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1 **The stoichiometric C-Fe ratio regulates glucose mineralization and stabilization via**
2 **microbial processes**

3

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24

25 **Abstract**

26 The association of soil organic matter (SOM) with iron (Fe) oxides by adsorption and/or co-
27 precipitation contributes to long term C stabilization in soil. While there is an understanding of
28 the relationship between soil carbon (C) and the biogeochemical cycling of Fe, a lack of
29 information exists on the role of Fe oxides on the accumulation of C in paddy soils. This study
30 aimed to assess the role of Fe (oxyhydr)oxides on mineralization and stabilization processes
31 following amendment of paddy soil with a labile C substrate (99 atom % ¹³C-glucose). The
32 study utilized 4 paddy soils with a total Fe concentration ranging from 13.7 to 55.8 g kg⁻¹. In
33 soils with 42.7 and 55.8 g kg⁻¹ Fe, the addition of glucose resulted in an Fe bound organic C:
34 Fe molar ratio (C:Fe molar ratio) ≥ 6, suggesting the formation of Fe-OM complexes mainly
35 via co-precipitation. The highest portion of ¹³C (13.8%) protected in Fe-OM complexes was
36 found in soil containing 55.8 g kg⁻¹ of Fe. The stabilization of the added labile C substrate was
37 shown, using random forest analysis, to be controlled by the C: Fe molar ratio, while substrate
38 mineralization was regulated by the core genera *Sphingomonas* and *Devosa* (r-strategists)
39 (affiliated to Proteobacteria) and C:N ratio. Substrate mineralization was 47 % lower in soil
40 containing 55.8 g Fe kg⁻¹ compared to 13.7 g Fe kg⁻¹, with a concomitant reduction in SOM
41 priming of 37 %. This reduction in substrate mineralization and the priming effect was likely
42 due to lower C substrate availability via the formation of Fe-OM complexes, thereby protecting
43 the C from mineralization. In conclusion, the Fe concentration in paddy soils plays a central
44 role in the abiotic stabilization of ‘new’ C through the formation of Fe-OM complexes via co-

45 precipitation, thereby limiting the availability of this C substrate for microbial mineralization,
46 and at the same time modulating the microbial community structure.

47

48 Keywords: Fe gradient, co-precipitation, Fe organic matter complexes, Bacterial and fungal
49 DNA amplification and sequencing, Shannon index, random forest analysis, structural equation
50 modeling

51

52 **1. Introduction**

53 Paddy soils play an important role in the storage of soil carbon C due to their unique
54 geochemical properties (Ge et al., 2012; Wiesmeier et al., 2019; Wei et al., 2019). Iron (Fe)
55 plays an important role in many physicochemical processes, including the stabilization of soil
56 organic matter (SOM) (Kaiser and Guggenberger, 2000; Boyd and Ellwood, 2010; Jeewani et
57 al., 2020); as well as biotic processes resulting in Fe adsorption, co-precipitation and mineral
58 transformation (Jones, 1998; Chen et al., 2014; Huang et al., 2018; Wan et al., 2019). Previous
59 studies have coupled the biogeochemical cycling of Fe with SOM (Ponnamperuma, 1972;
60 Huang et al., 2018; Wiesmeier et al., 2019; Liu et al., 2029). Unlike C/N/P stoichiometry, only
61 a limited number of studies have evaluated the mechanisms and effect of C:Fe molar ratio on
62 SOM accumulation in paddy soils (Zhou et al., 2009; Song et al., 2016). Therefore, a better
63 understanding of C-Fe cycling and the role of the soil microbial community is needed to
64 optimize C storage in paddy soils.

65 Iron oxides and oxyhydroxides (for simplicity, these are defined as Fe oxides
66 henceforth) constitute a range of pedogenic minerals that are involved in a range of
67 physicochemical processes associated with the stabilization of SOM (Chen et al., 2020; Cornell
68 and Schwertmann, 1996; Li et al., 2020a). Fe oxides, which contain abundant hydroxyl groups

69 at their surfaces, can form stable organo-mineral complexes by interacting with carboxyl
70 groups of SOM (Kleber et al., 2015), thus stabilising the SOM (Chen et al., 2020). Iron-organic
71 matter associations (Fe-OM) play an essential role in C sequestration (Silva et al., 2015; Wang
72 et al., 2017; Wiesmeier et al., 2019). They have been shown to stabilize 21.5% of the SOM in
73 ocean sediments as well as 37.8% of the organic C in forest soils (Lalonde et al., 2012; Zhao
74 et al., 2016; Xue et al., 2020). Importantly, the Fe bound C: Fe molar ratio (C:Fe molar ratio)
75 can elucidate the binding mechanism between SOM and Fe oxides in soil (Coward et al., 2018;
76 Chen et al., 2020). When C:Fe molar ratio > 1, SOM and Fe oxides may be associated via co-
77 precipitation mechanisms (Wagai et al., 2013). In contrast, C:Fe molar ratios < 1 indicate the
78 formation of Fe-OM associations via adsorptive mechanisms (Coward et al., 2018). Fe-OM
79 complexes can occur in paddy soil due to changes in pH or redox potential, where the Fe bound
80 C:Fe molar ratios of soil are 0.2-6 (Kato et al., 2004; Cheng et al., 2010; Henneberry et al.,
81 2012). This is largely due to the sorption capacity of Fe oxides towards dissolved organic C
82 (DOC) (Chen et al., 2014; Chen et al., 2020; Kaiser and Guggenberger, 2000) and the affinity
83 of dissolved Fe phases to form co-precipitates with DOC (Kleber et al., 2015). Co-precipitation
84 can change the surface properties of the minerals (e.g., specific surface area and surface charge)
85 and affect the interaction and stability of Fe-OM associations (Chen et al., 2014; Xue et al.,
86 2020). Recently, several studies have evaluated the mechanisms of formation, properties, and
87 reactivity of Fe-OM complexes derived from co-precipitation with different C sources, such as
88 rhizodeposits and forest-floor extracts (Chen et al., 2014; Mikutta et al., 2014; Jeewani et al.,
89 2020), humic and fulvic acids (Angelico et al., 2014) and polysaccharides (Mikutta et al.,
90 2008). It was shown that glucose could adsorb to Fe oxide via hydrogen bonding and co-
91 precipitation (Olsson et al., 2011; Chen et al., 2014).

92 The biochemical nature of SOM plays a critical role in mediating the source of energy
93 for microbial metabolism in soil (Baldock et al., 2004; Li et al., 2020b; Wang et al., 2017).

94 According to Huang et al. (2020), rhizodeposits and plant litter are the primary sources of C
95 for soil microbial activity, thus contributing to soil respiration and formation of SOM. Glucose
96 is a central metabolite and an abundant, available biomolecule that is easily and rapidly
97 mineralized by microorganisms in soils (Canarini et al., 2019; Liu et al., 2019; Van Hees et al.,
98 2005). Therefore, knowledge about the pathway of microbial metabolism and mineralization
99 of easily available substances in the soil is crucial to obtain a better understanding of SOM
100 formation and mineralization processes. However, few studies have evaluated the contribution
101 of Fe in paddy soils to the formation of Fe-OM complexes with a labile C substrate to mimic
102 rhizodeposition, where plant exudates are key contributors to the DOC pool.

103 Soil microorganisms are the critical mediators of the biogeochemical cycling of Fe in soil
104 (Whitman et al., 2018; Jeewani et al., 2020). Microbial diversity and community composition
105 strongly affect substrate mineralization (Kamble and Bååth, 2016; Li et al., 2018 Fu et al.,
106 2020). Generally, bacteria prefer to mineralize labile compounds and dominate in the initial
107 phases of mineralization (Garcia-Pausas and Paterson, 2011). Liang et al. (2014) noted that the
108 dominant bacterial phyla Proteobacteria, Firmicutes, and Actinobacteria play a crucial role in
109 the early mineralization process. Substrate addition to the soil can impact upon native SOM
110 decomposition, a process called the soil priming effect (PE) (Dimassi et al., 2014; Zhang et al.,
111 2019; Yu et al., 2018; Zhu et al., 2018; Lu et al., 2019), by modifying microbial activity and
112 community composition (Kuzyakov, 2002; Perveen et al., 2019). Thus there is a need to
113 understand the role of differing Fe concentrations in soil on the accessibility of labile C
114 substrates and the subsequent effect on microbial community composition.

