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

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26 Abstract

Rhizosphere compartments, including rhizosphere soil, rhizoplane soil, and the endosphere, 27 are comprised of specialized bacterial communities. For better understanding, effect of 28 goethite on modification of microbial composition of soil-microbes in rhizo-compartments of 29 maize (Zea mays L.), DNA sequencing was used. The objective of our study was to 30 31 determine the mechanisms by which goethite (α -FeOOH) shapes the bacterial communities in these various rhizosphere compartments. According to Linear Discriminant Effect Size 32 Analysis Acidimicrobiales (Actinobacteria) were relatively enriched in the rhizosphere, 33 Heminiimonas in the rhizoplane, and Enterobacteriales in the endosphere. Differential 34 Abundance Analysis revealed that goethite addition enriched Actinobacteria and depleted 35 Proteobacteria in all rhizosphere compartments. The goethite amendment enlarged the 36 difference of Shannon diversity between rhizosphere compartments, with much lower 37 diversity in endosphere and rhizoplane compared to rhizosphere soil, indicating a higher 38 selection of microbiome assemblage. This was confirmed by the beta Nearest Taxon Index 39 (β NTI) with the β NTI >+2, value indicating that changes in environmental conditions 40 progressively increase the strength of selection leading to variable selection (deterministic 41 42 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 43 rhizosphere compartments was regulated by specific edaphic variables, with the contributions 44 of goethite (62%), total C (52%), soil pH (50%), and FeOM (25%). Stabilization of 45

rhizosphere carbon at the presence of goethite would be the selective step for its accessibility 46 and consequent microbial community composition. For instance, the keystone 47 48 microorganisms e.g., *Pseudomonas* (Proteobacteria), had complex interactions within the cooccurrence network, indicating its narrow niche and wide colonization ability with other 49 microbes. Taken together, goethite narrows down the diversity towards the endosphere and 50 modulate the bacterial community composition. Our study also provided further evidence for 51 52 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 53 54 of rhizo-C.

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

57

58 Introduction

The rhizosphere is a complex micro-ecological zone that is enriched in C, energy, and 59 nutrients. It represents a highly dynamic and diverse zone for biochemical interactions 60 between plant roots and soil biota (Hinsinger et al. 2009). It is also characterized by a unique 61 bacterial community, which is impacted by plant-associated factors such as plant trait 62 variation/developmental stage in addition to the soil type and related edaphic factors such as 63 64 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 65 factor in affecting soil microbial composition and assembly process (Dini-Andreote et al. 66 2015). 67

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

71 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 72 organic substances and well recognized for their ability to stabilize rhizodeposits (Pii et al. 73 74 2016; Wan et al. 2019). It was reported that goethite stabilizes organic C through physicochemical interactions (Kaiser and Guggenberger 2007). Dissolved iron can be 75 transported through the soil profile and precipitated on root surfaces and it encounters unique 76 77 biogeochemical processes closer to root zone via modulating the microbiome community composition (Somenahally et al. 2011). Although the direct effects of goethite on the 78 79 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. 80

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Properties of the rhizosphere soil are modified by a range of factors (SOC content, pH) 82 83 (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 84 85 homogenous selection (Caruso et al. 2011; Dini-Andreote et al. 2015). The selective 86 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 87 variation in the selective environment. Further, it was demonstrated that microbial 88 89 community selection is occurred at the rhizosphere and that selectivity at the rhizocompartments might act effectively as a gate for controlling entry of the microbes into the 90 root endosphere via gate filtering effect. 91

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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

