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Abstract

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

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

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

1. Introduction

Soil management to increase soil organic matter (SOM) storage had been suggested as a countermeasure to mitigate the increasing CO_2 concentration in the atmosphere (Jeewani et al., 2020a; Lal, 2004; Minasny et al., 2017). Paddy soils account for approximately 40% of China's soil carbon (C) sequestration potential (Pan et al., 2004; Atere et al., 2020). SOM storage in paddy soils is promoted through enhanced physical protection within aggregates (Jastrow et al., 2007; Chen et al., 2020; Duan et al., 2020), physicochemical stabilization by the formation of organo-mineral complexes (Jeewani et al., 2020a; Kleber et al., 2015; Yu et al., 2017), and changes in the soil microbial community (Butler et al., 2003; Kandeler et al., 2008). The mechanisms for the physicochemical stabilization and concomitant changes of microbial processing of C in rice paddies, however, requires an improved understanding to optimize soil and SOM management.

An important physicochemical stabilization mechanism of SOM in the soil is through the interaction with Fe oxides (Kaiser and Guggenberger, 2000; Boyd and Ellwood, 2010; Jeewani et al., 2020a). There is a growing pool of literature showing that Fe(III) polycations bind with SOM to form Fe-organic matter complexes (Fe-OM), which stabilize both native SOM and new inputs of C in soil (Chen et al., 2020; Duan et al., 2020; Jeewani et al., 2020a). The formation of Fe-OM complexes is mainly attributed to two main processes, adsorption, and co-precipitation (Kleber et

al., 2015; Chen et al., 2020). The formation and stabilization Fe-OM complexes in paddy soil depend on pH, C:Fe molar ratio, and/or redox potential (Chen et al., 2014; Liu et al., 2019). The dominance of either co-precipitation vs. adsorption processes can be assessed by the C:Fe molar ratio of the Fe-OM complex. Ratios <1 indicate that C is stabilized via adsorption while ratios >6 are due to co-precipitation (Wang et al., 2017). The active Fe ratio is another important index that influences soil physicochemical properties such as organic matter retention, surface charge, specific surface area and aggregate formation (Duiker et al., 2003; Wan et al., 2019).

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

Although significant advances have been made to better understand the modulation of the microbial community composition and activity by Fe oxides in soil, a paucity of knowledge exists on the role of Fe oxides in SOC storage via mediating bidirectional processes of C decomposition and stabilization. Specifically, the contributions of abiotic (physicochemical) and biotic (microbial community) processes to mineralization and stabilization of C as governed by Fe oxides are poorly understood. The key mechanisms by which Fe oxides regulate C contents in soils include; i)

fostering soil aggregation and thus physically protecting SOM from biological degradation (Barral et al., 1998; Jeewani et al., 2020a), ii) stabilizing SOM by adsorption, and co-precipitation and iii) enabling microbially mediated electron transfer via the donation of electrons to oxidants and accepting an electron from microbes (Weber et al., 2006; Chen et al., 2020).

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

2. Materials and methods

2.1 Soil sampling

Soils were Gleyic Stagnic Anthrosol (FAO, 2015) developed under long-term rice (*Oryza sativa*) paddies (from the 0–20 cm layer) in the subtropical region of Hunan Province, China. The region has a mean annual temperature of 17.7 ^{o}C and rainfall of 1402 mm. The soils were chosen to achieve four different contents of Fe oxides and oxyhydroxides, thereafter termed as Fe oxides.

The concentrations of soil Fe oxides were 13.7 Fe g kg^{-1} (referred to as Fe-13), 25.8 g kg^{-1} (Fe-25), 42.7 g kg^{-1} (Fe-42), and 55.8 g kg^{-1} (Fe-55) (Table S1).

