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1	Abiotic and biotic regulation on carbon mineralization and stabilization in paddy soils along
2	iron oxide gradients
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25 Abstract

Iron (Fe) oxides in paddy soil regulate soil organic carbon (C) content via the balance between 26 stabilization by adsorption and/or co-precipitation, and mineralization by shifting the microbial 27 community. The contributions of abiotic and biotic mechanisms involved in C processes of 28 29 stabilization and decomposition, however, remains poorly understood. We examined the mineralization and stabilization of maize straw-derived C (δ^{13} C=5000 ‰), and soil priming effects 30 (PE), along with the soil microbial community structure in four paddy soils, with Fe oxide 31 concentrations ranging from 13.7 to 55.8 g kg⁻¹ (Fe-13, Fe-25, Fe-42, and Fe-55). Fe-55 stabilized 32 20.5 mg 13 C kg⁻¹ soil of the maize straw-derived C, being significantly greater (P<0.05) than Fe-33 13 (5 mg¹³C kg⁻¹ soil). The high C:Fe molar ratio of Fe-55 suggests the main pathway of 34 stabilization of the maize straw-derived C via co-precipitation as Fe-OM. The paddy soil with the 35 highest Fe content (Fe-55) had 12-16% lower straw mineralization and 8-11% lower PE than Fe-36 13 during the first 7 days of incubation. Soils with low Fe oxide concentration had a lower 37 microbial diversity but increased nodes and edges in their co-occurrence network and were 38 dominated mainly by Proteobacteria. Random forest analysis further revealed that Proteobacteria 39 and Actinobacteria (the keystone species, i.e., Gaiella) gave the largest contribution to maize-40 straw mineralization and priming, while fungi, i.e., genus Mortierella, contributed to the 41 stabilization of C by the development of aggregates through their filamentous hyphae. This study 42 confirmed that the concentration of Fe oxide in paddy soils plays a central role in C sequestration 43 via biotic and abiotic processes, including i) modulation of the community diversity and 44 composition, especially the abundance of the keystone microorganisms, and ii) physicochemical 45

stabilization of maize-straw derived C through the formation of Fe-OM complexes via coprecipitation, thereby limiting the availability of C substrate for microbial mineralization and
concomitantly lowering the soil priming effect.

49

Keywords: Fe organic matter complexes, Microbial co-occurrence network, O2PLS
 analysis, Random forest analysis, ¹³C labeled straw, Priming effects

52

53 **1. Introduction**

Soil management to increase soil organic matter (SOM) storage had been suggested as a 54 countermeasure to mitigate the increasing CO₂ concentration in the atmosphere (Jeewani et al., 55 56 2020a; Lal, 2004; Minasny et al., 2017). Paddy soils account for approximately 40% of China's soil carbon (C) sequestration potential (Pan et al., 2004; Atere et al., 2020). SOM storage in paddy 57 58 soils is promoted through enhanced physical protection within aggregates (Jastrow et al., 2007; Chen et al., 2020; Duan et al., 2020), physicochemical stabilization by the formation of organo-59 mineral complexes (Jeewani et al., 2020a; Kleber et al., 2015; Yu et al., 2017), and changes in the 60 soil microbial community (Butler et al., 2003; Kandeler et al., 2008). The mechanisms for the 61 physicochemical stabilization and concomitant changes of microbial processing of C in rice 62 paddies, however, requires an improved understanding to optimize soil and SOM management. 63

An important physicochemical stabilization mechanism of SOM in the soil is through the interaction with Fe oxides (Kaiser and Guggenberger, 2000; Boyd and Ellwood, 2010; Jeewani et al., 2020a). There is a growing pool of literature showing that Fe(III) polycations bind with SOM to form Fe-organic matter complexes (Fe-OM), which stabilize both native SOM and new inputs of C in soil (Chen et al., 2020; Duan et al., 2020; Jeewani et al., 2020a). The formation of Fe-OM complexes is mainly attributed to two main processes, adsorption, and co-precipitation (Kleber et al., 2015; Chen et al., 2020). The formation and stabilization Fe-OM complexes in paddy soil
depend on pH, C:Fe molar ratio, and/or redox potential (Chen et al., 2014; Liu et al., 2019). The
dominance of either co-precipitation vs. adsorption processes can be assessed by the C:Fe molar
ratio of the Fe-OM complex. Ratios <1 indicate that C is stabilized via adsorption while ratios >6
are due to co-precipitation (Wang et al., 2017). The active Fe ratio is another important index that
influences soil physicochemical properties such as organic matter retention, surface charge,
specific surface area and aggregate formation (Duiker et al., 2003; Wan et al., 2019).

The presence of Fe oxides (e.g., goethite) can shift the soil microbiome by redox processes 77 (Weber et al., 2006; Chen et al., 2020). Direct contact between the microorganism and the solid-78 Fe(III) oxide can form conductive cellular 'nanowires', creating a bridge for the reduction of 79 Fe(III) oxides by Geobacter (Weber et al., 2006). Both Geobacter and Shewanella are key 80 microbial genera that facilitate electron transfer by functioning as an electrical conduit to the 81 Fe(III) oxide surface (Weber et al., 2006). Fe has been shown to shift the rhizosphere community 82 from being dominated by bacteria to fungi in an Alfisol, impacting the mineralization of 83 rhizodeposits (Jeewani et al., 2020a; Jeewani et al., 2021). In particular, Fe oxides shift the 84 microbial community composition of soil dominated by the bacterial phyla Proteobacteria, 85 Firmicutes, and Actinobacteria (these phyla are recognized drivers of C mineralization) towards 86 K-strategists which have the ability to explore C sources in oligotrophic conditions (Garcia-Pausas 87 88 and Paterson, 2011; Chen et al., 2014; Jeewani et al., 2020a).