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

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

112

113 Materials and methods

114 Site description and sampling

The soil was collected (0-10-cm) from an experimental plot of the Zhejiang University research station located in Zhejiang province, China. This area experiences a temperate monsoon climate with an average annual rainfall of 1453 mm and a temperature of 23°C. The plant species were dominated by *Osmanthus fragrans* (Sweet osmanthus). After removing visible stones and plant residues, the soil was air-dried and sieved <2mm. The soil was classified as an Alfisol with a sandy clay loam texture and a pH (soil: H₂O_{deionized} 1:2.5) of
6.7. Total C content (28.6 g kg⁻¹) and total N content (1.04 g kg⁻¹) were determined by dry
combustion (Perkin Elmer EA 2400, Shelton, CT, USA). Total Fe content was 19.1 g kg⁻¹
and determined by inductively coupled plasma mass spectrometer (ICP-MS; Perkin Elmer,
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 dose approximately equivalent to 10% w/w of the original Total Fe content of the soil (Hori 128 et al. 2010). Goethite was evenly mixed (applied as finely ground powder form [<0.053mm] 129 130 (Sigma-Aldrich Chemistry Company, Germany) with soil. The control (n=3) and goethite amended soil (n=3) (1 kg dry weight equivalent) were placed into rhizoboxes (30 x 14 cm) 131 which had a layer of acid-washed quartz sand (250 g) at the bottom to assist drainage and to 132 minimize the potential development of anaerobic conditions. Maize (Zea mays. L) seeds were 133 surface disinfected using 30% H₂O₂ for 30 minutes and germinated in a nursery for one week. 134 135 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 day using sterilized deionized water. The rhizoboxes were then incubated in a greenhouse at a 137 constant temperature $(25^{\circ}C)$. 138

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 144 vigorously with sterile forceps to separate the soil from the root surfaces (Chen et al. 2016). 145 This soil (rhizosphere compartment) was stored at -80°C until DNA extraction. The roots 146 designated for rhizoplane collection were recovered from the previous process (i.e., to 147 remove the rhizosphere soil) and placed in a Falcon tube with 15 ml PBS, and tightly 148 adhering microbes at the root surface were removed by sonication (Edwards et al. 2015) (30 s 149 150 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 151 152 rhizoplane compartment. The roots designated for the endosphere collection were cleaned and sonicated as described before. Two additional sonication procedures using clean PBS 153 solution were used to ensure that all microbes were removed from the root surface. The 154 sonicated roots were then stored at -80°C until DNA extraction. 155

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157 DNA extraction from rhizo-compartments

The rhizosphere and rhizoplane soil were centrifuged for 30 sec at 10000 x G to concentrate 158 159 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 160 (Mini Bead beater, Bio spec Products). Microbial DNA extraction was conducted with 0.5 g 161 of moist soil using the Fast DNA Spin Kit (MP Biomedicals, Santa, Ana, CA, USA) 162 following the manufacturer's protocol. The extracted DNA was purified using an Ultra Clean 163 DNA purification Kit (MOBIO, Carlsbad, CA, USA). The isolated DNA was eluted in 50 µl 164 165 of TE Buffer. The DNA concentration was quantified using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Finally, DNA samples were stored at -166 80°C before molecular analysis. 167

169 Gene amplification and sequencing

170 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'-171 GTGCCAGCMGCCGCGGTAA-3') linked with a specific-sample 5-bp barcode sequence at 172 the 5'end of primer, and 806R (5'- GGACTACHVGGG TWTCTAAT -3') was used as the 173 reverse primer (Caporaso et al. 2011). Each sample was amplified in triplicate, and then the 174 175 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 176 across all samples at equimolar concentrations (20 ng μ l⁻¹) into a composite sample, and the 177 178 index sequencing of paired-end 250 bp was performed on an Illumina HiSeq 2000 platform. 179 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. 180 181 (Salter et al. 2014; Vestergaard et al. 2017).

182

183 Data processing and statistical analysis

The data of bacterial 16S rRNA was processed by the QIIME 180-dev pipeline (Caporaso et 184 al. 2012). Low-quality reads (quality score < 20 read length < 200 bp and sequence errors) 185 were discarded. Chimeric sequences were identified by UCHIME (Edgar 2010) and removed. 186 The remaining high-quality sequences were clustered into operational taxonomic units 187 188 (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 189 taxonomic assignment with the RDP classifier. Taxonomy is assigned to bacterial phylotypes 190 of the Green genes database. All data sets were rarefied to 39,000 sequences per sample for 191