115 The objective of the study was to elucidate the role of inherent soil Fe concentration on
116 glucose mineralization, the soil priming effect, and 'new' C stabilization. We hypothesize that:
117 i) substrate mineralization is lower and stabilization of substrate-derived C is higher with
118 increasing soil Fe oxide concentration due to the stabilization of substrate-derived C as Fe-OM

119 complexes, and ii) the increase in Fe oxide concentration will modulate the microbial
120 communities and their substrate utilization pattern associated with the geochemical cycling of
121 C and Fe. To test these hypotheses, we conducted a 56-day laboratory incubation using 4
122 similar paddy soils but with a natural Fe gradient. We supplied ^{13}C labeled glucose to represent
123 rhizodeposition, and investigated the mineralization and the stabilization of added substrate,
124 and the interactions with the soil microbial community composition.

125

126 **2. Materials and methods**

127 *2.1. Soil sampling and preparation*

128 Soils were sampled from the 0–20 cm (plough layer) of long-term rice (*Oryza sativa*)
129 paddies in Hunan province, China. Soil sampling was done at 4 sites along a 200 m transect
130 representing an Fe concentration gradient, with three replicates collected within a 1 m² plot at
131 each sampling location. Ethanol (70%) was used to sterilize equipment during sampling to
132 avoid microbial cross-contamination between soils. The sampling region had a mean annual
133 temperature of 17.7 °C, and a rainfall of 1402 mm (Table 1). The soil was classified as a Gleyic
134 Stagnic Anthrosol (FAO) (Iuss Working Group, 2015). The soils had Fe concentrations of 13.7
135 g Fe kg⁻¹ (referred to as Fe-13), 25.8 g Fe kg⁻¹ (Fe-25), 42.7 g Fe kg⁻¹ (Fe-42) and 55.8 g Fe kg⁻¹
136 (Fe-55) (Table 1). The soil samples had visible plant roots manually removed and were sieved
137 at field moisture (<2 mm) before being air-dried. Prior to a 14-day pre-incubation period (at
138 25°C), water holding capacity (WHC) of each soil was determined using a volumetric method
139 (see supplementary information). Soils were then sprayed with deionized water to obtain a
140 water content of 40% that of the WHC. This soil preparation protocol mimics the field situation,
141 where the soil stays mostly dry before rice cultivation and avoids the formation of reducing
142 conditions after addition of substrates during the incubation period.

143

144 *2.2. Experimental design*

145 An incubation experiment (over 56 days) was conducted to examine the priming effects
146 and substrate mineralization using a natural Fe gradient in soil. For each of the 4 soils, there
147 was a control (no glucose amendment) (n=3) and a ¹³C -glucose amendment (n=3), to make a
148 total of 24 incubation jars. Each incubation treatment consisted of a 500 mL glass jar, which
149 had a rubber bung seal. Within each jar, 40 g of oven-dry equivalent soil was contained within
150 a glass vessel along with a CO₂ trap (20 mL 1 M NaOH), which was replaced at several
151 sampling times. To maintain then humidity with in the jars, 10 mL water was maintained in the
152 base of the jar. Labeled glucose (99% -¹³C) (Cambridge isotope laboratories) was applied at
153 500 μg C g⁻¹ soil (1.5-2.9 % total C) soil by evenly mixing with each 40 g of soil. The jars were
154 sealed with a rubber bung and incubated at 25 °C for 56 days. Three blank jars containing only
155 water and CO₂ traps were also included. The incubation jars were opened periodically to
156 maintain aerobic conditions. After the 56 days of incubation, the soil was homogenized by
157 mixing and separated into two uniform batches, one being freeze-dried for the extraction of
158 DNA, and the second was allocated for further analysis. The ¹³C distribution was analyzed in
159 the bulk soil and Fe-OM complexes to estimate the mechanisms of substrate-derived C
160 stabilization along the Fe concentration gradient.

161

162 *2.3. Soil physical and chemical analyses*

163 Total soil C and N content (air-dried, milled <200 μm) was determined by dry
164 combustion using a Perkin Elmer EA2400, Shelton, CT, USA. Soil pH was measured at a soil:
165 water ratio of 1:2.5 (w/w) using a micro-electrode (Seven Compact, Mettler Toledo Inc.,
166 Switzerland). Microbial biomass C (MBC) was determined using the chloroform fumigation

167 extraction method (Vance et al., 1987). Fumigated and unfumigated portions of 15 g moist
168 soil were extracted with 30 ml 0.5M K₂SO₄. Organic C in the extracts was measured using a
169 TOC analyser (Multi N/C 3100, Analytik Jena AG, Jena, Germany). Microbial biomass C was
170 calculated as EC/kEC, where EC= (organic carbon extracted from fumigated soils) - (organic
171 carbon extracted from non-fumigated soils) and kEC=0.45 (Wu et al., 1990). DOC was
172 determined from the non-fumigated component of the soil. ¹³C in soil microbial biomass C was
173 determined as described by Luo et al. (2013). The natural abundance of δ¹³C in soils was
174 measured using air-dried and sieved (<200 μm) soil, which was accurately weighed (about 0.2
175 mg) into tin capsules prior to analysis using an isotope ratio mass spectrometer with IAEA-600
176 (Caffeine); δ¹³C=-27.771‰ as standard material (Meng et al., 2013) (Thermo Fisher Scientific,
177 DELTA V plus IRMS, Bremen, Germany) coupled with an elemental analyzer (EA NA1500 -
178 EA 1110 device, Carlo Erba and Thermo Fisher Scientific, Bremen, Germany).

179 The total Fe concentration of the soil sample was determined after digestion with a
180 concentrated acid mixture of HF, HClO₄, and HNO₃ (Zhou et al., 2007). Dithionite-citrate-
181 bicarbonate (DCB) extraction method was used to quantify the total extractable soil iron oxide
182 (Fe_d) concentration by determining the Fe concentration in the solution (Lalonde et al., 2012;
183 Wang et al., 2017; Wan et al., 2019). Freeze-dried soil (0.50 g) was mixed with 30 mL of buffer
184 solution (0.27 M trisodium citrate and 0.11 M sodium bicarbonate, pH 7.3) in 50 mL centrifuge
185 tubes, which was then placed in a water bath (80 °C). Sodium dithionite (0.50 g) was added to
186 the mixture, and it was maintained at 80 °C for 15 min. Soils were extracted by sodium chloride
187 (NaCl) instead of trisodium citrate and sodium dithionite, at an equivalent ionic strength as the
188 control under the same conditions. The suspension was then separated by centrifugation at 4000
189 x G for 10 min. The residue was thrice rinsed with ultra-pure H₂O (5 mL), centrifuged, and the
190 supernatant was carefully removed. The precipitate was freeze-dried overnight. Organically
191 complexed Fe oxides (Fe_p) and soil amorphous Fe oxides (Fe_o) were extracted with sodium

192 pyrophosphate and ammonium oxalate, respectively (Keiluweit et al., 2015; Wan et al., 2019).
193 The TC and TN in the extracted residue was analyzed using an elemental analyzer, and the
194 $\delta^{13}\text{C}$ isotopic composition of the residue was obtained by an isotope ratio mass spectrometer
195 (Thermo Fisher Scientific, DELTA V plus IRMS, Bremen, Germany) coupled with an
196 elemental analyzer (EA NA1500 - EA 1110 device, Carlo Erba and Thermo Fisher Scientific,
197 Bremen, Germany). For the analysis of the soluble Fe concentration, washings and supernatants
198 were combined and acidified to pH 2.0 before being filtered using a 0.45- μm PTFE membrane
199 filter. The Fe concentration in solution was determined using ICP-MS. The calculations are
200 found in the supplementary information.

201

202 *2.4. Analysis of $\delta^{13}\text{C}$ -CO₂*

203 The CO₂ traps were collected and changed after 1, 3, 7, 14, 28, and 56 days for the
204 determination of C mineralization. At sampling, a 5 mL aliquot of trap solution was diluted
205 with 10 mL water and titrated against standardized 0.5 M HCl using an Easy Plus auto titrator
206 (Mettler Toledo, Greifensee, Switzerland) to determine the total CO₂ evolved (Tinsley et al.,
207 1951). To determine the $\delta^{13}\text{C}$ -CO₂ from the trapped CO₂, an 8 mL aliquot of the trap solution
208 was added to 8 mL of 1.5 M BaCl₂ into 50 mL falcon tubes and incubated at 25 °C for 0.5 h to
209 allow precipitation. The resulting BaCO₃ precipitate was then filtered, rinsed three times with
210 deionized water, and freeze-dried overnight in a falcon tube (Luo et al., 2011; Luo et al., 2017).
211 The precipitates were scraped from the falcon tube, accurately weighed (about 0.2 mg) into tin
212 capsules, and analyzed using a stable isotope ratio mass spectrometer (IRMS, Thermo Fisher,
213 USA) (Aoyama et al., 2000).