Although significant advances have been made to better understand the modulation of the microbial community composition and activity by Fe oxides in soil, a paucity of knowledge exists on the role of Fe oxides in SOC storage via mediating bidirectional processes of C decomposition and stabilization. Specifically, the contributions of abiotic (physicochemical) and biotic (microbial community) processes to mineralization and stabilization of C as governed by Fe oxides are poorly understood. The key mechanisms by which Fe oxides regulate C contents in soils include; i) fostering soil aggregation and thus physically protecting SOM from biological degradation (Barral
et al., 1998; Jeewani et al., 2020a), ii) stabilizing SOM by adsorption, and co-precipitation and iii)
enabling microbially mediated electron transfer via the donation of electrons to oxidants and
accepting an electron from microbes (Weber et al., 2006; Chen et al., 2020).

99 The study aimed to elucidate the abiotic and biotic mechanisms by which Fe oxides in paddy soils control the decomposition and stabilization of ¹³C labeled substrate, maize-straw as a 100 proxy. Also, native SOM mineralization by maize-straw, i.e., priming effects, was determined as 101 well. We hypothesize that maize straw mineralization is lower, and its stabilization is greater with 102 103 increasing soil Fe oxide concentration due to co-precipitation resulting in Fe-OM complexes. We also hypothesize that increasing Fe oxide concentration in the soil will modulate the microbial 104 communities and their processes associated with the geochemical cycling of C. Finally, we 105 106 hypothesize that abiotic processes mainly account for straw stabilization, while C loss via mineralization is regulated by both biotic and abiotic variables. To test these hypotheses, we 107 conducted an incubation study with four paddy soils having a natural gradient of Fe oxide 108 109 concentrations to quantify the contribution of abiotic and biotic processes to C mineralization and stabilization. 110

111

112 **2. Materials and methods**

113 2.1 Soil sampling

114 Soils were Gleyic Stagnic Anthrosol (FAO, 2015) developed under long-term rice (*Oryza* 115 *sativa*) paddies (from the 0–20 cm layer) in the subtropical region of Hunan Province, China. The 116 region has a mean annual temperature of 17.7 °C and rainfall of 1402 mm. The soils were chosen 117 to achieve four different contents of Fe oxides and oxyhydroxides, thereafter termed as Fe oxides. The concentrations of soil Fe oxides were 13.7 Fe g kg⁻¹ (referred to as Fe-13), 25.8 g kg⁻¹ (Fe-25), 42.7 g kg⁻¹ (Fe-42), and 55.8 g kg⁻¹ (Fe-55) (Table S1).

120

121 2.2 Experimental design

The soil samples had visible plant roots, which were manually removed. Thereafter, the 122 soil was dried to 40% water holding capacity and sieved (<2 mm). The soils were pre-incubated 123 at 25°C for 14 days before starting the experiment. To investigate the mineralization, stabilization, 124 125 and priming effects of maize-straw derived C from the 4 paddy soils (i.e., 4 Fe oxides concentrations), a 56-day incubation experiment was established, where each soil had either a 126 control (no amendment) or ¹³C labeled straw amendment (n=3), to make a total of 24 incubation 127 128 jars. To establish the incubation, each beaker (100 mL) contained 40 g of oven-dry equivalent soil was placed within a 1L glass jar containing 10 mL water on the base to maintain humidity. The 129 incubation jar also contained a CO₂ trap (20 mL 1 M NaOH), which was replaced at several 130 sampling times (1, 3, 7, 14, 28, and 56 days). Labeled maize-straw with a ¹³C enrichment of 131 δ^{13} C=5000 ‰ was collected from a previous CO₂ labeling experiment. The ¹³C labeled maize-132 133 straw was oven-dried at 105°C for 2 h, then at 60°C for 48h, and thereafter ground to pass a 1mm sieve. Maize straw was applied at 500 µg C g⁻¹ soil by evenly mixing into the 40 g of soil, 134 representing an equivalent of 1275 kg dry maize-straw ha⁻¹. The jars were sealed with a rubber 135 136 bung and incubated at 25°C for 56 days. Three blank jars contained only water and CO₂ traps. The incubation jars were opened periodically to maintain aerobic conditions. 137

138

139 *2.3 Soil physical and chemical analyses*

Soil pH was measured at a soil: water ratio of 1:2.5 (w/w) using a micro-electrode (Seven
Compact, Mettler Toledo Inc., Switzerland), and microbial biomass C (MBC) and dissolved

organic carbon (DOC) were determined using the chloroform fumigation extraction method (Vance et al., 1987). Total soil C and N content (air-dried, milled <200 μ m) was determined by dry combustion (Perkin Elmer EA2400, Shelton, CT, USA). The natural δ^{13} C abundance of the soils (air-dried, milled <200 μ m accurately weighed about 0.2 mg into tin capsules) was determined using an isotope ratio mass spectrometer (Thermo Fisher Scientific, DELTA V plus IRMS, Bremen, Germany) coupled with an elemental analyzer (EA NA1500 - EA 1110 device, Carlo Erba and Thermo Fisher Scientific, Bremen, Germany) according to Meng et al. (2013).

The total Fe oxide concentration of each soil was determined after acid digestion with a 149 150 concentrated mixture of HF, HClO₄, and HNO₃ (Zhou et al., 2007). Dithionite-citrate-bicarbonate (DCB) extraction was used to quantify total reactive soil Fe oxides (Fed) (Lalonde et al., 2012; 151 Wang et al., 2017). In brief, freeze-dried soil (0.50 g) was mixed with 30 mL of buffer solution 152 153 (0.27 M trisodium citrate and 0.11 M sodium bicarbonate, pH 7.3) in 50 mL centrifuge tubes and placed in a water bath (80 °C). Sodium dithionite (0.50 g) was added to the mixture, and it was 154 maintained at 80°C for 15 min. As a control, soils were extracted by sodium chloride (NaCl) 155 156 instead of trisodium citrate and sodium dithionite, at an equivalent ionic strength under the same conditions. Then, the solution was separated by centrifugation at 4000 for 10 min. The residue 157 was washed three times, each with 5 mL of deionized water, and then freeze-dried. Total carbon 158 (TC) and total nitrogen (TN) and δ^{13} C isotopic composition in the precipitate were analyzed by 159 isotope ratio mass spectrometer (Thermo Fisher Scientific, DELTA V plus IRMS, Bremen, 160 161 Germany) coupled to an elemental analyzer (EA NA1500 - EA 1110 device, Carlo Erba and Thermo Fisher Scientific, Bremen, Germany). Organically complexed Fe oxides (Fe_p) were 162 extracted using sodium pyrophosphate (Keiluweit et al., 2015; Wang et al., 2017), and amorphous 163 164 soil Fe (Fe₀) was extracted with ammonium oxalate (Keiluweit et al., 2015; Wang et al., 2017). For the analysis of the soluble Fe, washings and supernatants were combined and acidified to pH 165 2.0. The combined samples were filtered using a pre-combusted 0.45-µm PTFE membrane filter, 166

and Fe was determined using ICP-MS. The calculations can be found in the supplementaryinformation.

