137 day using sterilized deionized water. The rhizoboxes were then incubated in a greenhouse at a
138 constant temperature (25°C).

139

140 **Soil sampling for rhizo-compartments**

141 The soil and plants were carefully removed from each rhizobox after 45 days of growth. The
142 excess soil was manually shaken from the roots leaving approximately 1 mm of soil still
143 attached to the roots. To collect rhizosphere soil roots from each rhizobox, they were placed

into a sterile flask with 50 ml of sterile phosphate-buffered saline (PBS) solution and stirred vigorously with sterile forceps to separate the soil from the root surfaces (Chen et al. 2016). This soil (rhizosphere compartment) was stored at -80°C until DNA extraction. The roots designated for rhizoplane collection were recovered from the previous process (i.e., to remove the rhizosphere soil) and placed in a Falcon tube with 15 ml PBS, and tightly adhering microbes at the root surface were removed by sonication (Edwards et al. 2015) (30 s at 50-60 Hz with output frequency 42 kHz, power 90 W, Branson Ultrasonics). The roots were then removed into another sterile flask, and the liquid PBS fraction was kept as the rhizoplane compartment. The roots designated for the endosphere collection were cleaned and sonicated as described before. Two additional sonication procedures using clean PBS solution were used to ensure that all microbes were removed from the root surface. The sonicated roots were then stored at -80°C until DNA extraction.

DNA extraction from rhizo-compartments

The rhizosphere and rhizoplane soil were centrifuged for 30 sec at 10000 x G to concentrate into a 2 ml tube. The supernatant was discarded, leaving only the soil fraction behind. The root fraction was pre-homogenized, before DNA extraction by bead beating for 1 minute (Mini Bead beater, Bio spec Products). Microbial DNA extraction was conducted with 0.5 g of moist soil using the Fast DNA Spin Kit (MP Biomedicals, Santa, Ana, CA, USA) following the manufacturer's protocol. The extracted DNA was purified using an Ultra Clean DNA purification Kit (MOBIO, Carlsbad, CA, USA). The isolated DNA was eluted in 50 µl of TE Buffer. The DNA concentration was quantified using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Finally, DNA samples were stored at -80^{o}C before molecular analysis.

Gene amplification and sequencing

The bacterial 16S rRNA gene fragments were amplified using primer sets targeting the V4-V5 variable region (Vandenkoornhuyse et al. 2007). The forward primer was 515F (5'-GTGCCAGCMGCCGCGGTAA-3') linked with a specific-sample 5-bp barcode sequence at the 5'end of primer, and 806R (5'- GGACTACHVGGG TWTCTAAT -3') was used as the reverse primer (Caporaso et al. 2011). Each sample was amplified in triplicate, and then the three reaction products were pooled and purified using Agencourt Ampure XP beads (USA) and quantified by real-time quantitative PCR (Eva Green TM). All amplicons were pooled across all samples at equimolar concentrations (20 ng μl^{-1}) into a composite sample, and the index sequencing of paired-end 250 bp was performed on an Illumina HiSeq 2000 platform. Adequate amounts of DNA were used for sequencing (mainly more than >5 ng) to reduce the potential biases that happened during downstream processes such as DNA preparation. (Salter et al. 2014;Vestergaard et al. 2017).

Data processing and statistical analysis

The data of bacterial 16S rRNA was processed by the QIIME 180-dev pipeline (Caporaso et al. 2012). Low-quality reads (quality score < 20 read length < 200 bp and sequence errors) were discarded. Chimeric sequences were identified by UCHIME (Edgar 2010) and removed. The remaining high-quality sequences were clustered into operational taxonomic units (OTUs) based on a 97% pairwise identity using the UCLUST algorithm (Edgar 2010). The representative sequences of each OTU were then chosen for subsequent alignment and taxonomic assignment with the RDP classifier. Taxonomy is assigned to bacterial phylotypes of the Green genes database. All data sets were rarefied to 39,000 sequences per sample for

192 the bacterial α- and β-diversity analyses to prevent potential bias caused by different
193 sequencing depth. For β-diversity analysis, the dissimilarity of bacterial communities was
194 calculated via principal coordinates analyses (PCoA). Beta nearest taxon index (βNTI) is the
195 number of standard deviations from the mean of the null distribution, βNTI <-2 or >+2
196 indicates less than or greater than expected phylogenetic turnover, for one pair-wise
197 comparison (Dini-Andreote et al. 2015). The βNTI was calculated with the online interface
198 Galaxy.

