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Abstract

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

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

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

1. Introduction

Paddy soils play an important role in the storage of soil carbon C due to their unique geochemical properties (Ge et al., 2012; Wiesmeier et al., 2019; Wei et al., 2019). Iron (Fe) plays an important role in many physicochemical processes, including the stabilization of soil organic matter (SOM) (Kaiser and Guggenberger, 2000; Boyd and Ellwood, 2010; Jeewani et al., 2020); as well as biotic processes resulting in Fe adsorption, co-precipitation and mineral transformation (Jones, 1998; Chen et al., 2014; Huang et al., 2018; Wan et al., 2019). Previous studies have coupled the biogeochemical cycling of Fe with SOM (Ponnamperuma, 1972; Huang et al., 2018; Wiesmeier et al., 2019; Liu et al., 1029). Unlike C/N/P stoichiometry, only a limited number of studies have evaluated the mechanisms and effect of C:Fe molar ratio on SOM accumulation in paddy soils (Zhou et al., 2009; Song et al., 2016). Therefore, a better understanding of C-Fe cycling and the role of the soil microbial community is needed to optimize C storage in paddy soils.

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

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

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

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

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

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

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

2. Materials and methods

2.1. Soil sampling and preparation

Soils were sampled from the 0–20 cm (plough layer) of long-term rice (*Oryza sativa*) paddies in Hunan province, China. Soil sampling was done at 4 sites along a 200 m transect representing an Fe concentration gradient, with three replicates collected within a 1 m^2 plot at each sampling location. Ethanol (70%) was used to sterilize equipment during sampling to avoid microbial cross-contamination between soils. The sampling region had a mean annual temperature of 17.7 ^oC, and a rainfall of 1402 mm (Table 1). The soil was classified as a Gleyic Stagnic Anthrosol (FAO) (Iuss Working Group, 2015). The soils had Fe concentrations of 13.7 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} (Fe-55) (Table 1). The soil samples had visible plant roots manually removed and were sieved at field moisture (<2 mm) before being air-dried. Prior to a 14-day pre-incubation period (at 25°C), water holding capacity (WHC) of each soil was determined using a volumetric method (see supplementary information). Soils were then sprayed with deionized water to obtain a water content of 40% that of the WHC. This soil preparation protocol mimics the field situation, where the soil stays mostly dry before rice cultivation and avoids the formation of reducing 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 ^{13}C -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 CO_2 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%-^{13}C) (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 ^{0}C for 56 days. Three blank jars containing only
155 water and CO_2 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 ^{13}C 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

extraction method (Vance et al., 1987). Fumigated and unfumigated portions of 15 g moist soil were extracted with 30 ml 0.5M K_2SO_4. Organic C in the extracts was measured using a TOC analyser (Multi N/C 3100, Analytik Jena AG, Jena, Germany). Microbial biomass C was calculated as EC/kEC, where EC= (organic carbon extracted from fumigated soils) - (organic carbon extracted from non-fumigated soils) and kEC=0.45 (Wu et al., 1990). DOC was determined from the non-fumigated component of the soil. ^{13}C in soil microbial biomass C was determined as described by Luo et al. (2013). The natural abundance of $\delta^{13}C$ in soils was measured using air-dried and sieved (<200 µm) soil, which was accurately weighed (about 0.2 mg) into tin capsules prior to analysis using an isotope ratio mass spectrometer with IAEA-600 (Caffeine); $\delta^{13}C$=-27.771‰ as standard material (Meng et al., 2013) (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).

The total Fe concentration of the soil sample was determined after digestion with a concentrated acid mixture of HF, $HClO_4$, and HNO_3 (Zhou et al., 2007). Dithionite-citrate-bicarbonate (DCB) extraction method was used to quantify the total extractable soil iron oxide (Fe_d) concentration by determining the Fe concentration in the solution (Lalonde et al., 2012; Wang et al., 2017; Wan et al., 2019). Freeze-dried soil (0.50 g) was mixed with 30 mL of buffer solution (0.27 M trisodium citrate and 0.11 M sodium bicarbonate, pH 7.3) in 50 mL centrifuge tubes, which was then placed in a water bath (80 °C). Sodium dithionite (0.50 g) was added to the mixture, and it was maintained at 80 °C for 15 min. Soils were extracted by sodium chloride (NaCl) instead of trisodium citrate and sodium dithionite, at an equivalent ionic strength as the control under the same conditions. The suspension was then separated by centrifugation at 4000 x G for 10 min. The residue was thrice rinsed with ultra-pure H_2O (5 mL), centrifuged, and the supernatant was carefully removed. The precipitate was freeze-dried overnight. Organically complexed Fe oxides (Fe_p) and soil amorphous Fe oxides (Fe_o) were extracted with sodium

pyrophosphate and ammonium oxalate, respectively (Keiluweit et al., 2015; Wan et al., 2019). The TC and TN in the extracted residue was analyzed using an elemental analyzer, and the $\delta^{13}C$ isotopic composition of the residue was obtained by 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). For the analysis of the soluble Fe concentration, washings and supernatants were combined and acidified to pH 2.0 before being filtered using a 0.45-μm PTFE membrane filter. The Fe concentration in solution was determined using ICP-MS. The calculations are found in the supplementary information.