169

170 2.4 Analysis of $\delta^{13}C$ -CO₂

For the determination of C mineralization, CO₂ was collected in traps filled with 1M 171 NaOH, which were changed after 1, 3, 7, 14, 28, and 56 days. At sampling, a 5 mL aliquot of trap 172 solution was diluted with 10 ml H₂O and titrated against standardized 0.5 M HCl using an Easy 173 174 Plus auto titrator (Mettler Toledo, Greifensee, Switzerland) to determine the total CO₂ evolved (Tinsley et al., 1951). To determine the δ^{13} C-CO₂ from the trapped CO₂, an 8 ml aliquot of the 175 trap solution was added to 8 mL of 1.5 M BaCl₂ into a 50 mL falcon tube and incubated at 25 °C 176 177 for 0.5 h to allow precipitation. The resulting BaCO₃ precipitate was then filtered, rinsed three times with deionized H₂O, and freeze-dried overnight in a falcon tube (Luo et al., 2011). The 178 precipitates were carefully removed from the falcon tube, accurately weighed (about 0.2 mg) into 179 tin capsules, and analyzed using a stable isotope ratio mass spectrometer (Thermo Fisher 180 Scientific, DELTA V plus IRMS, Bremen, Germany). 181

182

183 $2.5 \,\delta^{13}C$ calculations

184 The mineralization of the straw was distinguished from SOM mineralization based on the 185 changes of stable isotopic composition (δ^{13} C) over time as follows.

186
$$\delta^{13}C(\%_0) = [\left(\frac{\text{Rsample}}{\text{R}_{\text{VPDB}}}\right) - 1] \times 1000$$
 (1)

187 R_{sample} is the mass ratio of ¹³C to ¹²C of the sample, and R_{PDB} is the mass ratio of ¹³C to ¹²C of the 188 Vienna Peedee belemnite (PDB) standard (0.0112372). According to Luo et al. (2011), the labeled 189 ¹³C (%) was then estimated from:

190
$$CO_2^{13}C(\mu g g^{-1} \text{soil}) = CO_2^{13}C(\%) \times \text{Total evolved } CO_2 - C(\mu g g^{-1} \text{soil})/100$$
 (2)

191
$$CO_2^{13}C(\%) = (\delta C_{\text{treatment}} - \delta C_{\text{original}})/\delta C_{\text{straw}} - \delta C_{\text{original}}) * 100$$
 (3)

192 Where $CO_2^{13}C$ (%) is the proportion of evolved CO_2 from the added maize-straw, $\delta C_{\text{treatment}}$ is the

193 $\delta^{13}C$ (‰) of evolved CO₂ from soil amended with maize straw, $\delta C_{\text{original}}$ is the $\delta^{13}C$ (‰) of original

soil C before maize-straw was added. δC_{straw} is the $\delta^{13}C$ (‰) from the added maize-straw. Thus,

195 the CO₂-C produced by the straw during the incubation was calculated.

196
$$CO_2 - C_{\text{treatment}-1}(\mu g C g^{-1} \text{Soil}) = \text{Total } CO_2 - C_{\text{treatment}} - \text{Total } CO_2 - C_{\text{maize straw}}(4)$$

197 Where, CO₂-C_{treatment-1} is the non-isotopically labeled CO₂-C evolved from maize-straw amended

soil, Total CO₂-C treatment is the soil evolved CO₂ from the treatment, and Total CO₂-C maize straw

is the evolved CO_2 derived from the isotopically labelled maize-straw.

200 The primed soil CO₂-C with the addition of maize-straw was calculated from:

201 Primed soil
$$CO_2 - C(\mu g C g^{-1} Soil) = CO_2 - C_{treatment-1} - Total CO_2 C_{control}$$
 (5)

202 Where, CO_2 - $C_{control}$ is total CO_2 -C evolved from control.

203

204 2.6 DNA extractions sequencing and processing of sequencing data

After the incubation, 0.50 g of soil was freeze-dried and extracted using a Fast DNA Spin Kit (MP Biomedicals, Santa Ana, CA, USA). The extracted DNA was dissolved in 50 μl of TE buffer, and the concentrations of DNA were quantified using a Nanodrop 2000 (Thermo Scientific, Willmington, USA). The bacterial 16S rRNA gene fragments were amplified using primer sets targeting the V4-V5 variable region. The forward primer is 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 (Bates et al., 2011). The ITS1 region was amplified by PCR for fungal genes using the 5'-212 CTTGGTCATTTAGAGGAAAAGTAA-3' primer 5'-213 forward and 214 GCTGCGTTCTTCATCGATGC-3' reverse primer (Bates et al., 2011). Samples were amplified in three replicates, and the three reaction products were pooled and purified using Agincourt 215 216 Ampure XP beads (Indianapolis, USA). All amplicons were pooled across all samples at equimolar concentrations (20 ng μ l⁻¹) into a representative sample, and the index sequencing of 217 218 paired-end 250 bp was performed on an Illumina HiSeq 2000 platform. Bacterial and fungal DNA amplification and sequencing were performed by Major Bio, Inc. (Shanghai, China). 219