199

200 **Differential abundance analysis**

201 We used the R package "DESeq2" (Love et al. 2014) to calculate the differential abundance
202 (log_2-fold change in the relative abundance of each OTU) between the amendment and the
203 unamended rhizoboxes. We independently filtered out OTUs that were sparsely represented
204 across samples [i.e., those OTUs for which the DESeq2-normalized count across samples
205 ("base Mean") was less than 0.6]. Sparse OTUs did not contain sufficient sequence counts to
206 provide statistically significant results and were removed, thus reducing the number of
207 multiple comparisons performed. We adjusted the *p*-values with the Benjamini and Hochberg
208 (BH) correction method and selected a study-wide false discovery rate (FDR) of 10% to
209 denote statistical significance (Love et al. 2014). We defined "responding OTUs" as OTUs
210 with a differential abundance greater than one and an adjusted *p*-value of <0.1.

211 Changes in bacterial community composition between different compartments of goethite
212 amended soil was further tested by Linear Discriminant Effect Size Analysis (LEfSe) (Segata
213 et al. 2011). LEfSe analysis from the phylum to genus level was used to identify differentially
214 abundant features as well as to encode biological consistency and effect relevance. The LDA
215 scores higher than 3.0 were selected to find specialized bacterial groups enriched in response

to three rhizosphere compartments. The best multivariate distance-based linear modeling (distLM) analysis (Anderson and Legendre 1999) was applied for factors including Total C (TC), C: N ratio (C: N), Fe bound C content (FeOM), Total N (TN), pH and total Fe.

Co-occurrence network construction

Microbial network analyses are required caution in drawing to obtain appropriate conclusions. We combined the data of rhizo-compartments and develop the co-occurrence microbial network, to compare the co-occurrence pattern of goethite added with the control. Interpretation of net-work analysis based on microbial abundances should do concerning characterization of DNA to avoid the biases about sequence analyses of the target microbial taxa (Nannipieri et al. 2020). For network inference, all possible Spearman's rank correlations between OTUs with more than five sequences were calculated (2200 OTUs). This prior-filtering step removed poorly represented OTUs and reduced the complexity of the network, facilitating the determination of the core soil community. In addition, the network properties change by changing the network inference parameters and the studied system is simplified by the network representation (Faust and Raes 2012). We considered a valid co-occurrence event to be a robust correlation if the Spearman's correlation coefficient (r) was statistically significant (p-value <0.01). The nodes in the reconstructed network represent the OTUs at 90% identity, whereas the edges correspond to a significant and robust correlation between nodes. To describe the topology of the resulting network, by various measures (that is average path length, average node connectivity, diameter, clustering coefficient, cumulative degree distribution, and modularity) were calculated. All statistical analyses were carried out in the R environment using vegan (Oksanen et al. 2007) package. To increase prediction accuracy, Wang et al. (2017) proposed the combined use different inference tools: Co-occurrence Network Inference, Molecular Ecological Network Analysis, and extended

Local Similarity Analysis by using Cytoscape (v350) (Shannon et al. 2003) was used for network visualization and topological analysis and visualized with the interactive platform Gephi (Bastian et al. 2009).

The statistical analysis of all non-microbial data was performed in SPSS 20 (SPSS, Inc., Chicago, IL, USA). A one-way analysis of variance (ANOVA) was used to analyze data variance. Prior to one-way ANOVA, Normality and equality of variances were tested. When normality assumptions were not satisfied, a non-parametric test (Kruskal-Wallis test).

Results

Taxonomic analysis of the bacterial community

There are notable differences in the percentages of relative abundance of various phyla across the rhizosphere compartments. (Fig. 1a). Proteobacteria, Actinobacteria, Chloroflexi, and Acidobacteria dominated in both goethite amended and control soils (Fig. 1a). The endosphere of maize roots in the goethite amended soil showed a greater abundance of Cyanobacteria than the rhizosphere soil and rhizoplane soil. In contrast, Proteobacteria, Actinobacteria, and Acidobacteria were mostly depleted in the endosphere of the maize root. Actinobacteria and Choroflexi decreased in abundance towards the inner compartments for the control, and the goethite amended treatment (Fig. 1a).