2.2 Experimental design

The soil samples had visible plant roots, which were manually removed. Thereafter, the soil was dried to 40% water holding capacity and sieved (<2 mm). The soils were pre-incubated at 25°C for 14 days before starting the experiment. To investigate the mineralization, stabilization, and priming effects of maize-straw derived C from the 4 paddy soils (i.e., 4 Fe oxides concentrations), a 56-day incubation experiment was established, where each soil had either a control (no amendment) or ^{13}C labeled straw amendment (n=3), to make a total of 24 incubation jars. To establish the incubation, each beaker (100 mL) contained 40 g of oven-dry equivalent soil was placed within a 1L glass jar containing 10 mL water on the base to maintain humidity. The incubation jar also contained a CO_2 trap (20 mL 1 M NaOH), which was replaced at several sampling times (1, 3, 7, 14, 28, and 56 days). Labeled maize-straw with a ^{13}C enrichment of $\delta^{13}C$=5000 ‰ was collected from a previous CO_2 labeling experiment. The ^{13}C labeled maize-straw was oven-dried at 105°C for 2 h, then at 60°C for 48h, and thereafter ground to pass a 1mm sieve. Maize straw was applied at 500 μg C g^{-1} soil by evenly mixing into the 40 g of soil, representing an equivalent of 1275 kg dry maize-straw ha^{-1}. The jars were sealed with a rubber bung and incubated at 25°C for 56 days. Three blank jars contained only water and CO_2 traps. The incubation jars were opened periodically to maintain aerobic conditions.

2.3 Soil physical and chemical analyses

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

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

The total Fe oxide concentration of each soil was determined after acid digestion with a concentrated mixture of HF, $HClO_4$, and HNO_3 (Zhou et al., 2007). Dithionite-citrate-bicarbonate (DCB) extraction was used to quantify total reactive soil Fe oxides (Fe_d) (Lalonde et al., 2012; Wang et al., 2017). In brief, 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 and 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. As a control, soils were extracted by sodium chloride (NaCl) instead of trisodium citrate and sodium dithionite, at an equivalent ionic strength under the same conditions. Then, the solution was separated by centrifugation at 4000 for 10 min. The residue was washed three times, each with 5 mL of deionized water, and then freeze-dried. Total carbon (TC) and total nitrogen (TN) and $\delta^{13}C$ isotopic composition in the precipitate were analyzed by isotope ratio mass spectrometer (Thermo Fisher Scientific, DELTA V plus IRMS, Bremen, Germany) coupled to an elemental analyzer (EA NA1500 - EA 1110 device, Carlo Erba and Thermo Fisher Scientific, Bremen, Germany). Organically complexed Fe oxides (Fe_p) were extracted using sodium pyrophosphate (Keiluweit et al., 2015; Wang et al., 2017), and amorphous soil Fe (Fe_o) was extracted with ammonium oxalate (Keiluweit et al., 2015; Wang et al., 2017). For the analysis of the soluble Fe, washings and supernatants were combined and acidified to pH 2.0. The combined samples were filtered using a pre-combusted 0.45-μm PTFE membrane filter,

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

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

For the determination of C mineralization, CO_2 was collected in traps filled with 1M NaOH, which were changed after 1, 3, 7, 14, 28, and 56 days. At sampling, a 5 mL aliquot of trap solution was diluted with 10 ml H_2O 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 a 50 mL falcon tube 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 H_2O, and freeze-dried overnight in a falcon tube (Luo et al., 2011). The precipitates were carefully removed from the falcon tube, accurately weighed (about 0.2 mg) into tin capsules, and analyzed using a stable isotope ratio mass spectrometer (Thermo Fisher Scientific, DELTA V plus IRMS, Bremen, Germany).

2.5 $\delta^{13}C$ calculations

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

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

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 Peedee belemnite (PDB) standard (0.0112372). According to Luo et al. (2011), the labeled ^{13}C (%) was then estimated from:

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

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

Where $CO_2{}^{13}C$ (%) is the proportion of evolved CO_2 from the added maize-straw, $\delta C_{treatment}$ is the $\delta^{13}C$ (‰) of evolved CO_2 from soil amended with maize straw, $\delta C_{original}$ is the $\delta^{13}C$ (‰) of original soil C before maize-straw was added. δC_{straw} is the $\delta^{13}C$ (‰) from the added maize-straw. Thus, the CO_2-C produced by the straw during the incubation was calculated.