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

199

200 Differential abundance analysis

We used the R package "DESeq2" (Love et al. 2014) to calculate the differential abundance 201 (log₂-fold change in the relative abundance of each OTU) between the amendment and the 202 unamended rhizoboxes. We independently filtered out OTUs that were sparsely represented 203 204 across samples [i.e., those OTUs for which the DESeq2-normalized count across samples ("base Mean") was less than 0.6]. Sparse OTUs did not contain sufficient sequence counts to 205 provide statistically significant results and were removed, thus reducing the number of 206 multiple comparisons performed. We adjusted the *p*-values with the Benjamini and Hochberg 207 (BH) correction method and selected a study-wide false discovery rate (FDR) of 10% to 208 209 denote statistical significance (Love et al. 2014). We defined "responding OTUs" as OTUs with a differential abundance greater than one and an adjusted *p*-value of <0.1. 210

Changes in bacterial community composition between different compartments of goethite amended soil was further tested by Linear Discriminant Effect Size Analysis (LEfSe) (Segata et al. 2011). LEfSe analysis from the phylum to genus level was used to identify differentially abundant features as well as to encode biological consistency and effect relevance. The LDA 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.

219

220 **Co-occurrence network construction**

Microbial network analyses are required caution in drawing to obtain appropriate 221 conclusions. We combined the data of rhizo-compartments and develop the co-occurrence 222 microbial network, to compare the co-occurrence pattern of goethite added with the control. 223 224 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 225 taxa (Nannipieri et al. 2020). For network inference, all possible Spearman's rank 226 correlations between OTUs with more than five sequences were calculated (2200 OTUs). 227 This prior-filtering step removed poorly represented OTUs and reduced the complexity of the 228 229 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 230 simplified by the network representation (Faust and Raes 2012). We considered a valid co-231 232 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 233 OTUs at 90% identity, whereas the edges correspond to a significant and robust correlation 234 between nodes. To describe the topology of the resulting network, by various measures (that 235 is average path length, average node connectivity, diameter, clustering coefficient, 236 237 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 238 prediction accuracy, Wang et al. (2017) proposed the combined use different inference tools: 239 Co-occurrence Network Inference, Molecular Ecological Network Analysis, and extended 240

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).

248

249 **Results**

250 **Taxonomic analysis of the bacterial community**

There are notable differences in the percentages of relative abundance of various phyla across 251 the rhizosphere compartments. (Fig. 1a). Proteobacteria, Actinobacteria, Chloroflexi, and 252 Acidobacteria dominated in both goethite amended and control soils (Fig. 1a). The 253 254 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, 255 Actinobacteria, and Acidobacteria were mostly depleted in the endosphere of the maize root. 256 257 Actinobacteria and Choroflexi decreased in abundance towards the inner compartments for the control, and the goethite amended treatment (Fig. 1a). 258

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-265 compartments separate across the first principal coordinate, indicating that the largest source 266 of variation in root-associated microbial composition was in the rhizosphere. To test the 267 assembly process of the bacterial community in goethite amended soil, we calculated the 268 β NTI for paired samples (Fig. 1d). β NTI <-2 or >+2 is interpreted as the dominance of 269 homogeneous or variable selection, respectively. Changes in the soil environment 270 271 progressively increase the importance of assembly processes. Under the homogeneous selection scenario, the selective environment is spatially homogeneous within the period of 272 273 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, 274 taxa selected for in one place may be selected against in another place because of spatial 275 276 variation in the selective environment. Homogeneous and variable selection should cause less than and greater than expected community turnover, respectively. Our results demonstrated 277 that the β NTI score of goethite added soil was in the range of >+2 (Fig. 1d), which were 278 consistent with random phylogenetic turnover. These results indicated that bacterial 279 community assemblage processes in the goethite added soil was a variable assembly process. 280 β NTI scores of control were <+2, which indicates that stochastic processes were governed in 281 community dynamics. According to the distLM Analysis, soil bacterial community was 282 influenced by the soil variables Fe (62%), TC (52%), pH (50%), and FeOM (25%) (Table 283 284 S1).