There was no significant difference in α-diversity of the rhizosphere compartment in treatments. Nevertheless, α-diversity of the rhizoplane and endosphere showed considerable difference between control and goethite added treatments, where the diversity in the rhizoplane and endosphere of goethite amended soil was much lower than that of control (Fig. 1b). PCoA was performed to investigate the assembly of bacterial communities between all samples. The root-associated bacterial community between the goethite amended and the

control soil was separated along the second principal coordinate (Fig. 1c). The rhizo-compartments separate across the first principal coordinate, indicating that the largest source of variation in root-associated microbial composition was in the rhizosphere. To test the assembly process of the bacterial community in goethite amended soil, we calculated the βNTI for paired samples (Fig. 1d). βNTI <−2 or >+2 is interpreted as the dominance of homogeneous or variable selection, respectively. Changes in the soil environment progressively increase the importance of assembly processes. Under the homogeneous selection scenario, the selective environment is spatially homogeneous within the period of time and does not change significantly during the relatively short time. however, if the selective environment is spatially heterogeneous, leading to variable selection. In that case, taxa selected for in one place may be selected against in another place because of spatial variation in the selective environment. Homogeneous and variable selection should cause less than and greater than expected community turnover, respectively. Our results demonstrated that the βNTI score of goethite added soil was in the range of >+2 (Fig. 1d), which were consistent with random phylogenetic turnover. These results indicated that bacterial community assemblage processes in the goethite added soil was a variable assembly process. βNTI scores of control were <+2, which indicates that stochastic processes were governed in community dynamics. According to the distLM Analysis, soil bacterial community was influenced by the soil variables Fe (62%), TC (52%), pH (50%), and FeOM (25%) (Table S1).

Association of significantly enriched OTUs within the rhizosphere compartments

Differential OTU abundance analysis was used to identify enriched OTUs, with community separation between goethite amended soil and the control (Fig. 2). The goethite amendment soil had a high ratio of statistically significant OTUs that were depleted in all rhizosphere compartments. The rhizosphere soil was the most exclusive compartment enriching for 172 OTUs while depleting 243 OTUs (Fig. 2). In comparison, the rhizoplane was enriched for 77 and depleted for 35 OTUs, while the endosphere was enriched for only 33 OTUs and depleted of 68 OTUs. The majority of the OTUs enriched in the rhizosphere were simultaneously enriched in the rhizoplane and/or endosphere of goethite amended soils, and there were noteworthy overlaps in differentially abundant OTUs between the compartments (Fig. 2). The LEfSe results show that the phylum Actinobacteria was enriched in the rhizosphere relative to the other two compartments (Fig. S1). Within the Actinobacteria, taxonomic orders confirming enrichment in the rhizosphere bacterial genera include *Acidimicrobiales* and *Solirubrobacterales.* Within the Proteobacteria group, *Xanthomonadales* and *Nitrosomonadales* were enriched in the rhizosphere. *Enterobacteriaceae* (Proteobacteria) was enriched in the rhizoplane soil in the control and the goethite treatment. The bacterial lineage enriched in the endosphere was *Herminlimonas* from the Proteobacteria.

Construction of co-occurrence network

The construction of OTU networks explored the co-occurrence patterns in the root-associated bacterial communities. The co-occurrence networks differed between the treatment and the control. The dominant OTUs (30%) in both networks belonged to Proteobacteria (Table S3), whereas the percentage of OTUs from Actinobacteria was 28% in the goethite amended soil compared to 15% in control. In the goethite amended soil, approximately 28% of the nodes