220 The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered by trimmomatic, and connected by FLASH using the following steps. The 250 bp reads were cut-off 221 at the site, receiving an average quality score of less than 20 over a 50 bp sliding window, and the 222 223 cut-off reads shorter than 50 bp with the reads containing mismatch characters. Then, sequences longer than ten bp were assembled according to their overlapped sequence. The maximum 224 mismatch ratio of the overlap region was 0.2, and those mismatched reads were discarded. Finally, 225 samples were differentiated according to the barcode and primers, then adjust the sequence 226 direction and exact barcode matching. UPARSE (version 7.1, http://drive5.com/uparse) was used 227 228 to cluster the operational taxonomic units (OTUs) with 97% similarity cut-off. Chimeric 229 sequences were removed. The RDP classifier (http://rdp.cme.msu.edu/) was used to examine the 230 taxonomy of each OTU representative sequence against the 16S rRNA database using a 231 confidence threshold of 0.7 (Chen et al., 2017). Total datasets were rarefied to 39,000 sequences per sample for the bacterial α - and β -diversity analyses. α -diversity of bacterial and fungal 232 communities was calculated using the Shannon index. β-diversity was analyzed using principal 233 234 coordinate analyses (PCoA). Relative effects of soil variables such as TC, C:N ratio, Fe_d, C:Fe molar ratio, DOC, and pH on soil bacteria and fungi communities were analyzed by distance-235

based linear model multivariate analysis (distLM) using the distLM forward3 software (Anderson,
2003).

238

239 2.7 Bidirectional orthogonal partial least squares

The bidirectional orthogonal partial least squares (O2PLS) analysis has been used to uncover the large-scale organization of metabolic networks (Trygg, 2002). SIMCA-P 14 (Version 14.1.0.2047) software was used to determine the O2PLS analysis to correlate the microbial taxa to the priming, substrate mineralization, and substrate-derived C stabilization. The Y-matrix was designed as the C dynamics dataset, and the X-matrix was intended as the microbial community dataset (Trygg, 2002).

246

247 2.8 Co-occurrence network (Co-Net)

A co-occurrence network was established to visualize the co-occurrence pattern of 248 249 different genera using the Cytoscape. While constructing networks, the OTUs with relative 250 abundances greater than 0.01% were kept, and the dissimilarity threshold to the maximum value 251 of the KLD matrix and the Spearman's correlation threshold to 0.8 were calculated. For each edge and measure, permutation and bootstrap distributions were generated with 100 iterations. The 252 253 measure-specific P-value was computed as the area of the mean of the permutation distribution under a Gauss curve generated from the mean and standard deviation of the bootstrap distribution. 254 The *P*-values were adjusted using the Benjamini-Hochberg procedure (Benjamini and Hochberg, 255 1995). Finally, only edges supported by two measures and with adjusted P-values below 0.05 were 256 257 retained. The nodes in the constructed networks represent OTUs, and edges represent strong and 258 significant correlations between OTUs. Network visualizations were conducted using Gephi (Bastian et al., 2009) and Cytoscape 3.5.1 (Shannon et al., 2003). The Network Analyzer tool was 259

260 used to calculate the network topology parameters. Genera with the highest betweenness centrality scores were considered keystone species (Martín González et al., 2010). 261

262

2.9 Random forest analysis and structural equation modeling 263

The relative importance of edaphic factors on the substrate mineralization and stabilization 264 was evaluated using random forest analysis (Liaw and Wiener, 2002). Edaphic variables 265 validation of soil physicochemical variables (C:N ratio, Fed, C:Fe molar ratio, DOC, and pH) and 266 267 biological variables (bacterial diversity, fungal diversity, the composition of Proteobacteria, Actinobacteria, and Ascomycota) were used in the random forest analysis to assess their relative 268 contributions/influences to the substrate-derived C mineralization, priming, and stabilization. 269

Structural equation modeling (SEM) was conducted in Amos 18.0 (IBM, Chicago, IL, 270 271 USA) to investigate the pathways and drivers of stabilization and mineralization of substrate in the soils with respect to direction, magnitude, and effect relationships. In the SEM, chi-square 272 was used to evaluate model fitting, while a non-chi-squared test (P > 0.05) indicates a good fit of 273 the model to the data. The analysis of correlation metrics calculated the coefficients of each path. 274 The path in this model was considered significant with a P < 0.05. 275

276 SPSS 20 (SPSS, Inc., Chicago, IL, USA) was used to analyze all non-microbial data. Oneway ANOVA was used to assess the difference of mineralization between soils. We examined the 277 relations between variables by Pearson's correlation analysis (with a two-tailed test), after 278 279 performing a Kolmogorov-Smirnov (K-S) test to determine whether the data were normally distributed. Residues were analyzed for normal distribution and homogeneity by Shapiro-Wilk 280 and Levene's tests. If conditions were met, the Tukey Post-hoc test was performed to reveal 281 differences between the treatments. 282

284 **3. Results**

285 *3.1 CO*₂ *efflux*

The higher concentration of Fe oxides in paddy soil resulted in lower total soil CO₂ efflux following the addition of maize straw (Fig 1). The highest maize-straw derived ¹³CO₂ efflux from each incubation period was found in Fe-13 soil (P < 0.05), while the lowest (P < 0.01) substratederived CO₂ efflux, as well as the smallest PE, was observed in Fe-55 (Fig. 1). Maize-straw derived ¹³CO₂ efflux increased in Fe-13 (from 31% to 33%) between days 3 and 7.