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

Where, CO_2-$C_{treatment\text{-}1}$ is the non-isotopically labeled CO_2-C evolved from maize-straw amended soil, Total CO_2-$C_{\,treatment}$ is the soil evolved CO_2 from the treatment, and Total CO_2 -$C_{\,maize\ straw}$ is the evolved CO_2 derived from the isotopically labelled maize-straw.

The primed soil CO_2-C with the addition of maize-straw was calculated from:

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

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

2.6 DNA extractions sequencing and processing of sequencing data

After the incubation, 0.50 g of soil was freeze-dried and extracted using a Fast DNA Spin Kit (MP Biomedicals, Santa Ana, CA, USA). The extracted DNA was dissolved in 50 µl of TE buffer, and the concentrations of DNA were quantified using a Nanodrop 2000 (Thermo Scientific, Willmington, USA). The bacterial 16S rRNA gene fragments were amplified using primer sets targeting the V4-V5 variable region. The forward primer is 515F (5'-GTGCCAGCMGCCGCGGTAA-3') linked with a specific-sample 5-bp barcode sequence at the 5'end of primer, and 806R (5'- GGACTACHVGGG TWTCTAAT -3') was used as the reverse

primer (Bates et al., 2011). The ITS1 region was amplified by PCR for fungal genes using the 5'-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).

The raw 16S rRNA gene sequencing reads were demultiplexed, quality-filtered by trimmomatic, and connected by FLASH using the following steps. The 250 bp reads were cut-off 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. Then, sequences longer than ten bp were assembled according to their overlapped sequence. The maximum mismatch ratio of the overlap region was 0.2, and those mismatched reads were discarded. Finally, samples were differentiated according to the barcode and primers, then adjust the sequence direction and exact barcode matching. UPARSE (version 7.1, 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 Shannon index. β-diversity was analyzed using principal coordinate analyses (PCoA). Relative effects of soil variables such as TC, C:N ratio, Fe_d, C:Fe molar ratio, DOC, and pH on soil bacteria and fungi communities were analyzed by distance-

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

2.7 Bidirectional orthogonal partial least squares

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

2.8 Co-occurrence network (Co-Net)

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

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

2.9 Random forest analysis and structural equation modeling

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

Structural equation modeling (SEM) was conducted in Amos 18.0 (IBM, Chicago, IL, USA) to investigate the pathways and drivers of stabilization and mineralization of substrate in the soils with respect to direction, magnitude, and effect relationships. In the SEM, chi-square 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 20 (SPSS, Inc., Chicago, IL, USA) was used to analyze all non-microbial data. One-way ANOVA was used to assess the difference of mineralization between soils. We examined the relations between variables by Pearson's correlation analysis (with a two-tailed test), after performing a Kolmogorov-Smirnov (K-S) test to determine whether the data were normally distributed. Residues were analyzed for normal distribution and homogeneity by Shapiro-Wilk and Levene’s tests. If conditions were met, the Tukey Post-hoc test was performed to reveal differences between the treatments.

3. Results

3.1 CO_2 efflux

The higher concentration of Fe oxides in paddy soil resulted in lower total soil CO_2 efflux following the addition of maize straw (Fig 1). The highest maize-straw derived $^{13}CO_2$ efflux from each incubation period was found in Fe-13 soil ($P < 0.05$), while the lowest ($P < 0.01$) substrate-derived CO_2 efflux, as well as the smallest PE, was observed in Fe-55 (Fig. 1). Maize-straw derived $^{13}CO_2$ efflux increased in Fe-13 (from 31% to 33%) between days 3 and 7.