belonged to Actinobacteria. The network also showed an enrichment of Proteobacteria (30%) and Acidobacteria (17%). As indicated by the edge number and density of the network in goethite, amended soil was more complicated interaction than that of the control. The average path length and modularity were very close in both networks, while the clustering coefficient was higher in the goethite amended network (Table S2). The bacterial community exhibited 96% (975) of significant correlations (positive links) of 152 OTUs (nodes) in the root-associated compartments in the goethite amended the soil, and 76% (194) links of 145 nodes in control (Table S2). The keystone genera that were calculated using the highest betweenness centrality of co-occurrence network in the rhizosphere of the goethite amended soil was *Solirubrobacter*, *Pseudomonas,* and *Nitrosomonas,* while *Rhizobales* and *Comamonadaceae* were in the control soil (Fig. 3b). Most of the negative links derived from *Pseudomonas* (Proteobacteria) were connected with other bacterial phyla in the rhizosphere of the goethite amended soil. These differences link composition in rhizosphere networks between the treatment and control that were consistent with those in the network nodes. However, the composition of nodes and links varied within each network. In particular, Actinobacterial nodes were higher in goethite amended soil than those in the rhizosphere network of the control. Simultaneously, Actinobacteria accounted for more links than Actinobacteria in the control soil (Fig. 3a).

Discussion

Microbial community of rhizo-compartments

The present study has shown that the amendment of goethite with an Alfisol has a significant effect on the bacterial community composition within the rhizosphere, rhizoplane, and the endosphere (Fig. 1a). The relative abundance of Proteobacteria dominated in all rhizosphere compartments, while the relative abundance of Acidobacteria and Actinobacteria decreased

from the rhizosphere to the endosphere. A similar pattern has also been observed for rice (Edwards et al. 2015) and Arabidopsis (Bulgarelli et al. 2012; Peiffer et al. 2013), suggesting that different rhizosphere compartments create a significant influence on the microbial community.

By the differential abundance analysis, we observed that the rhizosphere serves as a combined zone of bacterial OTUs (Fig. 2). The majority of OTUs belonging Actinobacteria enriched in the rhizosphere were also enriched in the rhizoplane and endosphere. Conversely, the vast majority of OTUs (Proteobacteria) depleted in the rhizosphere were also depleted in the rhizoplane and endosphere revealing that there was selective colonization in the rhizosphere, and it acted as a gate selection for the microbial colonization in the endosphere (Edwards et al. 2015; Peiffer et al. 2013). The LEfSe analysis further explained microbial lineages that are tightly linked to compartments (Fig. S1). It showed the enrichment of *Acidimicrobiales, Hemilimonas,* and *Enterobacteriales* in the rhizosphere, rhizoplane, and endosphere, respectively. *Acidimicrobiales* (Actinobacteria) can produce various secondary metabolites with antibiotic properties (Silva-Lacerda et al. 2016). Proteobacteria are considered as fast growers with the ability to utilize a majority of rhizosphere C substrates (Philippot et al. 2013). Within the phylum of Proteobacteria, one of the most abundant order was *Enterobacteriales,* which was shown to preferentially colonize in the rhizosphere of several plants and was known for their beneficial effects on plant growth and/or protection against pathogens (el Zahar Haichar et al. 2008). The highest relative abundance of Cyanobacteria was found in the endosphere (Fig. 1a). Cyanobacteria lives in symbiosis with other organisms by secreting polysaccharides and N fixating (Karthikeyan et al. 2007) and this can inhibit the growth of pathogenic bacteria. Modulations of the root-associated microbiome across the three rhizo-compartments provide evidence for the rapid selection of root-associated microbiomes from the soil.

Goethite effects on the microbiome in rhizosphere compartments

Selective power acting on bacterial assemblages has likely shaped well-adapted bacterial communities in rhizosphere compartments. However, a paucity of information exists on the role of goethite on controlling bacterial composition around the rhizosphere, rhizoplane, and endosphere of maize. While there is evidence that goethite can stabilize SOC in the soil through physiochemical reactions (Kaiser and Guggenberger 2007; Wan et al. 2019), it is unclear how these abiotic changes (changing C availability) can influence soil biotic processes.