291

3.2 Influence of Fe oxides on the stabilization of maize-straw derived C in soil

To quantify the straw-derived C stabilization in soil, the content of ¹³C in the bulk soil and 293 on the Fe-OM complex was assessed (Fig. 2). With increasing concentrations Fe oxides of the 294 soil, ¹³C retention in both bulk soil and on the Fe-OM complex increased (Fig. 2A). The maize 295 straw amendment resulted in a ¹³C content in Fe-OM of 20.5 mg kg⁻¹ in Fe-55, which was 4.1-296 fold greater than in the Fe-13 soil. Concurrently, the Fe-bound C content increased with increasing 297 concentration of Fe oxide in soil (Fig. 2B), indicating enhanced Fe-OM complexation. C:Fe molar 298 299 ratios with values <1 and >6 were designated complexes derived by sorption and co-precipitation, respectively (Wang et al., 2017). The C:Fe molar ratio of Fe-OM complexes of soils varied 300 between 4.08 and 6.5 (Fig. 2C), and for the Fe-42 and Fe-55 soils, C:Fe molar ratios of 6.2 and 301 6.5 suggested that co-precipitation was the dominant process in the formation of Fe-bound OC. 302

303

304 *3.3 Soil microbial community along with the Fe concentration*

Both bacterial (Fig. 3A) and fungal (Fig. 3B) diversity (Shannon index) increased with
increasing Fe oxide concentration of the soil. Compositional dissimilarities of microorganisms

between treatments were assessed by PCoA analysis, and it showed the loadings of PC1 39.2%
and PC2 24.2% for bacteria, and PC1 31.5% and PC2 21.2% for fungi (Figs. 3C and 3D). A
considerable variation in microbial communities occurred across the four soils with different Fe
oxide concentrations. The microbial groups in both Fe-13 and Fe-25 were grouped and separated
from Fe-42 and Fe-55.

The relative abundance of bacterial and fungal communities at the phylum level showed 312 313 notable differences between soils with different concentrations of Fe oxides (Figs. 3E and 3F). Proteobacteria was the most dominant phylum in all soils, which accounted for 41% of the whole 314 315 bacterial sequences in Fe-13 and 27% in Fe-55. Bacterial phyla of Acidobacteria, Actinobacteria, and Rokubacteria sere also prominent, and their relative abundance increased with increasing 316 concentrations of Fe oxide (Fig. 3E). Among the fungal phyla, Ascomycota was dominant in all 317 soils, and its relative abundance increased from 57% in Fe-13 to 74% in Fe-55. There was a 318 decrease in the relative abundance of the phylum Basidiomycota with increasing concentrations 319 of Fe oxide, while a phylum Unclassified-K-Fungi increased with increasing Fe oxide 320 concentration (Fig. 3F). 321

DistLM analysis showed that the soil bacterial community was affected by TC (21%), C:N (15%), Fe (14%), C:Fe molar ratio (13%), and pH (13%). The fungal community was influenced by TC (23%), C:N ratio (15%), Fe_d (15%) C:Fe molar ratio (12%), and DOC (7%). Fe oxide concentration in soil contributed to both bacteria and fungi, with an influence of 27% (Table 2).

326

327 *3.4 Co-occurrence network*

In the analysis of co-occurrence networks, increasing concentrations of Fe oxides modified the interactions amongst bacteria and fungi (Fig. 4). Fewer links were observed in the network of Fe-42 and Fe-55 (high Fe oxide concentration) than in Fe-13 and Fe-25 (low Fe oxide

concentration) (Table S2). The number of positive links decreased, and negative links increased 331 in the bacterial network in soils containing higher Fe oxide concentrations (Table S2). Based on 332 the 'betweenness centrality scores', the top-three genera identified as keystone taxa in soil with 333 high Fe oxide concentrations were subgroup 6 (affiliated to phylum Acidobacteria) and the order 334 Rokubacteriales (affiliated with the phylum Rokubacteria). Keystone genera in the bacterial 335 network from Fe-13 and Fe-25 were Gaiella (affiliated to phylum Actinobacteria) and 336 337 Methylogigellaceae (affiliated to phylum Proteobacteria). In contrast to the bacterial network, greater positive links, and fewer negative links in the fungal co-occurrence network were found 338 339 in Fe-45 and Fe-55 compared to Fe-13 and Fe-25. Cercophora (affiliated with the phylum Ascomycota) and Unclassified-K-Fungi were keystone genera for Fe-13 and Fe-25. In contrast, 340 Mycoarthris (affiliated with the phylum Ascomycota) and Mortierella (affiliated with the phylum 341 342 Mortierellomycota) were keystones in the high Fe containing co-occurrence network.

343

344 3.5 Association between C dynamics and the keystone microbiota

345 O2PLS analysis was used to identify the microbial community associated with maizestraw derived C mineralization and the PE. The following conditions were considered for analysis: 346 (i) variable influence projection (VIP) value ≥ 1.3 ; (ii) correlation coefficient (P < 0.05); (iii) the 347 number of microbial taxa being highly correlated ($r \ge 0.7$). Based on those criteria, seven genera 348 were selected as key genera related to the PE and maize-straw mineralization (Table 3). The 349 maize-straw derived C mineralization was correlated with the bacterial genera Nitrosospira, 350 351 MND1, Gaiella, and fungal genera belonging to the family Glomeraceae. The PE was highly correlated with the bacterial genera Gaiella, Rokubacteriales, Nitrosospira, and the genera of 352 *Unclassified_K_Fungi* (Table 3). 353

356 There were significant correlations between Fe oxides (Fe_d, Fe_o, and Fe_p), Fe-bound OC, and the molar ratio of C:Fe, suggesting that the inherent Fe oxide content has marked effects on 357 the preservation of maize-straw derived C (Table 1). There were significant correlations between 358 soil pH and Fe bound OC, Fe_o:Fe_d, and the molar ratio of C:Fe. Similarly, there was a significant 359 positive correlation between TC and the amount of Fe-bound OC (r = 0.987, P < 0.01) and Fe 360 361 bound C:Fe molar ratio (r = 0.939, P < 0.01). Significant positive correlations were also observed between Fe_d with the amount of Fe-bound OC (r = 0.745, P = 0.01) and the C:Fe molar ratio (r = 0.745, P = 0.01) and the C:Fe molar ratio (r = 0.745, P = 0.01) and the C:Fe molar ratio (r = 0.745, P = 0.01) and the C:Fe molar ratio (r = 0.745, P = 0.01) and the C:Fe molar ratio (r = 0.745, P = 0.01) and the C:Fe molar ratio (r = 0.745, P = 0.01) and the C:Fe molar ratio (r = 0.745, P = 0.01) and the C:Fe molar ratio (r = 0.745, P = 0.01) and the C:Fe molar ratio (r = 0.745, P = 0.01) and the C:Fe molar ratio (r = 0.745, P = 0.01) and the C:Fe molar ratio (r = 0.745, P = 0.01) and the C:Fe molar ratio (r = 0.745, P = 0.01) and the C:Fe molar ratio (r = 0.745, P = 0.01) and the C:Fe molar ratio (r = 0.745) and P = 0.01 (r = 0.745) (r = 0.74362 0.979, *P* < 0.05). 363