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

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

3.3 Soil microbial community along with the Fe concentration

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

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

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

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

3.4 Co-occurrence network

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

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

3.5 Association between C dynamics and the keystone microbiota

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

355 *3.6 Correlations between microbial communities and Fe-related edaphic variables*

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

364 Random forest analysis allowed us to investigate the relative importance of
365 physicochemical and biological factors on substrate mineralization, the PE, and maize-straw
366 derived C stabilization. It showed that maize-straw mineralization and the PE were mainly
367 governed by the involvement of soil biological factors such as bacterial and fungal diversity.
368 Maize-straw-derived C stabilization was regulated mainly by Fe_d (8.9% increase of the mean
369 squared error (IncMSC), C:Fe molar ratio (7.7% IncMSC), C:N ratio (6.7% IncMSC), and DOC
370 (5.9% IncMSC) (Fig. 5). SEM also supported the notion that the key pathways of substrate-
371 derived C stabilization and mineralization were controlled by physicochemical processes and
372 microbial interactions (Fig. S1). C:Fe molar ratio exerted a direct positive effect on substrate
373 stabilization, as measured by CO_2 efflux (+0.81, $P < 0.001$) (Fig. S1). Fe_d exerted a positive effect
374 on bacterial diversity (+0.69, $P < 0.001$), network co-occurrence (+0.59, $P < 0.001$) and fungal
375 diversity (+0.76, $P < 0.01$). The bacterial diversity, network co-occurrence, and fungal diversity
376 had a positive influence on substrate mineralization (+0.71, $P < 0.001$) and soil priming (+0.54, P
377 < 0.05). However, bacterial diversity (-0.43, $P < 0.01$) and fungal diversity (-0.89, $P < 0.05$) had
378 a negative impact on substrate stabilization.

4. Discussion

4.1 Contribution of abiotic variables on maize-straw stabilization

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

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

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

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

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

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

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

Random forest analysis confirmed our hypothesis that maize-straw derived C stabilization was governed mainly via abiotic mechanisms, while decomposition was regulated via both biotic and abiotic variables (Fig. 5). Further, SEM analysis revealed that maize-straw mineralization was controlled by bacterial diversity, pH, and C:N ratio of the soil (Fig. S1). It was reported that the mineralization of SOM is not only directly governed by interactions between the effects of microbial community structure but also substrate availability (Juarez et al., 2013; Mohanty et al.,

2013). Moreover, Fe_d and C:Fe molar ratios have indirect effects on maize-straw mineralization by limiting the C resources to microbial utilization. Hence, both abiotic protection and effects on the community structure resulted in lower maize-straw mineralization in Fe-55 (32.5% mineralized) compared to Fe-13 (57.5% mineralized).

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

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

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

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

486 *Gaiella* (affiliated with the phylum Actinobacteria) was the key genera responsible for
487 maize-straw mineralization and the PE (Table 3). *Gaiellales* is a deep-branching lineage of
488 Actinobacteria, which can adapt to resource-poor oligotrophic conditions by having the potential
489 to fix CO_2 (McCarthy and Williams, 1992; Holmalahti et al., 1994; Severino et al., 2019).
490 Actinobacteria are a functionally diverse group of organisms that are known to be involved in C
491 cycling in soils (McCarthy and Williams, 1992; Holmalahti et al., 1994). They exhibit a high
492 substrate versatility and metabolic diversity and can survive by utilizing diverse C and N resources
493 (e.g., by upregulating cellulolytic activity) in both copiotrophic and oligotrophic environments.
494 Genera belonging to *Glomeraceae* (phylum Glomeromycota) contributed to maize-straw
495 mineralization, and certain genera appear to preferentially associate with Fe oxide surfaces
496 (Whitman et al., 2018) and access nitrogen from SOM (Veresoglou et al., 2012). Their role in soil
497 C processes reflects their mycelial growth habit, which makes it possible to explore the soil in
498 search of resources (Jia et al., 2019).

499

5. Conclusions

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

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713

714 **Figure and Table captions**

715 **Fig. 1.** CO_2 efflux of basal soil-derived, maize-straw-derived, and maize-straw primed CO_2 during
716 the first 7 days of incubation with the addition of maize-straw. Basal soil-derived represents the

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

726

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

734

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

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

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

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

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

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

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

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