285

286 Association of significantly enriched OTUs within the rhizosphere compartments

Differential OTU abundance analysis was used to identify enriched OTUs, with community 288 separation between goethite amended soil and the control (Fig. 2). The goethite amendment 289 soil had a high ratio of statistically significant OTUs that were depleted in all rhizosphere 290 compartments. The rhizosphere soil was the most exclusive compartment enriching for 172 291 OTUs while depleting 243 OTUs (Fig. 2). In comparison, the rhizoplane was enriched for 77 292 and depleted for 35 OTUs, while the endosphere was enriched for only 33 OTUs and 293 294 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 295 296 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 297 rhizosphere relative to the other two compartments (Fig. S1). Within the Actinobacteria, 298 taxonomic orders confirming enrichment in the rhizosphere bacterial genera include 299 Acidimicrobiales Solirubrobacterales. Within Proteobacteria 300 and the group, 301 *Xanthomonadales* and Nitrosomonadales were enriched in the rhizosphere. Enterobacteriaceae (Proteobacteria) was enriched in the rhizoplane soil in the control and the 302 goethite treatment. The bacterial lineage enriched in the endosphere was Herminlimonas from 303 the Proteobacteria. 304

305

306 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%) 312 and Acidobacteria (17%). As indicated by the edge number and density of the network in 313 goethite, amended soil was more complicated interaction than that of the control. The average 314 path length and modularity were very close in both networks, while the clustering coefficient 315 was higher in the goethite amended network (Table S2). The bacterial community exhibited 316 96% (975) of significant correlations (positive links) of 152 OTUs (nodes) in the root-317 318 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 319 320 betweenness centrality of co-occurrence network in the rhizosphere of the goethite amended soil was Solirubrobacter, Pseudomonas, and Nitrosomonas, while Rhizobales and 321 Comamonadaceae were in the control soil (Fig. 3b). Most of the negative links derived from 322 Pseudomonas (Proteobacteria) were connected with other bacterial phyla in the rhizosphere 323 of the goethite amended soil. These differences link composition in rhizosphere networks 324 between the treatment and control that were consistent with those in the network nodes. 325 However, the composition of nodes and links varied within each network. In particular, 326 Actinobacterial nodes were higher in goethite amended soil than those in the rhizosphere 327 network of the control. Simultaneously, Actinobacteria accounted for more links than 328 Actinobacteria in the control soil (Fig. 3a). 329

330

331 Discussion

332 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 341 combined zone of bacterial OTUs (Fig. 2). The majority of OTUs belonging Actinobacteria 342 343 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 344 345 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 346 (Edwards et al. 2015; Peiffer et al. 2013). The LEfSe analysis further explained microbial 347 lineages that are tightly linked to compartments (Fig. S1). It showed the enrichment of 348 Acidimicrobiales, Hemilimonas, and Enterobacteriales in the rhizosphere, rhizoplane, and 349 endosphere, respectively. Acidimicrobiales (Actinobacteria) can produce various secondary 350 metabolites with antibiotic properties (Silva-Lacerda et al. 2016). Proteobacteria are 351 considered as fast growers with the ability to utilize a majority of rhizosphere C substrates 352 (Philippot et al. 2013). Within the phylum of Proteobacteria, one of the most abundant order 353 was Enterobacteriales, which was shown to preferentially colonize in the rhizosphere of 354 several plants and was known for their beneficial effects on plant growth and/or protection 355 356 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 357 other organisms by secreting polysaccharides and N fixating (Karthikeyan et al. 2007) and 358 this can inhibit the growth of pathogenic bacteria. Modulations of the root-associated 359 microbiome across the three rhizo-compartments provide evidence for the rapid selection of 360 root-associated microbiomes from the soil. 361

363 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.

371

372 Although microbial α -diversity pattern holds the same pattern from the rhizosphere to the 373 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 374 goethite likely depends on a "filtration effect" (Dibbern et al. 2014). Here, the rhizosphere 375 preferentially selects microbiome by recruitment to the vicinity of the root and followed by a 376 transition from the rhizosphere to the endosphere (Edwards et al. 2015; Valverde et al. 2014). 377 The ordination of beta diversity results revealed a clear separation between the different 378 rhizosphere compartments as well as between the control and the goethite treatments. This 379 380 indicates that selective changes in the bacterial community structure in the rhizosphere compartments were influenced by heterogeneity (availability and accessibility of C sources) 381 of soil. Recent studies revealed that bacterial community associated with root is not random, 382 383 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 384 process of bacterial community assemblages in goethite added to the soil (Fig. 1d). 385