Random forest analysis allowed us to investigate the relative importance of 364 physicochemical and biological factors on substrate mineralization, the PE, and maize-straw 365 derived C stabilization. It showed that maize-straw mineralization and the PE were mainly 366 governed by the involvement of soil biological factors such as bacterial and fungal diversity. 367 Maize-straw-derived C stabilization was regulated mainly by Fed (8.9% increase of the mean 368 369 squared error (IncMSC), C:Fe molar ratio (7.7% IncMSC), C:N ratio (6.7% IncMSC), and DOC 370 (5.9% IncMSC) (Fig. 5). SEM also supported the notion that the key pathways of substratederived C stabilization and mineralization were controlled by physicochemical processes and 371 372 microbial interactions (Fig. S1). C:Fe molar ratio exerted a direct positive effect on substrate 373 stabilization, as measured by CO₂ efflux (+0.81, P < 0.001) (Fig. S1). Fe_d exerted a positive effect 374 on bacterial diversity (+0.69, P < 0.001), network co-occurrence (+0.59, P < 0.001) and fungal diversity (+0.76, P < 0.01). The bacterial diversity, network co-occurrence, and fungal diversity 375 had a positive influence on substrate mineralization (+0.71, P < 0.001) and soil priming (+0.54, P376 377 < 0.05). However, bacterial diversity (-0.43, P < 0.01) and fungal diversity (-0.89, P < 0.05) had a negative impact on substrate stabilization. 378

379

380 **4. Discussion**

381 *4.1 Contribution of abiotic variables on maize-straw stabilization*

We evaluated the role of Fe oxide on the stabilization of maize-straw derived ¹³C and 382 showed that increasing natural concentrations of Fe oxides from 13.7 to 55.8 g Fe kg⁻¹ soil resulted 383 in a 3.2-fold increase in ¹³C stabilization across the 56-day incubation (Figs. 2A and 2B). 384 Increasing concentrations of Fe oxides also resulted in an increased C:Fe molar ratio, supporting 385 386 the hypothesis that higher concentrations of Fe oxides can stabilize a greater quantity of new C. Soil organic carbon can be associated with iron oxides through surface adsorption and co-387 precipitation (Chen et al., 2014). The molar ratio of Fe-bound OC to total Fe oxides (C:Fe) was 388 389 proposed to quantify the organic carbon bound to iron oxides (Chen et al., 2014; Lalonde et al., 2012). C:Fe molar ratios with values <1 and >6 were designated complexes derived by sorption 390 and co-precipitation, respectively (Wang et al., 2017). Overall, the C:Fe molar ratio of Fe-OM 391 complexes of soils varied between 4.08 and 6.5 (Fig. 2C), and for the Fe-42 and Fe-55 soils, C:Fe 392 393 molar ratios of 6.2 and 6.5 suggested that co-precipitation was the dominant process in the 394 formation of Fe-bound OC. Our results are consistent with prior observations that the retention of maize-straw derived C via co-precipitation with Fe strongly increased with increasing C:Fe molar 395 ratio (Sodano et al., 2017). Henneberry et al. (2012) reported that SOM was strongly bound due 396 397 to co-precipitation with Fe, but the binding strength was controlled by the presence of carboxylic acid functional groups. Therefore, co-precipitation likely represents the key process in controlling 398 C retention in Fe oxide rich soils and is of particular interest for our understanding of SOM 399 400 stabilization and turnover mechanisms in paddy soils (Fig. 6). According to the correlation matrix for the soil properties, we found that the molar ratio of C:Fe had a significant correlation with TC, 401 Fed, and Feo:Fed (Table 1). This result suggests that the presence of TC, Fe oxides (Fed), and active 402

Fe ratio (Fe₀:Fe_d) control the amount of Fe-bound OC in soil. It is generally assumed that a large 403 C content might facilitate the formation of Fe-bound OC compounds (Schwertmann and Latham, 404 405 1986; Jeewani et al., 2020a). Our study also showed that the amount of C sequestered in the paddy soils was related to the content of the Fed. This result is consistent with recent findings that suggest 406 407 the formation of Fe-OM complexes is an effective physicochemical stabilization mechanism in paddy soil (Chen et al., 2020; Jeewani et al., 2020a). Our results support the hypothesis that maize-408 409 straw develops Fe-OM complexes, and these are enhanced by increasing background concentrations of Fe in soil. 410

411

412 4.2 Concentration of Fe oxide influences the soil microbial community in paddy soil

413 Distance-based multivariate (DISTLM) analysis was used to predict the effects of edaphic variables on microbial community structure. Total C, C:N, Fed, and C:Fe molar ratio were the 414 415 most influential factors for microbial community composition (Table 2). According to Aciego 416 Pietri and Brookes, (2009), the direct effects of TC are derived from substrate inputs on microbial 417 biomass, activity, and community structure. Further, it was reported that microbial biomass and microbial activities were increased with an increased TC content and C:N ratio (Cleveland and 418 Liptzin, 2007; Goyal et al., 1999). Fe oxide minerals can also directly modulate microbial 419 community composition, providing niche-specific conditions that select for similar microbial taxa 420 across a Fe gradient (Ernest Chi Frua and Danielle, 2012; Whitman et al., 2018). 421

Bacteria and fungi showed higher diversity in paddy soil with a greater content of Fe oxide (Fig. 3), a result previously reported in other agricultural soils (Weber et al., 2006; Azam and Finneran, 2013). This finding was also supported by the increased fungal diversity after the addition of goethite (Jeewani et al., 2020b). Furthermore, the increase in fungal diversity is likely due to competitive advantages over the bacteria through more competitive C accessibility.