214

215 *2.5 $\delta^{13}\text{C}$ calculations*

216 The mineralization of the substrate was distinguished from SOM mineralization based
 217 on the changes of stable isotopic composition ($\delta^{13}\text{C}$) over time as follows.

$$218 \quad \delta^{13}\text{C}(\text{‰}) = \left[\left(\frac{R_{\text{sample}}}{R_{\text{VPDB}}} \right) - 1 \right] \times 1000 \quad (1)$$

219 R_{sample} is the mass ratio of ^{13}C to ^{12}C of the sample, and R_{VPDB} is the mass ratio of ^{13}C to ^{12}C of
 220 the Vienna Pee Dee belemnite (VPDB) standard (0.0112372). The labeled ^{13}C (%) was then
 221 estimated from:

$$222 \quad \text{CO}_2^{13}\text{C}(\mu\text{g g}^{-1}\text{soil}) = \text{CO}_2^{13}\text{C}(\%) \times \text{Total evolved CO}_2\text{C}(\mu\text{g g}^{-1}\text{soil})/100 \quad (2)$$

$$223 \quad \text{CO}_2^{13}\text{C}(\%) = (\delta^{13}\text{C}_{\text{treatment}} - \delta^{13}\text{C}_{\text{original}}) / (\delta^{13}\text{C}_{\text{substrate}} - \delta^{13}\text{C}_{\text{original}}) * 100 \quad (3)$$

224 Where CO_2^{13}C (%) is the proportion of evolved ^{13}C in CO_2 from the added substrate (glucose)
 225 $\delta^{13}\text{C}_{\text{treatment}}$ is the $\delta^{13}\text{C}$ (‰) of evolved CO_2 from substrate added to the soil, $\delta^{13}\text{C}_{\text{control}}$ is the
 226 $\delta^{13}\text{C}$ (‰) of original soil C without substrate was added. $\delta^{13}\text{C}_{\text{substrate}}$ is the $\delta^{13}\text{C}$ (‰) from the
 227 added substrate. Thus, the CO_2^{13}C resulting from the mineralization of the substrate (glucose)
 228 during the incubation (substrate-derived C) was calculated.

229 The primed soil CO_2C , resulting from enhanced decomposition of SOM with the addition of
 230 glucose was calculated from:

$$231 \quad \text{Priming soil CO}_2\text{C}(\mu\text{g C g}^{-1}\text{soil}) = \text{CO}_2\text{C}_{\text{treatment-1}} - \text{CO}_2\text{C}_{\text{control-1}} \quad (4)$$

232 Where, $\text{CO}_2\text{C}_{\text{treatment-1}}$ is the non-isotopically labeled C in CO_2 evolved from substrate amended
 233 soil, and $\text{CO}_2\text{-C}_{\text{control-1}}$ is the C in total CO_2 evolved from the control.

234 Substrate-derived C represents the CO_2 evolved from glucose (based on $^{13}\text{CO}_2$ measurements).

235 Primed soil C signifies CO_2 evolved from native soil, primed by added glucose. Basal soil-

236 derived C accounts for the total CO₂ evolved from the respective control soil without glucose
237 addition.

238

239 *2.6. DNA extractions and sequencing*

240 DNA of 0.50 g of freeze-dried soil was extracted using a Fast DNA Spin Kit (MP
241 Biomedicals, Santa Ana, CA, USA). The extracted DNA was dissolved in 50 µL of TE
242 buffer, and the concentrations of DNA were quantified using a Nanodrop 2000 (Thermo
243 Scientific, Willmington, USA). The bacterial 16S rRNA gene fragments were amplified using
244 primer sets targeting the V4-V5 variable region. The forward primer is 515F (5'-
245 GTGCCAGCMGCCGCGGTAA-3') linked with a specific-sample 5-bp barcode sequence at
246 the 5' end of primer, and 806R (5'- GGACTACHVGGG TWTCTAAT -3') was used as the
247 reverse primer (Bates et al., 2011). The ITS1 region was amplified by PCR for fungal genes
248 using the 5'- CTTGGTCATTTAGAGGAAAAGTAA-3' forward primer and 5'-
249 GCTGCGTTCTTCATCGATGC-3' reverse primer (Bates et al., 2011). Samples were
250 amplified in three replicates, and the three reaction products were pooled and purified using
251 Agincourt Ampure XP beads (Indianapolis, USA). All amplicons were pooled across all
252 samples at equimolar concentrations (20 ng µl⁻¹) into a representative sample, and the index
253 sequencing of paired-end 250 bp was performed on an Illumina HiSeq 2000 platform. Bacterial
254 and fungal DNA amplification and sequencing were performed by Major Bio, Inc. (Shanghai,
255 China).

256

257 *2.7. Processing of sequencing data and related statistical analysis*

258 The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered by
259 Trimmomatic, and connected by FLASH using the following steps. Firstly, the 250 bp reads
260 were cut-off at the site, receiving an average quality score of less than 20 over a 50 bp sliding
261 window, and the cut-off reads shorter than 50 bp with the reads containing mismatch characters.
262 Secondly, sequences that were longer than ten bp were assembled according to their overlapped
263 sequence. The maximum mismatch ratio of the overlap region is 0.2, and those mismatched
264 reads were discarded. Thirdly, samples were differentiated according to the barcode and
265 primers. Finally, sequence direction adjustment and barcode matching was undertaken.
266 Between 4,682 and 51,150, valid sequences were obtained per sample for all experimental
267 samples. UPARSE (version7.1, <http://drive5.com/uparse>) was used to cluster the operational
268 taxonomic units (OTUs) with 97% similarity cut-off. Chimeric sequences were removed. The
269 RDP classifier (<http://rdp.cme.msu.edu/>) was used to examine the taxonomy of each OTU
270 representative sequence against the 16S rRNA database using a confidence threshold of 0.7
271 (Chen et al., 2017). Total datasets were rarefied to 39,000 sequences per sample for the
272 bacterial α - and β -diversity analyses. α -diversity of bacterial and fungal communities was
273 calculated using the alpha diversity index (herein referred to as the Shannon index) that
274 represents both the richness and the evenness of the community. Principal coordinates analyses
275 (PCoA) were used to analyze the β -diversity analysis. Relative effects of soil variables such as
276 TC, TN, C:N ratio, C:Fe molar ratio, DOC, Fe-OM, and pH on soil bacteria and fungi
277 communities were analyzed by distance-based linear model multivariate analysis (distLM)
278 using the distLM forward3 software (Anderson, 2003). SIMCA-P 14 (Version 14.1.0.2047)
279 software used to determine the two-way orthogonal partial least squares (O2PLS) analysis to
280 correlate the microbial taxa to the priming, substrate mineralization, and substrate-derived C
281 stabilization. The Y-matrix was designed as the C dynamics datasets, and the X-matrix was
282 intended as the microbial community datasets (Trygg and Wold, 2003). The relative

283 importance of edaphic factors on the substrate mineralization and stabilization was quantified
284 using random forest analysis (Liaw and Wiener, 2002). Soil physicochemical variables (C:N
285 ratio, Fe concentration, C: Fe molar ratio, DOC, pH, TN, TC, and TP; total phosphorus) and
286 biological variables (bacterial diversity, fungal diversity, Actinobacteria, Proteobacteria,
287 Ascomycota and Basidiomycota) were used in the random forest analysis to assess their relative
288 contributions to the substrate derived C mineralization and stabilization. The pathways and
289 drivers of stabilization and mineralization of substrate in the soils were investigated by
290 structural equation modelling (SEM), which can determine the direction, magnitude, and effect
291 relationships. SEM was conducted using AMOS 21.0 to confirm possible causal relationships
292 between abiotic variables and the biotic community on C dynamics. In the SEM, chi-square
293 was used to evaluate model fitting, while a non-chi-squared test ($P>0.05$) indicates a good fit
294 of the model to the data. The analysis of correlation metrics calculated the coefficients of each
295 path. The path in this model was considered significant with a $P<0.05$. SPSS software version
296 20.0 for Windows (SPSS, Chicago, IL, USA) was used for statistical tests via a one-way
297 analysis of variance (ANOVA) on the soil biogeochemical data to assess the effect of substrate
298 (glucose) addition. Residues were analyzed for normal distribution and homogeneity by
299 Shapiro-Wilk and Levene's tests. If conditions were met, the Tukey Post-hoc test was
300 performed to determine the differences between the treatments. PCoA of the microbial
301 community based on the sequencing data was performed using SPSS 20.0 for Windows and
302 was visualized using Origin Pro 9.1 (Origin Lab, Northampton, MA, USA).