Changes in bacterial composition are related to the heterogeneity of soil that allowed 387 microbes to exploit spatially structured unique niches and communities towards the selective 388 environment (Dini-Andreote et al. 2015; Mendes et al. 2014). It is well established that 389 edaphic factors (e.g., soil pH, SOC) were defined the micro-environment for microorganisms, 390 thus determining their population dynamics and community composition (Dini-Andreote et 391 al. 2015; Nuccio et al. 2016). Our analysis showed that goethite built up unique niches with 392 393 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 394 395 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 396 present study support that variable selection of microbial composition occurs due to the 397 398 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 399 rhizosphere microbiota. 400

401

402 Goethite modified Co-occurrence network of rhizosphere

Network analysis was used to determine the interactions between microbial communities 403 (Freilich et al., 2011). Rhizobales and Comamonadaceae were the keystone microorganisms 404 405 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 406 Solirubrobacter, Pseudomonas, and Nitrosomonas, which had the highest number of central 407 nodes observed in the rhizosphere of goethite amended soil (Fig. 3; Table S2). Goethite 408 409 modulated keystone genera were associated with rapid C cycling (Hinsinger 2009; Whipps 2001). Importantly, *Pseudomonas* (within Proteobacteria) had greater betweenness centrality 410 and degree of negative links with most other taxa in the goethite amended the soil (Fig. 3; 411

Table 2). The selection and competitive colonization power of bacteria depend on metabolic 412 capacities [the ability to utilize root exudates (e.g., amino acids and polysaccharides)] and 413 growth rate (Lugtenberg and Dekkers 1999; Walker et al. 2004). It was reported that 414 formation of interconnected microbial assemblages with keystone species such as 415 Pseudomonas was due to (i) competition for space and nutrients (ii) modification of the host 416 physiology (iii) modulation of microbial community composition, providing a competitive 417 418 advantage to other organisms in the rhizosphere (Hassani et al. 2018; Lugtenberg and Dekkers 1999; Tyc et al. 2017). Moreover, Pseudomonas act as an important colonizer that 419 420 can colonized with specific bacterial families including Oxalobacteraceae, Bacillaceae, and Burkholderiaceae (Hassani et al. 2018; Lugtenberg and Dekkers 1999). Thus, interactions of 421 rhizodeposits with goethite by creating the C limited conditions affected the keystone 422 423 bacterial genera with a narrow niche.

424

425 Conclusions

This is the first study showing the effect of the goethite amendment in soil on the 426 composition of bacteria and network complexity in different rhizosphere compartments, 427 including rhizosphere rhizoplane and endosphere. Bacterial OTUs enrichment in each 428 rhizosphere compartment was reduced towards the endosphere, revealing selective 429 430 colonization in the rhizosphere and that it acted as a gate selection for the bacterial colonization in the rhizoplane and endosphere. Cyanobacteria were highly abundant in the 431 endosphere, indicating symbiosis associations with other organisms by secretions of 432 polysaccharides and N fixation. Variable selection processes dominated the assemblage of 433 bacterial communities in goethite added to the soil by the modulation of the bacterial 434 community. Total C, total Fe, and FeOM were the main soil properties that caused a spatially 435 heterogeneous environment that leads to the variable selection process. Bacterial 436

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.

443

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Fig. 1 Relative to bacterial abundance of community composition (a). α -diversity index 579 across the rhizosphere, rhizoplane, and endosphere. α -diversity was calculated by Shannon 580 index (b). The horizontal bars within boxes represent median. The tops and bottoms of boxes 581 represent 50th and 50th quartiles, respectively. Bacterial community differentiation between 582 all samples of principal component analysis plots (PCoA) for goethite and control treatments, 583 across the rhizosphere, rhizoplane, and endosphere (c). Distribution of beta Nearest Taxon 584 585 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 586 587 horizontal dotted blue line shows the 95% confidence intervals around the expectation under neutral community assembly. 588

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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.

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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.