To decipher coexisting interactions between microbial genera across the paddy soils with 427 increasing concentrations of Fe oxides, co-occurrence network analysis was conducted (Fig. 4). 428 The percentage of mutual-exclusion represented by negative links among bacterial taxa were 429 increased from 11% to 39% while decreasing the co-presence by 88% to 60% from Fe-13 to Fe-430 55. This implies that the limited C accessibility in soils with higher concentrations of Fe oxides 431 modulated the community (Table 2). In contrast, the positive links representing the co-presence 432 433 of fungal networks were increased from 83% to 97% with increasing total concentrations of Fe oxide, and negative links among fungi were decreased from 16% to 3% (Table S2). This indicates 434 435 a competitive advantage for fungi in their ability to access C sources in soils with higher concentrations of Fe oxides. This would be explained through the ability of fungi to explore the 436 soil in search of C sources and their adaptation as K-strategists to oligotrophic conditions (Janusz 437 438 et al., 2017; Jeewani et al., 2020a). In turn, the assembly of fungal communities in Fe rich soils seems to be a result of the environmental selection of soil fungal communities related to their 439 functions as described by the theory of competitive exclusion and habitat differentiation (Schimel 440 and Schaeffer, 2012). 441

442

443 4.3 Contributions of abiotic and biotic variables to maize-straw mineralization, priming, and
444 stabilization

Random forest analysis confirmed our hypothesis that maize-straw derived C stabilization was governed mainly via abiotic mechanisms, while decomposition was regulated via both biotic and abiotic variables (Fig. 5). Further, SEM analysis revealed that maize-straw mineralization was controlled by bacterial diversity, pH, and C:N ratio of the soil (Fig. S1). It was reported that the mineralization of SOM is not only directly governed by interactions between the effects of microbial community structure but also substrate availability (Juarez et al., 2013; Mohanty et al., 2013). Moreover, Fed and C:Fe molar ratios have indirect effects on maize-straw mineralization
by limiting the C resources to microbial utilization. Hence, both abiotic protection and effects on
the community structure resulted in lower maize-straw mineralization in Fe-55 (32.5%
mineralized) compared to Fe-13 (57.5% mineralized).

Soil organic carbon priming decreased by 14% between Fe-13 to Fe-55 (Fig. 1A). A recent 455 study conducted under oxic conditions, with the addition of Fe and DOC, observed that reactive 456 457 Fe(III) phases suppress DOC and OC mineralization by 35% and 47%, respectively (Chen et al., 2020). A study had also observed a substantial decrease in OM decomposition when glucose or 458 459 fulvic acid sorbed to Fe minerals such as ferrihydrite or goethite was added to soils (Hall et al., 2016). Thus, it is likely that Fe oxides protect recent substrate-derived DOC via co-precipitation 460 under oxic conditions, decreasing DOC availability for microbial growth and suppressing the 461 462 priming of native SOM (Adhikari et al., 2019; Chen et al., 2020). Thereby, our hypothesis that higher Fe content in the soil would lower the PE was supported. SEM (Fig. S1) and random forest 463 analysis (Fig. 5b) further support the notion that soil priming was directly affected by both 464 bacterial and fungal diversity, while DOC derived from maize-straw directly co-precipitated as 465 indicated by the C:Fe ratio. 466

The limitation of easily accessible labile-C due to physiochemical protection favored the 467 dominance of genera such as most of the fungi and some bacteria like Actinobacteria, that possess 468 the ability to explore C and nutrients via hyphae. Through their ability to prime SOM it allows 469 them to dominate and survive in oligotrophic conditions (Dini-Andreote et al., 2015; De la Cruz-470 Barrón et al., 2017; Jeewani et al., 2020). For instance, fungi are well adapted to nutrient-poor soil 471 and can degrade complex C substrates supplied to the soil as well as native SOM (Lundell et al., 472 473 2014). Our previous work also found that fungal genera such as Cystofilobasidium dominated in oligotrophic condition, e.g., Fe oxide enriched soils (Jeewani et al., 2020a). 474

476 *4.4 Keystone taxa involved in maize-straw mineralization and priming*

Further analysis using O2PLS showed that the genera of Nitrosospira, MND1 were 477 primarily responsible for maize-straw mineralization, and *Rokubacteriales* was the keystone 478 479 genera associated with the PE (Table 3). A previous study has reported that Rokubacteriales has a generalist metabolic strategy in oligotrophic environments (Anantharaman et al., 2016; Hug et 480 al., 2016). Rokubacteria can utilize different sources of C for energy (Becraft et al., 2017; Zhu et 481 482 al., 2019). It encodes multiple C transport proteins for the TCA cycle, indicating its broad range of metabolic activities (Meyling and Eilenberg, 2007; De la Cruz-Barrón et al., 2017). We, 483 therefore, suggest that Rokubacteria might dominate and contribute to C mineralization in Fe rich 484 paddy soils due to its high metabolic diversity and ability to utilize a wide range of substrates. 485

Gaiella (affiliated with the phylum Actinobacteria) was the key genera responsible for 486 maize-straw mineralization and the PE (Table 3). Gaiellales is a deep-branching lineage of 487 488 Actinobacteria, which can adapt to resource-poor oligotrophic conditions by having the potential to fix CO₂ (McCarthy and Williams, 1992; Holmalahti et al., 1994; Severino et al., 2019). 489 Actinobacteria are a functionally diverse group of organisms that are known to be involved in C 490 cycling in soils (McCarthy and Williams, 1992; Holmalahti et al., 1994). They exhibit a high 491 substrate versatility and metabolic diversity and can survive by utilizing diverse C and N resources 492 (e.g., by upregulating cellulolytic activity) in both copiotrophic and oligotrophic environments. 493 Genera belonging to Glomeraceae (phylum Glomeromycota) contributed to maize-straw 494 mineralization, and certain genera appear to preferentially associate with Fe oxide surfaces 495 496 (Whitman et al., 2018) and access nitrogen from SOM (Veresoglou et al., 2012). Their role in soil C processes reflects their mycelial growth habit, which makes it possible to explore the soil in 497 search of resources (Jia et al., 2019). 498