Although microbial α-diversity pattern holds the same pattern from the rhizosphere to the endosphere in both treatment, goethite addition enlarged the α-diversity difference between there different compartments (Fig. 1b). The changes of α-diversity with root proximity by goethite likely depends on a "filtration effect" (Dibbern et al. 2014). Here, the rhizosphere preferentially selects microbiome by recruitment to the vicinity of the root and followed by a transition from the rhizosphere to the endosphere (Edwards et al. 2015; Valverde et al. 2014). The ordination of beta diversity results revealed a clear separation between the different rhizosphere compartments as well as between the control and the goethite treatments. This indicates that selective changes in the bacterial community structure in the rhizosphere compartments were influenced by heterogeneity (availability and accessibility of C sources) of soil. Recent studies revealed that bacterial community associated with root is not random, but rather controlled by specific assembly processes and edaphic factors (Dini-Andreote et al. 2015; Fan et al. 2017). Here, the variable selection process is identified as the dominant process of bacterial community assemblages in goethite added to the soil (Fig. 1d).

Changes in bacterial composition are related to the heterogeneity of soil that allowed microbes to exploit spatially structured unique niches and communities towards the selective environment (Dini-Andreote et al. 2015; Mendes et al. 2014). It is well established that edaphic factors (e.g., soil pH, SOC) were defined the micro-environment for microorganisms, thus determining their population dynamics and community composition (Dini-Andreote et al. 2015; Nuccio et al. 2016). Our analysis showed that goethite built up unique niches with specific variables of total Fe, TC, and FeOM that modulate the composition of bacterial communities (Table S1). The co-precipitation of rhizosphere C as a result of the goethite amendment substantially lowers the availability of labile C sources in the rhizosphere, thus influencing community composition (Doetterl et al. 2015; Vogel et al. 2015). Results of the present study support that variable selection of microbial composition occurs due to the heterogeneity of soil created by goethite through physicochemical changes in soil (Table S1). It indicates that soil heterogeneity interacts to shape the spatial scaling of the maize rhizosphere microbiota.

Goethite modified Co-occurrence network of rhizosphere

Network analysis was used to determine the interactions between microbial communities (Freilich et al., 2011). *Rhizobales* and *Comamonadaceae* were the keystone microorganisms in control that keep utilizing a broad range of rhizo-C substrates with other microbial species (Denison and Kiers 2004; Fierer et al. 2007). A number of keystone genera were *Solirubrobacter*, *Pseudomonas,* and *Nitrosomonas,* which had the highest number of central nodes observed in the rhizosphere of goethite amended soil (Fig. 3; Table S2). Goethite modulated keystone genera were associated with rapid C cycling (Hinsinger 2009; Whipps 2001). Importantly, *Pseudomonas* (within Proteobacteria) had greater betweenness centrality and degree of negative links with most other taxa in the goethite amended the soil (Fig. 3;

412 Table 2). The selection and competitive colonization power of bacteria depend on metabolic
413 capacities [the ability to utilize root exudates (e.g., amino acids and polysaccharides)] and
414 growth rate (Lugtenberg and Dekkers 1999; Walker et al. 2004). It was reported that
415 formation of interconnected microbial assemblages with keystone species such as
416 *Pseudomonas* was due to (i) competition for space and nutrients (ii) modification of the host
417 physiology (iii) modulation of microbial community composition, providing a competitive
418 advantage to other organisms in the rhizosphere (Hassani et al. 2018; Lugtenberg and
419 Dekkers 1999; Tyc et al. 2017). Moreover, *Pseudomonas* act as an important colonizer that
420 can colonized with specific bacterial families including *Oxalobacteraceae, Bacillaceae,* and
421 *Burkholderiaceae* (Hassani et al. 2018; Lugtenberg and Dekkers 1999). Thus, interactions of
422 rhizodeposits with goethite by creating the C limited conditions affected the keystone
423 bacterial genera with a narrow niche.
424
425 **Conclusions**
426 This is the first study showing the effect of the goethite amendment in soil on the
427 composition of bacteria and network complexity in different rhizosphere compartments,
428 including rhizosphere rhizoplane and endosphere. Bacterial OTUs enrichment in each
429 rhizosphere compartment was reduced towards the endosphere, revealing selective
430 colonization in the rhizosphere and that it acted as a gate selection for the bacterial
431 colonization in the rhizoplane and endosphere. Cyanobacteria were highly abundant in the
432 endosphere, indicating symbiosis associations with other organisms by secretions of
433 polysaccharides and N fixation. Variable selection processes dominated the assemblage of
434 bacterial communities in goethite added to the soil by the modulation of the bacterial
435 community. Total C, total Fe, and FeOM were the main soil properties that caused a spatially
436 heterogeneous environment that leads to the variable selection process. Bacterial