303

304 **3. Results**

305 *3.1. Sources of CO₂ efflux*

306 The respired CO₂ during the first 0-7 days was partitioned into substrate derived, basal
307 soil-derived, and primed soil CO₂, compared across the gradient of soil Fe concentrations and
308 glucose amendment (Fig 1). Total CO₂ efflux in the Fe-13 and Fe-25 soil was greater than that
309 of the Fe-42 and Fe-55 soil. There was a general trend with the glucose addition that higher Fe
310 concentrations resulted in lower CO₂ efflux. The highest primed soil C and substrate-derived
311 C efflux with glucose amendment was reported for Fe-13, and lowest substrate-derived C and
312 primed soil C was from the Fe-55 soil (Fig. 1).

313

314 *3.2. Fe induced distribution pattern of substrate-derived C in the soil after incubation*

315 Retention of ¹³C in soil increased (58%) with the increase of Fe concentrations from
316 Fe-13 to Fe-55, relative to the total ¹³C content (Fig. 2A). The ¹³C distribution was analyzed to
317 quantify the ¹³C stabilization within the Fe-OM complexes. The ¹³C accumulation in the Fe-
318 OM complexes was controlled by the Fe concentrations in the soil, with the higher Fe
319 concentrations having higher retention of ¹³C (Fig. 2A). The glucose addition resulted in a ¹³C
320 content in Fe-OM of 3.8 mg kg⁻¹ in Fe-55, which was 4.2-fold greater than in the Fe-13 soil.

321 The ratio of Fe_p:Fe_d increased with the increase in soil Fe concentration (Fig. 2D),
322 suggesting enhanced Fe-OM complexation with increasing Fe concentration. The Fe bound
323 organic C:Fe ratio (C:Fe molar ratio) of Fe-OM complexes of the four soils varied between
324 3.4-6.5 (Figs. 2B and 2C) suggesting that co-precipitation (C:Fe > 6) (Chen et al., 2014; Wang
325 et al., 2017) was the key process in the Fe-42, and Fe-55 soil.

326

327 *3.3. Linkages between the bacterial and fungal diversity and composition with substrate* 328 *addition*

329 Microbial community composition at the completion of the incubation shows that
330 Proteobacteria (varied between 30-40%) and Ascomycota (varied between 40-80%) were most
331 abundant in all four soils, irrespective of treatment (Figs. 3A and 3B). Actinobacteria increased
332 by 7% and Basidiomycota by 50% in the soil with highest Fe concentration (Fe-55 soil). Fungal
333 diversity strongly increased along with the Fe concentrations from Fe-13 to Fe 55 (Fig. 4B).
334 Compositional dissimilarities of microbes between treatments were assessed by PCoA
335 analysis, and it showed the loadings of PC1 26.5% and PC2 19.2% for bacteria, and PC1 36.4%
336 and PC2 14.2% for fungi (Figs. 4C and 4D). Considerable variation in microbial communities
337 were observed with increasing Fe concentration, as indicated by the separation along the PC1.
338

339 *3.4. Correlations between C dynamics and microbial communities related to edaphic variables*

340 The distLM analysis was used to analyze the contributions of edaphic variables,
341 including the C:Fe ratio, TC, C:N, DOC, pH, TN, and Fe-OM, to the microbial community
342 (Anderson and Legendre, 1999). The soil bacterial community was affected by TC (16%), C:Fe
343 molar ratio (14%), C:N (18%), and DOC (13%). The variables related to C and N dynamics
344 (TC, TN, C:N, and DOC) accounted for 61% of the total variation in the bacterial community.
345 The fungal community was influenced by TC (26%), C:Fe molar ratio (18%), DOC (6%), and
346 TN (6%). The C: Fe molar ratio was the second most important factor contributing the soil
347 microbial community after TC in Fe rich soils (14% for bacteria and 18% fungi) (Table 2). Fe
348 concentration in soil exerted a contribution of 10% to bacteria and 12% for fungi (Table 2).

349 O2PLS analysis was used to identify the microbial community associated with
350 substrate-derived C mineralization and the PE. The following conditions were considered for
351 analysis: (i) variable influence projection (VIP) value ≥ 1.6 ; (ii) correlation coefficient
352 ($P < 0.05$); (iii) the number of microbial taxa being highly correlated ($r \geq 0.7$). Based on those

353 criteria, six genera were selected as key genera related to the PE and substrate mineralization
354 (Table 3). Most importantly, Proteobacteria positively correlated with a high VIP value for
355 substrate mineralization. The genera affiliated to the phyla Actinobacteria, Ascomycota, and
356 Basidiomycota were positively correlated with the PE. The substrate-derived C mineralization
357 was positively correlated with the genera including *Sphingomonas* and *Devosia* (affiliated to
358 phylum Proteobacteria), while *Sordariomycetes* (affiliated to phylum Ascomycota),
359 *Cryptococcus* (affiliated to the phylum Basidiomycota), *Gaiella* and *Streptomyces* (affiliated
360 to the phylum Actinobacteria) were highly correlated with the PE (Table 3).

361 Proteobacteria (*Sphingomonas* and *Devosia*) was positively correlated ($P>0.001$ and
362 $P>0.01$, respectively) with a high VIP value for substrate mineralization. Random forest
363 analysis showed that substrate mineralization was mainly governed by the involvement of soil
364 biological factors such as Proteobacteria and bacterial diversity. Whereas substrate-derived C
365 stabilization was largely regulated by physicochemical properties such as Fe, C:Fe molar ratio,
366 followed by the Fe concentration and TC (Fig. 5). While substrate stabilization was strongly
367 influenced by the C:Fe ratio (Figs. 5 and 6), it was negatively associated with microbial
368 composition, and positively associated with bacterial diversity (Fig. 6). Variations in Fe
369 concentration in soil positively influenced priming and substrate mineralization via fungal
370 diversity, with a lesser influence from bacterial diversity. The effect of the C:N ratio on
371 substrate mineralization was positively influenced by the involvement of Proteobacteria.
372 Whereas, the PE was largely determined by fungal diversity, mainly associated with
373 Ascomycota.

374

375 **4. Discussion**

376 *4.1. Effect of C:Fe molar ratio on soil C stabilization*

377 The accumulation of ^{13}C from the added glucose was higher in soils containing high
378 Fe concentration (i.e. Fe-55) (Fig. 2A), with a concomitant increase in the C:Fe molar ratio
379 (Fig. 2B). The C:Fe molar ratio has recently been considered to reflect the binding mechanisms
380 between SOM and Fe oxides (Coward et al., 2018). Sodano et al. (2017) emphasized that the
381 complexation of organic carbon with Fe, forming Fe-OM complexes, is an important C
382 sequestration mechanism in paddy soils. More specifically, when the C:Fe >6, the formation
383 of Fe-OM is via co-precipitation or aggregation (Wagai et al., 2013; Atere et al., 2020). In
384 contrast, C:Fe ratios <1 represent the formation of Fe-OM associations via inner-sphere
385 adsorptive processes (Coward et al., 2018). In our study, the C:Fe molar ratio in the Fe-13 and
386 Fe-25 soils was between 3.4 and 6.5 whereas the Fe-42 and Fe-55 soils had ratios >6 (Fig. 2B).
387 These results support the observations of previous research where a greater retention of forest
388 floor-derived DOC, and plant root-derived rhizodeposits was through co-precipitation rather
389 than by adsorption, where the initial C:Fe molar ratio >1 (Wan et al., 2019; Jeewani et al.,
390 2020). Similarly, Wissing et al. (2014) reported direct interactions between DOC-Fe(III)
391 compounds and chemical protection via the Fe-OM complexes. Although co-precipitation led
392 to robust and selective retention of aromatic constituents, the initial complexation of Fe^{2+} by
393 aliphatic carboxylic compounds and co-precipitation as C-rich Fe-OM associations contributed
394 to the overall retention of C in soil, particularly at higher solution C:Fe ratios (Chen et al.,
395 2014). As such, co-precipitation represents the main process in DOC retention in Fe oxide rich
396 soils and is of particular interest in understanding SOM stabilization and turnover mechanisms,
397 in particular, their role in controlling the C source and sink of paddy soils.