2.4. Analysis of $\delta^{13}C$-CO_2

The CO_2 traps were collected and changed after 1, 3, 7, 14, 28, and 56 days for the determination of C mineralization. At sampling, a 5 mL aliquot of trap solution was diluted with 10 mL water and titrated against standardized 0.5 M HCl using an Easy Plus auto titrator (Mettler Toledo, Greifensee, Switzerland) to determine the total CO_2 evolved (Tinsley et al., 1951). To determine the $\delta^{13}C$-CO_2 from the trapped CO_2, an 8 mL aliquot of the trap solution was added to 8 mL of 1.5 M $BaCl_2$ into 50 mL falcon tubes and incubated at 25 ^{o}C for 0.5 h to allow precipitation. The resulting $BaCO_3$ precipitate was then filtered, rinsed three times with deionized water, and freeze-dried overnight in a falcon tube (Luo et al., 2011; Luo et al., 2017). The precipitates were scraped from the falcon tube, accurately weighed (about 0.2 mg) into tin capsules, and analyzed using a stable isotope ratio mass spectrometer (IRMS, Thermo Fisher, USA) (Aoyama et al., 2000).

2.5 $\delta^{13}C$ calculations

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

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

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

$$CO_2\,{}^{13}_{\square}C(\mu g\, g^{-1} soil) = CO_2\,{}^{13}_{\square}C(\%) \times \text{Total evolved } CO_2C(\mu g\, g^{-1} soil)/100 \quad (2)$$

$$CO_2\,{}^{13}_{\square}C(\%) = (\delta\,{}^{13}_{\square}C_{treatment} - \delta\,{}^{13}_{\square}C_{original})/\delta\,{}^{13}_{\square}C_{substrate} - \delta\,{}^{13}_{\square}C_{original}) * 100 \quad (3)$$

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

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

$$\text{Priming soil } CO_2C(\mu g\ C\ g^{-1} soil) = CO_2C_{treatment\text{-}1} - CO_2C_{control\text{-}1} \quad (4)$$

Where, $CO_2C_{treatment\text{-}1}$ is the non-isotopically labeled C in CO_2 evolved from substrate amended soil, and CO_2-$C_{control\text{-}1}$ is the C in total CO_2 evolved from the control.

Substrate-derived C represents the CO_2 evolved from glucose (based on $^{13}CO_2$ measurements). Primed soil C signifies CO_2 evolved from native soil, primed by added glucose. Basal soil-

derived C accounts for the total CO_2 evolved from the respective control soil without glucose addition.

2.6. DNA extractions and sequencing

DNA of 0.50 g of freeze-dried soil was 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'- CTTGGTCATTTAGAGGAAAAGTAA-3' forward primer and 5'-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 Ampure XP beads (Indianapolis, USA). All amplicons were pooled across all samples at equimolar concentrations (20 ng μl^{-1}) into a representative sample, and the index sequencing of 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).

2.7. Processing of sequencing data and related statistical analysis

The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered by Trimmomatic, and connected by FLASH using the following steps. Firstly, the 250 bp reads were cut-off at the site, receiving an average quality score of less than 20 over a 50 bp sliding window, and the cut-off reads shorter than 50 bp with the reads containing mismatch characters. Secondly, sequences that were longer than ten bp were assembled according to their overlapped sequence. The maximum mismatch ratio of the overlap region is 0.2, and those mismatched reads were discarded. Thirdly, samples were differentiated according to the barcode and primers. Finally, sequence direction adjustment and barcode matching was undertaken. Between 4,682 and 51,150, valid sequences were obtained per sample for all experimental samples**.** UPARSE (version7.1, http://drive5.com/uparse) was used to cluster the operational taxonomic units (OTUs) with 97% similarity cut-off. Chimeric sequences were removed. The RDP classifier (http://rdp.cme.msu.edu/) was used to examine the taxonomy of each OTU representative sequence against the 16S rRNA database using a 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 communities was calculated using the alpha diversity index (herein referred to as the Shannon index) that represents both the richness and the evenness of the community. Principal coordinates analyses (PCoA) were used to analyze the β-diversity analysis. Relative effects of soil variables such as TC, TN, C:N ratio, C:Fe molar ratio, DOC, Fe-OM, and pH on soil bacteria and fungi communities were analyzed by distance-based linear model multivariate analysis (distLM) using the distLM forward3 software (Anderson, 2003). SIMCA-P 14 (Version 14.1.0.2047) software used to determine the two-way orthogonal partial least squares (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 datasets, and the X-matrix was intended as the microbial community datasets (Trygg and Wold, 2003). The relative

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

3. Results

3.1. Sources of CO_2 efflux

306 The respired CO_2 during the first 0-7 days was partitioned into substrate derived, basal
307 soil-derived, and primed soil CO_2, compared across the gradient of soil Fe concentrations and
308 glucose amendment (Fig 1). Total CO_2 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 CO_2 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 ^{13}C in soil increased (58%) with the increase of Fe concentrations from
316 Fe-13 to Fe-55, relative to the total ^{13}C content (Fig. 2A). The ^{13}C distribution was analyzed to
317 quantify the ^{13}C stabilization within the Fe-OM complexes. The ^{13}C 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 ^{13}C (Fig. 2A). The glucose addition resulted in a ^{13}C
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*

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

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

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

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

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

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

4. Discussion

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

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

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

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

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

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

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

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

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

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

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

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

5. Conclusion

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

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719

720 **Figure and Table captions**

721 **Fig. 1.** Cumulative basal soil-derived (A), substrate-derived (B) and primed soil (C) CO_2 during
722 first 7 days of incubation following the addition of glucose. 'Basal soil-derived C' signifies the
723 total CO_2 evolved from the native soil (non labeled C) from the glucose added treatment.
724 'Substrate-derived' signifies the CO_2 evolved from added labeled glucose (based on $^{13}CO_2$
725 measurements). 'Primed soil' C signifies CO_2 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 ^{13}C 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).

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

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

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