communities in the goethite amended soil had a more complex co-occurrence network, and *Solirubrobacter, Pseudomonas,* and *Nitrosomonas* were the keystone genera that have a narrow niche, demonstrating competitive interactions to occur with the C limited rhizosphere. Taken together, the goethite amendment strengthens the selective power of the bacterial assembly process in rhizosphere compartments likely due to biotic competition with abiotic habitat filtering by rhizosphere C accessibility.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (41671233 41721001 41807017)

References

Anderson MJ, Legendre P (1999) An empirical comparison of permutation methods for tests of partial regression coefficients in a linear model. J Stat Computation Simulation 62:271-303

Bastian M, Heymann S, Jacomy M (2009) Gephi: an open-source software for exploring and manipulating networks. Third international AAAI conference on weblogs and social media. Paris, France.

Blankenberg D, Von Kuster G, Coraor N, Ananda G, Lazarus R, Mangan M, Nekrutenko A, Taylor J (2010) Galaxy: a web-based genome analysis tool for experimentalists. Current Protocols in Molecul Biol 19:19-1021

458 Bulgarelli D, Rott M, Schlaeppi K van, Themaat EVL, Ahmadinejad N, Assenza F, Rauf
459 P, Huettel B, Reinhardt R, Schmelzer E (2012) Revealing structure and assembly cues for
460 Arabidopsis root-inhabiting bacterial microbiota. Nature 488:91–95
461 Caporaso JG, Lauber CL, Walters WA, Berglyons D, Huntley J, Fierer N, Owens SM,
462 Betley J, Fraser L, Bauer M (2012) Ultra-high-throughput microbial community analysis
463 on the Illumina HiSeq and MiSeq platforms. ISME J 6:1621-1624
464 Caruso T, Chan Y, Lacap DC, Lau MCY, McKay CP, Pointing, SB, (2011) Stochastic
465 and deterministic processes interact in the assembly of desert microbial communities on a
466 global scale. ISME J 5:1406-1413.
467 Chen C, Dynes JJ, Wang J, Sparks DL (2014) Properties of Fe-organic matter
468 associations via coprecipitation versus adsorption. Environ Sci Technol 48:13751-13759
469 Chen L, Brookes PC, Xu J, Zhang J, Zhang C, Zhou X, Luo Yu (2016) Structural and
470 functional differentiation of the root-associated bacterial microbiomes of perennial
471 ryegrass. Soil Biol Biochem 98:1-10
472 Denison RF, Kiers ET (2004) Lifestyle alternatives for rhizobia: mutualism parasitism
473 and forgoing symbiosis. FEMS Microbiol Lett 237:187-193
474 Dini-Andreote F, Stegen JC, van Elsas, JD, Salles, JF (2015) Disentangling mechanisms
475 that mediate the balance between stochastic and deterministic processes in microbial
476 succession. Proc Natl Acad Sci U S A 112:E1326-E1332
477 Dibbern D, Schmalwasser A, Lueders T, Totsche KU (2014) Selective transport of plant
478 root-associated bacterial populations in agricultural soils upon snowmelt. Soil Biol
479 Biochem 69:187-196
480 Doetterl S, Stevens A, Six J, Merckx R, Oost KV, Pinto MC, Casanovakatny A, Muñoz
481 C, Boudin M, Venegas EZ (2015) Soil carbon storage controlled by interactions between
482 geochemistry and climate. Nature Geoscience 8:780-783

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST Bioinformatics. 26:2460-2461

Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S Eisen JA, Sundaresan V (2015) Structure variation and assembly of the root-associated microbiomes of rice. Proc Natl Acad Sci U S A 112:E911-E920

el Zahar Haichar F, Marol C, Berge O, Rangel-Castro JI, Prosser JI, Balesdent Jm, Heulin T, Achouak W, (2008) Plant host habitat and root exudates shape soil bacterial community structure. ISME J 2:1221.