398 Importantly, our data has revealed the mechanisms in which glucose is stabilized in Fe-
399 OM complexes. The higher C retention obtained in Fe-42 and Fe-55 soils (Figs. 2A and 2B)
400 by co-precipitation was most probably due to the complexation of Fe compounds with low-
401 molecular-weight organic compounds, followed by co-precipitation as Fe-OM complexes

402 (Sodano et al., 2017; Wan et al., 2019). It was reported that glucose could adsorb to Fe oxides by
403 hydrogen bonding (Olsson et al., 2011), or associate via co-precipitation processes (Eusterhues
404 et al., 2011; Henneberry et al., 2012; Wan et al., 2019). Kleber et al. (2015) showed that the
405 association of labile-C molecules with Fe minerals via adsorption or co-precipitation, or the
406 formation of multi-layer organic bonds with mineral stabilized organic compounds, can
407 significantly lower the availability of labile C to microbes. Our results on the accumulation of
408 C from glucose in Fe-OM complexes support evidence for the role of Fe oxides in paddy soils
409 in stabilizing labile C (e.g., rhizodeposits) under unsaturated conditions.

410

411 *4.2. Microbial community assembly is associated with substrate availability in Fe rich paddy* 412 *soils*

413 The soil microbial community structure can be modified by the complex
414 physicochemical characteristics of soil (Fig. 3). The microbial community composition is
415 displayed by the relative abundance (Figs. 3A and 3B) as a function of the Fe concentration.
416 In soils with a greater Fe concentration, Actinobacteria dominated, while Proteobacteria and
417 Bacteroidetes had a lower abundance (Fig. 3A). These are recognized as copiotrophs (Fierer et
418 al., 2007), which grow rapidly and are usually more prevalent in soils with a high C availability.
419 Further, it was confirmed that *Gaiella* and *Streptomyces* (affiliated with phylum Actinobacteria)
420 were core genera responsible for the PE (Table 3). Actinobacteria has a broad metabolic
421 diversity and is thus better able to adapt to resource-poor oligotrophic conditions, when the
422 bioavailability of labile C is limited (McCarthy and Williams, 1992; Holmalahti et al., 1994;
423 Wei et al., 2020). It can therefore be concluded that decreasing the bioavailability of labile C
424 in soil, as a result of the protection mechanisms (e.g., Fe-OM) lowering glucose availability,
425 modified the bacterial community to favour the phylum Actinobacteria.

426 It is well known that filamentous fungi belong to the phyla Ascomycota and
427 Basidiomycota, and are well adapted to degrade SOM. Thus it is not surprising that fungal
428 diversity increased with increasing Fe concentrations (Fig. 4B), where the availability of labile
429 C decreased. Further, SEM and O2PLS analysis supported the notion that Ascomycota and
430 fungal diversity directly influence the PE (Fig. 6 and Table 3). It was reported that sequences
431 assigned to Ascomycota were dominant at different stages of decomposition, revealing that
432 they are key drivers responsible for residue degradation (Lundell et al., 2014).

433 The distLM analysis revealed that TC, C:Fe ratio, C: N ratio, DOC and Fe concentration
434 were major edaphic factors contributing to the bacterial and fungal communities (Table 2),
435 confirming the role of C in the community assembly. Specifically, the limited glucose C
436 availability in the soil rich in Fe oxides modulated the community that was dominated by K-
437 strategists, including *Gaiella*, *Streptomyces* (affiliated to the phylum Actinobacteria),
438 *Sordariomycetes* (affiliated to Ascomycota) and *Cryptococcus* (affiliated to Basidiomycota)
439 (Figs. 3A and 3B). The limitation of C and nutrients favored K-strategists that possess the
440 ability to explore the soil for C, being able to survive in both copiotrophic and oligotrophic
441 soils. Therefore, as Basidiomycota, Ascomycota, and Actinobacteria exhibit higher substrate
442 versatility and metabolic diversity, they can therefore survive in both copiotrophic and
443 oligotrophic conditions. Their ability to prime SOM provides a competitive advantage (Cui et
444 al., 2020; Dini-Andreote et al., 2015; De la Cruz-Barrón et al., 2017).

445

446 *4.3. Glucose mineralization and the priming effect is regulated by Fe concentration and* 447 *associated core microbes*

448 Glucose had higher mineralization in the soils with lower Fe concentration (Fig. 1).
449 Blagodatskaya et al. (2009) demonstrated that the input of small amounts of readily available

450 substrates (i.e., glucose or root exudates) significantly increased the growth rates of soil
451 microorganisms by between 13-20 %. Similarly, Wang et al. (2016) demonstrated that glucose-
452 stimulated microbial activity with a rapid shift from K-strategists and towards r-strategies. Our
453 random forest analysis showed that Proteobacteria (e.g., *Sphingomonas*; r-strategist) and
454 bacterial diversity had the greatest influence on substrate-derived C mineralization (Fig. 5 and
455 Table 3). As supported by SEM, substrate-derived C mineralization was mainly governed by
456 the C:N ratio with the involvement of Proteobacteria and bacterial diversity (Figs. 5 and 6).
457 Therefore, the higher substrate-derived C efflux reported in soils with the lower Fe
458 concentration was due to the stimulation of r-strategists resulting from the access to labile C
459 (i.e., glucose).

460 A higher positive PE was found in paddy soil with the lowest Fe concentration (i.e., Fe-
461 13), with the PE abating with higher Fe concentration (Fig. 1). Ascomycota (K-strategist) and
462 Actinobacteria (r-strategist) were the dominant phyla in the F-13 soil. The corresponding
463 genera (*Sordariomycetes*, *Gaiella*, and *Streptomyces*) are likely to have responded rapidly to
464 the added glucose, resulting in further microbial co-metabolism by increasing the
465 mineralization of native SOM (i.e., positive PE), especially in the soil with a low Fe
466 concentration. In contrast, in soil with the higher Fe concentrations (i.e., Fe-42 and Fe-55), the
467 glucose-derived C was directly co-precipitated by Fe-OM phases with the involvement of
468 physicochemical factors such as the C:Fe ratio and Fe concentration (Figs. 5 and 6), thereby
469 lowering the microbial availability of glucose. Thus, mineralization of glucose and priming of
470 native SOM were strongly controlled by the Fe concentration of soil by limiting the
471 accessibility of C by co-precipitation.

472

473 **5. Conclusion**

474 This study has established the importance of Fe concentration in paddy soils in
475 stabilizing C. The development of Fe-OM complexes, the main C stabilization mechanism,
476 increased in soils with higher Fe concentration. Paddy soil with an Fe concentration of 55.8 g
477 kg⁻¹ had 44.7% lower mineralization of glucose compared to soil with an Fe concentration of
478 13.7 g kg⁻¹ at day 1 of the incubation. At the same time, the priming of SOM was 37.4% lower
479 in soil with the highest Fe concentration. The addition of glucose resulted in the proliferation of the
480 r-strategists *Sphingomonas* and *Devosa*, while positive priming of SOM was mostly caused by genus
481 *Gaiella*, *Sordariomycetes*, and *Cryptococcus* (K strategists). We conclude that inherent Fe
482 concentration exceeding around 42 g kg⁻¹, have a molar C:Fe ratio of >6 and promote Fe-OM
483 complexes via co-precipitation. This limits the availability of the substrate for microbial
484 mineralization, thus allowing the accumulation of C in paddy soils.

485

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489

490 **7. References**

491 Anderson, M., 2003. DISTLM forward: a FORTRAN computer program to calculate a
492 distance-based multivariate analysis for a linear model using forward selection. Department of
493 Statistics, University of Auckland, New Zealand.

494 Anderson, M.J., Legendre, P., 1999. An empirical comparison of permutation methods for tests
495 of partial regression coefficients in a linear model. *J. Stat. Computat. Simulat.* 62, 271-303.