500 **5. Conclusions**

Paddy soil Fe-55 with the highest Fe content (55.8 g Fe kg⁻¹ soil) resulted in more than a 501 502 3-fold increase in the stabilization of C from supplied maize straw, compared to Fe-13 (with 13.7 g Fe kg⁻¹ soil). In the Fe-rich soil, 4.1% of maize-straw derived C was bound to Fe, and the C:Fe 503 molar ratio was 6.5, suggesting that co-precipitation was the dominant process in the stabilization 504 505 of this new C. Supporting our hypotheses, the paddy soils with a higher concentration of Fe oxides also had lower soil priming effects, and maize-straw derived mineralization than soils with a lower 506 507 concentration of Fe oxides. This decrease in decomposition is a result of lower accessibility of maize-straw derived C due to co-precipitation. In addition to abiotic processes, maize-straw 508 mineralization was regulated by the Fe mediated bacterial community, including their diversity, 509 interactions, and the keystone microbiome, i.e., Gaiella (phylum of Actinobacteria). It was further 510 confirmed by random forest analysis that phyla, including Proteobacteria and Actinobacteria gave 511 the largest contribution to both substrate mineralization and priming. This study revealed that the 512 513 concentration of Fe oxides in paddy soils plays a central role in long-term C preservation through the formation of Fe-OM complexes via co-precipitation, thereby limiting C availability to 514 microbes and concomitantly modulating the microbial community. 515

516

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714 Figure and Table captions

Fig. 1. CO₂ efflux of basal soil-derived, maize-straw-derived, and maize-straw primed CO₂ during
the first 7 days of incubation with the addition of maize-straw. Basal soil-derived represents the

717 total CO₂ evolved from the native soil (non-labeled C) from the maize-straw added treatment. Straw-derived represents the CO₂ evolved from added labeled maize-straw (based on 718 measurements of ${}^{13}CO_2$). Primed CO₂ represents the CO₂ evolved from the soil, primed by the 719 added maize-straw. Soil with the total concentration of Fe oxides of 13.7 g kg⁻¹ is referred to as 720 (Fe-13), soil with total concentration of Fe oxides 25.8 g kg⁻¹ (Fe-25), soil with total concentration 721 of Fe oxides 42.7 g kg⁻¹ (Fe-42), soil with total concentration of Fe oxides 55.8 g kg⁻¹ (Fe-55). 722 Error bars represent the standard deviation of the means (n=3). The different letters (a-d) above 723 the columns indicate a significant difference (P < 0.05) based on the analysis of variance with 724 725 Tukey's Post-hoc test.

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Fig. 2. Content of ¹³C derived from the added straw in bulk soil and in the Fe-OM complexes (A), total Fe-bound soil organic carbon (Fe-bound OC) content (B), corresponding molar ratio of Febound OC to Fe oxides (C), Fe-bound soil organic carbon (Fe-bound OC) content (C), and the ratio of organic matter (OM)-complexed Fe oxides to total reactive Fe oxides (Fe_d:Fe_p) indicating the degree of Fe complexation with organic matter (D) after 56 days of incubation. Values show means (n=3) \pm standard deviation. The different letters (a-d) above the columns indicate a significant difference (P < 0.05) based on the analysis of variance with Tukey's Post-hoc test.

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Fig. 3. Shannon index (alpha diversity) of soil bacterial (A) and fungal (B) community, plots of principle coordinate analysis (PCoA) representing beta diversity based on Bray-Curtis dissimilarity of bacterial (C) and fungal (D) communities and relative abundances of main bacterial (E) and fungal (F) phyla after 56 days of incubation.

Fig. 4. The network of co-occurring bacterial (A, B) and fungal (C, D) genera based on a correlation analysis (Spearman's P > 0.6) with a significance (P < 0.01) after 56 days of incubation. Low concentration of Fe oxides networks represents Fe-13, and Fe-25 soils, and high concentration of Fe oxides networks represent Fe-42 and Fe-55 soils. The nodes in networks are colored according to phyla, while the edges in networks are colored concerning modularity class. Node size is proportional to the betweenness centrality of each genus, and edge thickness is proportional to the weight of each correlation.

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Fig. 5. Random forest analysis representing the relative importance of soil physicochemical (C:N ratio, Fe_d, C:Fe ratio, DOC, and pH) and biological variables (bacterial diversity, fungal diversity, the composition of Proteobacteria, Actinobacteria, and Ascomycota) for substrate (labeled maizestraw) mineralization (A), soil priming effect (B) and substrate stabilization (C) after 56 days of incubation. Abbreviations: Fe_d, total reactive Fe oxides, C:Fe ratio, the molar ratio of Fe-bound organic carbon to total iron oxides; DOC, dissolved organic carbon.

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Fig 6. Proposed concept diagram for substrate stabilization and mineralization, and induced rhizosphere priming effects: High concentrations of Fe oxides soils (Fe-55) retard C mineralization due to accessibility limitation of the substrate through interaction between coprecipitation (right) and substrate mineralization by the keystone microbes in less Fe containing soil (Fe-13) (left). The thickness of the arrows is proportional to the magnitude of C flow.

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Table 1. Pearson correlation matrix for the concentration of Fe bound organic carbon, and Febound C:Fe molar ratio with soil properties.

Table 2. Contributions of edaphic variables to the bacterial and fungal community as analyzed by
distance-based linear modeling (distLM) analysis.

Table 3. The core functional genera (with variable influence projection (VIP) >1.3) involved in
C dynamics, including maize-straw mineralization and priming effect (PE) estimated by the twoway orthogonal partial least squares analysis.