Fan K, Cardona C, Li Y, Shi Y, Xiang X, Shen C, Wang H, Gilbert JA, Chu H (2017) Rhizosphere-associated bacterial network structure and spatial distribution differ significantly from bulk soil in wheat crop fields. Soil Biol Biochem 113:275-284

Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. Ecology 88:1354-1364

Gorelova O (2006) Communication of cyanobacteria with plant partners during association formation. Microbiol 75:465-469

Gougoulias C, Clark JM, Shaw LJ (2014) The role of soil microbes in the global carbon cycle: tracking the below-ground microbial processing of plant-derived carbon for manipulating carbon dynamics in agricultural systems J Sci Food Agricul 94:2362-2371

Hassani MA, Durán P, Hacquard S (2018) Microbial interactions within the plant holobiont. Microbiome 6:58

Hinsinger P, Bengough AG, Vetterlein D, Young IM (2009) Rhizosphere: biophysics biogeochemistry and ecological relevance. Plant Soil 321:117-152

Huang X, Zhou X, Zhang J, Cai Z (2019) Highly connected taxa located in the microbial network are prevalent in the rhizosphere soil of healthy plant. Biol Fert Soils 55 299-312

Jones DL (1998) Organic acids in the rhizosphere–a critical review. Plant Soil 205:25-44

508 Kaiser K, Guggenberger G (2007) Sorptive stabilization of organic matter by
509 microporous goethite: sorption into small pores vs surface complexation. Eur J Soil Sci
510 58:45-59
511 Karthikeyan N, Prasanna R, Nain L, Kaushik BD (2007) Evaluating the potential of plant
512 growth promoting cyanobacteria as inoculants for wheat. Eur J Soil Biol 43:23-30
513 Love MI, Huber W, Anders S (2014) Moderated estimation of fold change and dispersion
514 for RNA-seq data with DESeq2. Genome Biol 15:550
515 Lugtenberg BJ, Dekkers LC (1999) What makes Pseudomonas bacteria rhizosphere
516 competent? Environ Microbiol 1:9-13
517 Mendes LW, Kuramae EE, Navarrete AA, van Veen JA, Tsai SM (2014) Taxonomical
518 and functional microbial community selection in soybean rhizosphere. ISME J 8:1577
519 Nuccio EE, Anderson-Furgeson J, Estera KY, Pett-Ridge J, de Valpine P, Brodie EL,
520 Firestone MK (2016) Climate and edaphic controllers influence rhizosphere community
521 assembly for a wild annual grass. Ecology 97:1307-1318
522 Oksanen J, Kindt R, Legendre P, O'Hara B, Stevens MHH, Oksanen MJ, Suggests M
523 (2007) The vegan package. Community Ecology Package 10:631-637
524 Peiffer JA, Spor A, Koren O, Jin Z, Tringe SG, Dangl JL, Buckler ES, Ley RE (2013)
525 Diversity and heritability of the maize rhizosphere microbiome under field conditions.
526 Proc Natl Acad Sci U S A 110:6548-6553
527 Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH (2013) Going back to
528 the roots: the microbial ecology of the rhizosphere. Nature Rev Microbiol 11:789
529 Pii Y, Borruso L, Brusetti L, Crecchio C, Cesco S, Mimmo T (2016) The interaction
530 between iron nutrition plant species and soil type shapes the rhizosphere microbiome.
531 Plant Physiol Biochem 99:39-48

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. The Isme Journal 4, 1340-1351.

Schreiter S, Ding G-C, Heuer H, Neumann G, Sandmann M, Grosch R, Kropf S Smalla K (2014) Effect of the soil type on the microbiome in the rhizosphere of field-grown lettuce. Front Microbiol 5:144

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W.S., Huttenhower, C., 2011. Metagenomic biomarker discovery and explanation. Genome biology 12, R60.

Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Research 13:2498-2504

Silva-Lacerda G, Santana R, Vicalvi-Costa M, Solidônio E, Sena K, Lima G, Araújo J (2016) Antimicrobial potential of actinobacteria isolated from the rhizosphere of the Caatinga biome plant Caesalpinia pyramidalis Tul. Genet Mol Res 15:1-12:

Somenahally AC, Hollister EB, Yan W, Gentry TJ, Loeppert RH (2011) Water Management Impacts on Arsenic Speciation and Iron-Reducing Bacteria in Contrasting Rice-Rhizosphere Compartments. Environ Sci Technol 45:8328-8335

Tyc O, Song C, Dickschat JS, Vos M, Garbeva P (2017) The Ecological Role of Volatile and Soluble Secondary Metabolites Produced by Soil Bacteria. Trends Microbiol 25:280-292

Valverde A, Makhalanyane TP, Cowan DA (2014) Contrasting assembly processes in a bacterial metacommunity along a desiccation gradient. Front Microbiol 5:668

556 Vandenkoornhuyse P, Mahé S, Ineson P, Staddon P, Ostle N, Cliquet J-B, Francez A-J,
557 Fitter AH, Young JPW (2007) Active root-inhabiting microbes identified by rapid
558 incorporation of plant-derived carbon into RNA. Proc Natl Acad Sci U S A 104:16970-
559 16975
560 Vestergaard G, Schulz S, Schöler A, Schloter M (2017) Making big data smart—how to
561 use metagenomics to understand soil quality. Biol Fertil Soils 53:479-484
562 Vogel C, Heister K, Buegger F, Tanuwidjaja I, Haug S, Schloter M, Kögel-Knabner I
563 (2015) Clay mineral composition modifies decomposition and sequestration of organic
564 carbon and nitrogen in fine soil fractions. Biol Fertil Soils 51:427-442
565 Walker TS, Bais HP, Déziel E, Schweizer HP, Rahme LG, Fall R, Vivanco JM (2004)
566 Pseudomonas aeruginosa-plant root interactions Pathogenicity biofilm formation and root
567 exudation. Plant Physiol 134:320-331
568 Wan D, Ye T, Lu Y, Chen W, Cai P, Huang Q (2019) Iron oxides selectively stabilize
569 plant-derived polysaccharides and aliphatic compounds in agricultural soils. Euro J Soil
570 Sci 70:1153-1163
571 Wang, H., Wei, Z., Mei, L., Gu, J., Yin, S., Faust, K., Raes, J., Deng, Y., Wang, Y., Shen,
572 Q., 2017. Combined use of network inference tools identifies ecologically meaningful
573 bacterial associations in a paddy soil. Soil Biology and Biochemistry 105, 227-235.
574 Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. J
575 Experimental Botany 52:487-511
576 Yang C-H, Crowley DE (2000) Rhizosphere microbial community structure in relation to
577 bacyerial abundance and plant iron nutritional status. Appl Environ Microbiol 66:345-351
578

Fig. 1 Relative to bacterial abundance of community composition (a). α-diversity index across the rhizosphere, rhizoplane, and endosphere. α-diversity was calculated by Shannon index (b). The horizontal bars within boxes represent median. The tops and bottoms of boxes represent 50th and 50th quartiles, respectively. Bacterial community differentiation between all samples of principal component analysis plots (PCoA) for goethite and control treatments, across the rhizosphere, rhizoplane, and endosphere (c). Distribution of beta Nearest Taxon Index (βNTI) according to the spatial distance in control and goethite added soil (d). Positive βNTI values indicate greater (less) than expected turnover in phylogenetic composition. The horizontal dotted blue line shows the 95% confidence intervals around the expectation under neutral community assembly.

Fig. 2 Enriched and depleted OTUs of three different rhizosphere compartments Enrichments and depletion of rhizosphere compartments compared with control (without goethite addition) as determined by differential abundance analysis (a). Each point represents an individual OTU, and the position along the y axis represents the abundance fold change compared with control. The highest differential abundance bacterial phylum was named at the bottom of each compartments.

Fig. 3 Microbial networks are showing the co-occurrence patterns within the rhizosphere microbiomes. In the networks for the goethite amended (a) and control (b), colored nodes assign if corresponding OTUs assigned to major phyla/subphyla. The size of each node is proportional to its average relative abundance. The blue and red links indicate significant positive and negative correlations between two nodes, respectively.

603