496 Angelico, R., Ceglie, A., He, J.-Z., Liu, Y.-R., Palumbo, G., Colombo, C., 2014. Particle size,
497 charge and colloidal stability of humic acids coprecipitated with Ferrihydrite. *Chemosphere*
498 99, 239-247.

499 Aoyama, M., Angers, D., N'dayegamiye, A., Bissonnette, N., 2000. Metabolism of ¹³C-labeled
500 glucose in aggregates from soils with manure application. *Soil. Biol. Biochem.* 32, 295-300.

501 Atere, C.T., Gunina, A., Zhu, Z., Xiao, M., Liu, S., Kuzyakov, Y., Chen, L., Deng, Y., Wu, J.,
502 Ge, T., 2020. Organic matter stabilization in aggregates and density fractions in paddy soil
503 depending on long-term fertilization: Tracing of pathways by ¹³C natural abundance. *Soil.*
504 *Biol. Biochem.* 107931.

505 Blagodatskaya, E.V., Blagodatsky, S.A., Anderson, T.H., Kuzyakov, Y., 2009. Contrasting
506 effects of glucose, living roots and maize straw on microbial growth kinetics and substrate
507 availability in soil. *Euro. J. Soil Sci.* 60, 186-197.

508 Baldock, J., Masiello, C., Gelin, Y., Hedges, J., 2004. Cycling and composition of organic
509 matter in terrestrial and marine ecosystems. *Marine. Chem.* 92, 39-64.

510 Bates, S.T., Berglyons, D., Caporaso, J.G., Walters, W.A., Knight, R., Fierer, N., 2011.
511 Examining the global distribution of dominant archaeal populations in soil. *ISME. J.* 5, 908-
512 917.

513 Boyd, P.W., Ellwood, M.J., 2010. The biogeochemical cycle of iron in the ocean. *Nature.*
514 *Geosci.* 3, 675-682.

515 Canarini, A., Kaiser, C., Merchant, A., Richter, A., Wanek, W., 2019. Root exudation of
516 primary metabolites: mechanisms and their roles in plant responses to environmental stimuli.
517 *Frontiers in Plant Science* 10, 157

518 Chen, C., Dynes, J.J., Wang, J., Sparks, D.L., 2014. Properties of Fe-Organic Matter
519 Associations via Co-precipitation versus Adsorption. *Environ. Sci. Tech.* 48, 13751-13759.

520 Chen, Z., Zheng, Y., Ding, C., Ren, X., Yuan, J., Sun, F., Li, Y., 2017. Integrated metagenomics
521 and molecular ecological network analysis of bacterial community composition during the
522 phytoremediation of cadmium-contaminated soils by bioenergy crops. *Ecotoxicol. Environ.*
523 *Safety.* 145, 111-118.

524 Cheng, L., Zhu, J., Chen, G., Zheng, X., Oh, N.H., Rufty, T., Richter, D.d., Hu, S., 2010.
525 Atmospheric CO₂ enrichment facilitates cation release from soil. *Ecol. Lett.* 13, 284-291.

526 Cornell, R., Schwertmann, U., 1996. *The Iron Oxides*, VCH. Weinheim.

527 Coward, E.K., Ohno, T., Plante, A.F., 2018. Adsorption and molecular fractionation of
528 dissolved organic matter on iron-bearing mineral matrices of varying crystallinity. *Environ.*
529 *Sci. Tech.* 52, 1036-1044.

530 Cui, J., Zhu, Z., Xu, X., Liu, S., Jones, D.L., Kuzyakov, Y., Shibistova, O., Wu, J., Ge, T.,
531 2020. Carbon and nitrogen recycling from microbial necromass to cope with C: N
532 stoichiometric imbalance by priming. *Soil. Biol. Biochem.* 142, 107720.

533 De la Cruz-Barrón, M., Cruz-Mendoza, A., Navarro-Noya, Y.E., Ruiz-Valdiviezo, V.M.,
534 Ortíz-Gutiérrez, D., Ramírez-Villanueva, D.A., Luna-Guido, M., Thierfelder, C., Wall, P.C.,
535 Verhulst, N.J.M.e., 2017. The bacterial community structure and dynamics of carbon and
536 nitrogen when maize (*Zea mays* L.) and its neutral detergent fibre were added to soil from
537 Zimbabwe with contrasting management practices. *Microb. Ecol.* 73, 135-152.

538 Dimassi, B., Mary, B., Wylleman, R., Labreuche, J., Couture, D., Piraux, F., Cohan, J.-P., 2014.
539 Long-term effect of contrasted tillage and crop management on soil carbon dynamics during
540 41 years. *Agricul. Ecosys. Environ.* 188, 134-146.

541 Dini-Andreote, F., Stegen, J.C., van Elsas, J.D., Salles, J.F., 2015. Disentangling mechanisms
542 that mediate the balance between stochastic and deterministic processes in microbial
543 succession. *Proc. Nat. Acad. Sci.* 112, E1326.

544 Eusterhues, K., Rennert, T., Knicker, H., Kögel-Knabner, I., Totsche, K.U., Schwertmann, U.,
545 2011. Fractionation of Organic Matter Due to Reaction with Ferrihydrite: Coprecipitation
546 versus Adsorption. *Environ. Sci. Technol.* 45, 527-533.

547 Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an ecological classification of soil
548 bacteria. *Ecology* 88, 1354-1364.

549 Fu, X., Wang, J., Xie, M., Zhao, F., Doughty, R., 2020. Increasing temperature can modify the
550 effect of straw mulching on soil C fractions, soil respiration, and microbial community
551 composition. *PloS One* 15, e0237245.

552 Garcia-Pausas, J., Paterson, E., 2011. Microbial community abundance and structure are
553 determinants of soil organic matter mineralisation in the presence of labile carbon. *Soil Biol.*
554 *Biochem.* 43, 1705-1713.

555 Ge, T., Yuan, H., Zhu, H., Wu, X., Nie, S.a., Liu, C., Tong, C., Wu, J., Brookes, P., 2012.
556 Biological carbon assimilation and dynamics in a flooded rice – Soil system. *Soil Biol.*
557 *Biochem.* 48, 39-46.

558 Giannetta, B., Zaccone, C., Plaza, C., Siebecker, M.G., Rovira, P., Vischetti, C., Sparks, D.L.,
559 2019. The role of Fe (III) in soil organic matter stabilization in two size fractions having
560 opposite features. *Sci.Total Environ.* 653, 667-674.

561 Henneberry, Y.K., Kraus, T.E.C., Nico, P.S., Horwath, W.R., 2012. Structural stability of
562 coprecipitated natural organic matter and ferric iron under reducing conditions. *Org. Geochem.*
563 48, 81-89.

564 Holmalahti, J., von Wright, A., Raatikainen, O.J.L.i.a.m., 1994. Variations in the spectra of
565 biological activities of actinomycetes isolated from different soils. *Lett. Applied. Microbiol.*
566 18, 144-146.

567 Huang, J., Liu, W., Deng, M., Wang, X., Wang, Z., Yang, L., Liu, L., 2020. Allocation and
568 turnover of rhizodeposited carbon in different soil microbial groups: A meta-analysis. *Soil*
569 *Biol. Biochem.* 107973.

570 Huang, L.M., Jia, X.X., Zhang, G.L., Thompson, A., Huang, F., Shao, M.A., Chen, L.M., 2018.
571 Variations and controls of iron oxides and isotope compositions during paddy soil evolution
572 over a millennial time scale. *Chem. Geol.* 476, 340-351.

573 Iuss Working Group, W., 2015. World reference base for soil resources 2014, update 2015:
574 International soil classification system for naming soils and creating legends for soil maps.
575 FAO Rome, 192.

576 Jeewani, P.H., Chen, L., Van Zwieten, L., Shen, C., Guggenberger, G., Luo, Y., Xu, J., 2020.
577 Shifts in the bacterial community along with root-associated compartments of maize as affected
578 by goethite. *Biol. Fertil. Soils*.

579 Jeewani, P.H., Gunina, A., Tao, L., Zhu, Z., Kuzyakov, Y., Van Zwieten, L., Guggenberger,
580 G., Shen, C., Yu, G., Singh, B.P., Pan, S., Luo, Y., Xu, J., 2020. Rusty sink of rhizodeposits
581 and associated keystone microbiomes. *Soil Biol. Biochem.* 107840.

582 Jones, D.L., 1998. Organic acids in the rhizosphere—a critical review. *Plant and Soil* 205, 25-
583 44.

584 Kaiser, K., Guggenberger, G., 2000. The role of DOM sorption to mineral surfaces in the
585 preservation of organic matter in soils. *Org. Geochem.* 31, 711-725.

586 Kamble, P.N., Bååth, E., 2016. Comparison of fungal and bacterial growth after alleviating
587 induced N-limitation in soil. *Soil Biol. Biochem.* 103, 97-105.

588 Katoh, M., Murase, J., Hayashi, M., Matsuya, K., Kimura, M., 2004. Nutrient leaching from
589 the plow layer by water percolation and accumulation in the subsoil in an irrigated paddy field.
590 *Soil Sci. Plant Nutrit.* 50, 721-729.

591 Keiluweit, M., Bougoure, J.J., Nico, P.S., Pett-Ridge, J., Weber, P.K., Kleber, M., 2015.
592 Mineral protection of soil carbon counteracted by root exudates. *Nature Climate Change* 5,
593 588-595.

594 Kleber, M., Eusterhues, K., Keiluweit, M., Mikutta, C., Mikutta, R., Nico, P., 2015. Mineral–
595 Organic Associations: Formation, Properties, and Relevance in Soil Environments. *Advances*
596 *Agron.* 130, 1-140.

597 Kuzyakov, Y., 2002. Factors affecting rhizosphere priming effects. *J. Plant Nutrit. Soil Sci.*
598 165, 382-396.

599 Lalonde, K., Mucci, A., Ouellet, A., Gélinas, Y.J.N., 2012. Preservation of organic matter in
600 sediments promoted by iron. *Nature* 483, 198-200.

601 Li, Y., Shahbaz, M., Zhu, Z., Chen, A., Nannipieri, P., Li, B., Deng, Y., Wu, J., Ge, T., 2020a.
602 Contrasting response of organic carbon mineralisation to iron oxides addition under conditions
603 of low and high microbial biomass in anoxic paddy soil, *Biol. Fert. Soils*.

604 Li, Y., Liu, Y., Pan, H., Hernández, M., Guan, X., Wang, W., Zhang, Q., Luo, Y., Di, H., Xu,
605 J. 2020b. Impact of grazing on shaping abundance and composition of active methanotrophs
606 and methane oxidation activity in a grassland soil. *Biol. Fert. Soils*. 1-12.

607 Li, Y., Yu, S., Strong, J., Wang, H., 2012. Are the biogeochemical cycles of carbon, nitrogen,
608 sulfur, and phosphorus driven by the “Fe III–Fe II redox wheel” in dynamic redox
609 environments? *J. Soils. Sediments.* 12, 683-693.

610 Li, Y.C., Li, Y.F., Chang, S.X., Yang, Y.F., Fu, S.L., Jiang, P.K., Luo, Y., Yang, M., Chen,
611 Z.H., Hu, S.D., Zhao, M.X., Liang, X., Xu, Q.F., Zhou, G.M., Zhou, J.Z., 2018. Biochar
612 reduces soil heterotrophic respiration in a subtropical plantation through increasing soil organic
613 carbon recalcitrancy and decreasing carbon-degrading microbial activity. *Soil Biol. Biochem.*
614 122, 173–185.

615 Liu, H.Y., Ding, Y., Zhang, Q.C., Liu, X.M., Xu, J.M., Li, Y. and Di, H.J. 2019. Heterotrophic
616 nitrification and denitrification are the main sources of nitrous oxide in two paddy soils. *Plant*
617 *Soil*. 445(1-2), 39-53.

618 Liu, Y., Dong, Y., Ge, T., Hussain, Q., Wang, P., Wang, J., Li, Y., Guggenberger, G., Wu, J. 2019.
619 Impact of prolonged rice cultivation on coupling relationship among C, Fe, and Fe-reducing
620 bacteria over a 1000-year paddy soil chronosequence: *Biol. Fert. Soils*, DOI: 10.1007/s00374-
621 019-01370-x.

622 Lu, X.H., Li, Y.F., Wang, H.L., Singh, B.P., Hu, S.D., Luo, Y., Li, J.W., Xiao, Y.H., Cai, X.Q.,
623 Li, Y.C., 2019. Responses of soil greenhouse gas emissions to different application rates of
624 biochar in a subtropical Chinese chestnut plantation. *Agr. Forest Meteorol.* 271,
625 168–179Lundell, T.K., Mäkelä, M.R., de Vries, R.P., Hildén, K.S., 2014. Genomics, lifestyles
626 and future prospects of wood-decay and litter-decomposing basidiomycota, *Advances in*
627 *Botanical Res.* 329-370.

628 Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Brookes, P.C., 2011. Short term soil priming
629 effects and the mineralisation of biochar following its incorporation to soils of different pH.
630 *Soil Biol. Biochem.* 43, 2304-2314.

631 Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Devonshire, B.J., Brookes, P.C., 2013.
632 Microbial biomass growth, following incorporation of biochars produced at 350 °C or 700 °C,
633 in a silty-clay loam soil of high and low pH. *Soil Biol. Biochem.* 57, 513-523.

634 Luo, Y., Zang, H., Yu, Z., Chen, Z., Gunina, A., Kuzyakov, Y., Xu, J., Zhang, K., Brookes,
635 P.C., 2017. Priming effects in biochar enriched soils using a three-source-partitioning
636 approach: ¹⁴C labelling and ¹³C natural abundance. *Soil Biol. Biochem.* 106, 28-35.

637 McCarthy, A.J., Williams, S.T.J.G., 1992. Actinomycetes as agents of biodegradation in the
638 environment-a review. *Gene* 115, 189-192.

639 Meng, F., Dungait, J.A.J., Zhang, X., He, M., Guo, Y., Wu, W., 2013. Investigation of
640 photosynthate-C allocation 27 days after ¹³C-pulse labeling of *Zea mays* L. at different growth
641 stages. *Plant.Soil.* 373, 755-764.

642 Mikutta, C., Mikutta, R., Bonneville, S., Wagner, F., Voegelin, A., Christl, I., Kretzschmar, R.,
643 2008. Synthetic coprecipitates of exopolysaccharides and ferrihydrite. Part I: Characterization.
644 *Geochim. Cosmochim. Acta* 72, 1111-1127.

645 Mikutta, R., Lorenz, D., Guggenberger, G., Haumaier, L., Freund, A., 2014. Properties and
646 reactivity of Fe-organic matter associations formed by co-precipitation versus adsorption:
647 Clues from arsenate batch adsorption. *Geochim. Cosmochim. Acta* 144, 258-276.

648 Neff, J.C., Townsend, A.R., Gleixner, G., Lehman, S.J., Turnbull, J., Bowman, W.D., 2002.
649 Variable effects of nitrogen additions on the stability and turnover of soil carbon. *Nature* 419,
650 915-917.

651 Olsson, R., Giesler, R., Persson, P.J.J.O.C., science, i., 2011. Adsorption mechanisms of
652 glucose in aqueous goethite suspensions. *J. Colloid. Interface. Sci.* 353, 263-268.

653 Ponnamperna, C.A., 1972. *The origins of life.* Dutton, New York.

654 Perveen, N., Barot, S., Maire, V., Cotrufo, M.F., Shahzad, T., Blagodatskaya, E., Stewart, C.E.,
655 Ding, W., Siddiq, M.R., Dimassi, B., 2019. Universality of priming effect: an analysis using
656 thirty five soils with contrasted properties sampled from five continents. *Soil Biol.*
657 *Biochem.*134, 162-171.

658 Silva, L.C., Doane, T.A., Corrêa, R.S., Valverde, V., Pereira, E.I., Horwath, W.R., 2015. Iron-
659 mediated stabilization of soil carbon amplifies the benefits of ecological restoration in degraded
660 lands. *Ecol. Applicat.* 25, 1226-1234.

661 Sodano, M., Lerda, C., Nisticò, R., Martin, M., Magnacca, G., Celi, L., Said-Pullicino, D.J.G.,
662 2017. Dissolved organic carbon retention by co-precipitation during the oxidation of ferrous
663 iron. *Geoderma* 307, 19-29.

664 Song, X., Li, L., Zhang, X., Zheng, J., Zheng, J., Hussain, Q., Pan, G., 2016. Molecular changes
665 of ferric oxide bound soil humus during the decomposition of maize straw. *Chem.*
666 *Biol. Technol. Agri.* 3, 27.

667 Sutton, R., Sposito, G., 2005. Molecular structure in soil humic substances: the new view.
668 *Environ. Sci. Technol.* 39, 9009-9015.

669 Tinsley, J., Taylor, T.G., Moore, J.H., 1951. The determination of carbon dioxide derived from
670 carbonates in agricultural and biological materials. *The Analyst* 76, 300-310.

671 Van Hees, P.A.W., Jones, D.L., Finlay, R., Godbold, D.L., Lundström, U.S., 2005. The carbon
672 we do not see—the impact of low molecular weight compounds on carbon dynamics and
673 respiration in forest soils: a review. *Soil Biol. Biochem.* 37, 1-13.

674 Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil
675 microbial biomass C. *Soil Biol. Biochem.* 19, 703-707.

676 Wagai, R., Mayer, L.M., Kitayama, K., Shirato, Y., 2013. Association of organic matter with
677 iron and aluminum across a range of soils determined via selective dissolution techniques
678 coupled with dissolved nitrogen analysis. *Biogeochem.* 112, 95-109.

679 Wan, D., Ye, T., Lu, Y., Chen, W., Cai, P., Huang, Q., 2019. Iron oxides selectively stabilize
680 plant-derived polysaccharides and aliphatic compounds in agricultural soils. *Euro. J. Soil. Sci.*
681 70, 1153-1163.

682 Wang, Y., Wang, H., He, J.-S., Feng, X., 2017. Iron-mediated soil carbon response to water-
683 table decline in an alpine wetland. *Nature Comm.* 8, 15972.

684 Wei, X., Zhu, Z., Liu, Y., Luo, Y., Deng, Y., Xu, X., Liu, S., Richter, A., Shibistova, O.,
685 Guggenberger, G., 2020. C: N: P stoichiometry regulates soil organic carbon mineralization
686 and concomitant shifts in microbial community composition in paddy soil. *Biol. Fert. Soils.*

687 Wei, X., Zhu, Z., Wei, L., Wu, J., Ge, T. 2019. Biogeochemical cycles of key elements in the
688 paddy-rice rhizosphere: microbial mechanisms and coupling processes: *Rhizosphere*, 100145.

689 Whitman, T., Neurath, R., Perera, A., Chu-Jacoby, I., Ning, D., Zhou, J., Nico, P., Pett-Ridge,
690 J., Firestone, M.J.E.m., 2018. Microbial community assembly differs across minerals in a
691 rhizosphere microcosm. *Environ. Microbiol.* 20, 4444-4460.

692 Wiesmeier, M., Urbanski, L., Hobbey, E., Lang, B., von Lützow, M., Marin-Spiotta, E., van
693 Wesemael, B., Rabot, E., Ließ, M., Garcia-Franco, N., 2019. Soil organic carbon storage as a
694 key function of soils-A review of drivers and indicators at various scales. *Geoderma* 333, 149-
695 162.

696 Wissing, L., Kölbl, A., Schad, P., Bräuer, T., Cao, Z.-H., Kögel-Knabner, I., 2014. Organic
697 carbon accumulation on soil mineral surfaces in paddy soils derived from tidal wetlands.
698 *Geoderma* 228, 90-103.

699 Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement
700 of soil microbial biomass C by fumigation-extraction—an automated procedure. *Soil Biol.*
701 *Biochem.* 22, 1167-1169.

702 Xue, B., Huang, L., Huang, Y., Ali Kubar, K., Li, X., Lu, J., 2020. Straw management
703 influences the stabilization of organic carbon by Fe (oxyhydr)oxides in soil aggregates.
704 *Geoderma* 358, 113987.

705 Yu, Z., Chen, L., Pan, S., Li, Y., Kuzyakov, Y., Xu, J., Brookes, P., Luo, Y., 2018. Feedstock
706 determines biochar-induced soil priming effects by stimulating the activity of specific
707 microorganisms. *Eur. J. Soil Sci.* 69, 521-534.

708 Zhang, Z., Wang, W., Qi, J., Zhang, H., Tao, F., Zhang, R., 2019. Priming effects of soil organic
709 matter decomposition with addition of different carbon substrates. *J. Soils. Sediments.* 19,
710 1171-1178.

711 Zhou, J.-M., Dang, Z., Cai, M.-F., Liu, C.-Q., 2007. Soil Heavy Metal Pollution Around the
712 Dabaoshan Mine, Guangdong Province, China. *Pedosphere* 17, 588-594.

713 Zhou, P., Song, G., Pan, G., Li, L., Zhang, X., Wu, L., 2009. SOC enhancement in three major
714 types of paddy soils in a long-term agro-ecosystem experiment in South China. II. Chemical
715 binding and protection in micro-aggregate size fractions. *Acta Pedologica Sinica* 46, 263-273.
716 Zhu, Z., Ge, T., Luo, Y., Liu, S., Xu, X., Tong, C., Shibistova, O., Guggenberger, G., Wu, J.
717 2018. Microbial stoichiometric flexibility regulates rice straw mineralization and its priming
718 effect in paddy soil: *Soil Biol. Biochem.* 121: 67-76.

719

720 **Figure and Table captions**

721 **Fig. 1.** Cumulative basal soil-derived (A), substrate-derived (B) and primed soil (C) CO₂ during
722 first 7 days of incubation following the addition of glucose. ‘Basal soil-derived C’ signifies the
723 total CO₂ evolved from the native soil (non labeled C) from the glucose added treatment.
724 ‘Substrate-derived’ signifies the CO₂ evolved from added labeled glucose (based on ¹³CO₂
725 measurements). ‘Primed soil’ C signifies CO₂ evolved from the mineralization of soil organic
726 matter. Error bars represent standard deviation of the means (n= 3).

727

728 **Fig. 2.** Content of ¹³C derived from the addition of labile C substrate in bulk soil and in the Fe-
729 OM complexes (A) and corresponding molar ratio of Fe-bound OC to dithionite-extractable
730 iron (Fe_d) (B). Fe-bound soil organic carbon (Fe-bound OC) content (C) and ratio of Fe_p:Fe_d
731 indicating degree of Fe complexation with organic matter (D) at the completion of the 56 day
732 incubation. Values show means (n=3) ± standard deviation.

733

734 **Fig. 3.** Relative abundance plots showing the relative distribution of various bacterial (A) and
735 fungal (B) phyla.

736

737 **Fig. 4.** Shannon index of soil bacterial (A) and fungal (B) alpha diversity and PCoA plots
738 representing beta diversity based on Bray-Curtis dissimilarity of bacterial (C) and fungal (D)
739 communities.

740

741 **Fig. 5.** Random forest analysis represents relative importance of soil physicochemical (C:N
742 ratio, Fe, C:Fe ratio, DOC, pH, TC, TN and TP) and biological variables (Bacterial diversity,
743 Fungal diversity, and composition of Actinobacteria, Proteobacteria, Ascomycota and
744 Basidiomycota) for substrate (labeled glucose) mineralization (A) and stabilization (B).

745

746 **Fig. 6.** Path analysis showing the underlying relationships between soil physicochemical
747 factors and microbial community diversity and composition for substrate stabilization, priming
748 effect and substrate mineralization in the soil. All the values present were statistically
749 significant (*** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$). Thickness of lines are proportionate to
750 regression weights. Red lines represent negative values; blue lines represent positive values.
751 The bacterial and fungal diversities are represented by the Shannon index. Standardized path
752 coefficients are listed beside each path. (Model fit :Chi-square value=64.72, $df=13$, $P=0.052$).

753

754 **Table 1.**

755 **Table 1.** Properties of four paddy soils. Soil with total Fe concentration of 13.7 g kg⁻¹ is referred
756 to as (Fe-13), soil with total Fe concentration 25.8 g kg⁻¹ (Fe-25), soil with total Fe
757 concentration 42.7 g kg⁻¹ (Fe-42), soil with total Fe concentration 55.8 g kg⁻¹ (Fe-55).

758 Abbreviations: TC; total carbon, TN; total nitrogen, TP; total phosphorous, WHC; water
759 holding capacity, MC; moisture content

760

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

763

764 **Table 3.** Two-way orthogonal partial least squares analysis to reveal the core functional genera
765 (with variable influence projection (VIP) >1.6) involved in C dynamics including priming
766 effect (PE) and substrate mineralization

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