

Supplement of Biogeosciences, 16, 1433–1445, 2019
<https://doi.org/10.5194/bg-16-1433-2019-supplement>
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Supplement of

Iron minerals inhibit the growth of *Pseudomonas brassicacearum* J12 via a free-radical mechanism: implications for soil carbon storage

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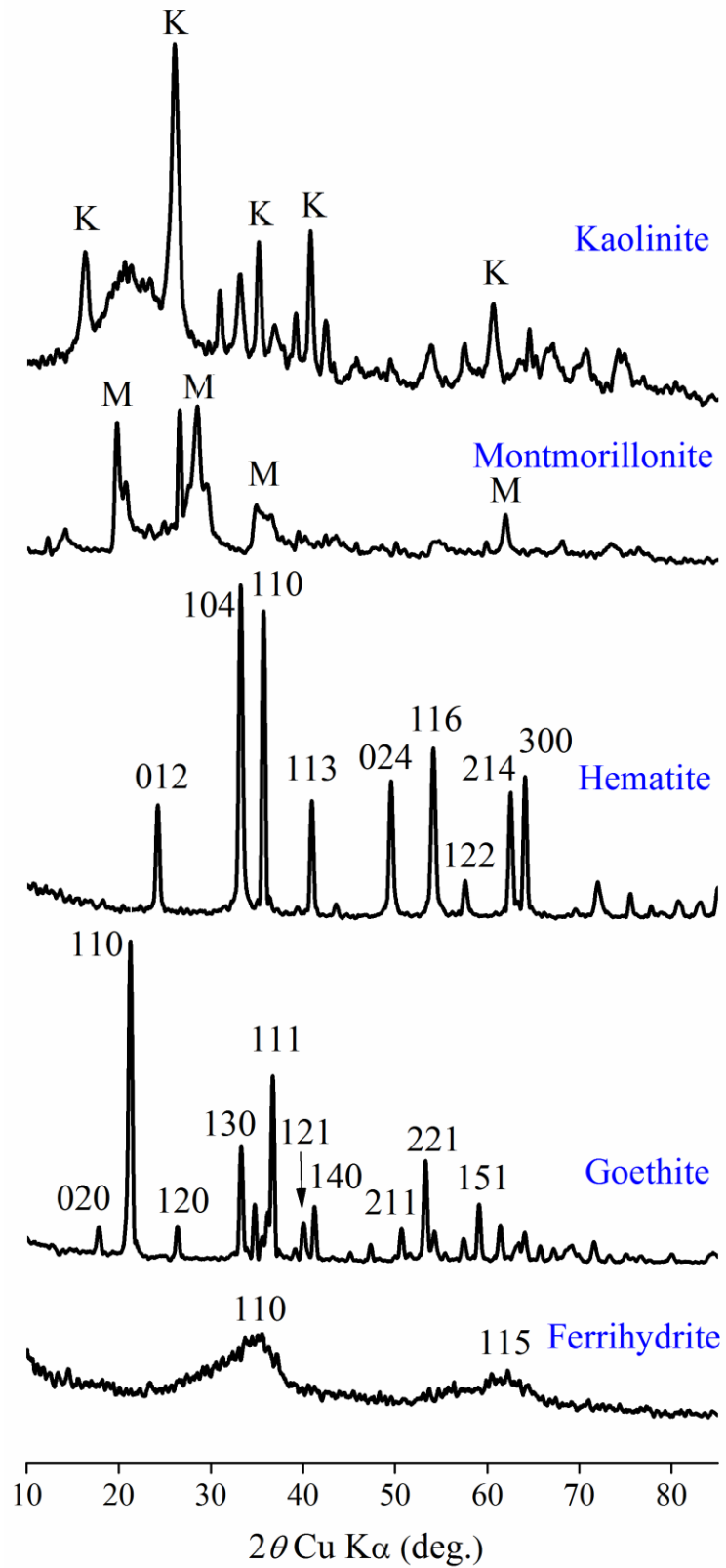
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20 **Supporting Methods**

X-ray diffraction (XRD). The synthetic Fe(III)-containing minerals used in this study were analyzed using X-ray diffraction (XRD) (Nanjing Normal University). Diffraction patterns were recorded from 3° to 60° 2-theta using Ni-filtered Cu K α radiations and counting for 1 s per step with a step size of 0.02° (Li et al., 2016).

25 *Analysis of FTIR Spectroscopy.* Samples were prepared as a mixture of 1 mg of minerals and 100 mg of potassium bromide (KBr, IR grade), and this mixture was then ground and homogenized. A subsample was then compressed between two clean, polished iron anvils twice in a hydraulic press at 20 000 psi to form a KBr window. The FTIR spectra were obtained by collecting 200 scans with a Nicolet 370 FTIR
30 spectrometer (Yu et al., 2012).

Analysis of amino acids. To test the effect of produced hydroxyl radicals on substrate availability, the concentration of free and total amino acids was detected by Amino Acid Analyzer (L-8900, Hitachi High-Technologies Corporation, Tokyo, Japan). As for raw culture and a cultivation time of 12 h with montmorillonite and ferrihydrite,
35 respectively, portions of the samples were filtered through a 0.22 μm membrane filter. First, 1 mL of the samples was processed with 5 mL 6M HCl. Second, the mixtures were placed in the oven for 22 h at 110 °C and then vacuum drying. Finally, the 1 mL ultrapure water was used to redissolve the precipitate and filtered through a 0.22 μm membrane filter. The concentration of free and total amino acids for samples were
40 analyzed by Amino Acid Analyzer and the values are expressed as the mean \pm SE (n = 3). A standard amino acid mixture of cysteic acid and methionine sulfone (20 $\mu\text{L mL}^{-1}$) was diluted to 100 $\mu\text{mol L}^{-1}$ for amino acid quantification and calibration. An external standard was used to calculate the concentration of each amino acid.



45 **Figure S1.** Powder X-ray diffraction (XRD) pattern of the minerals used in this study.

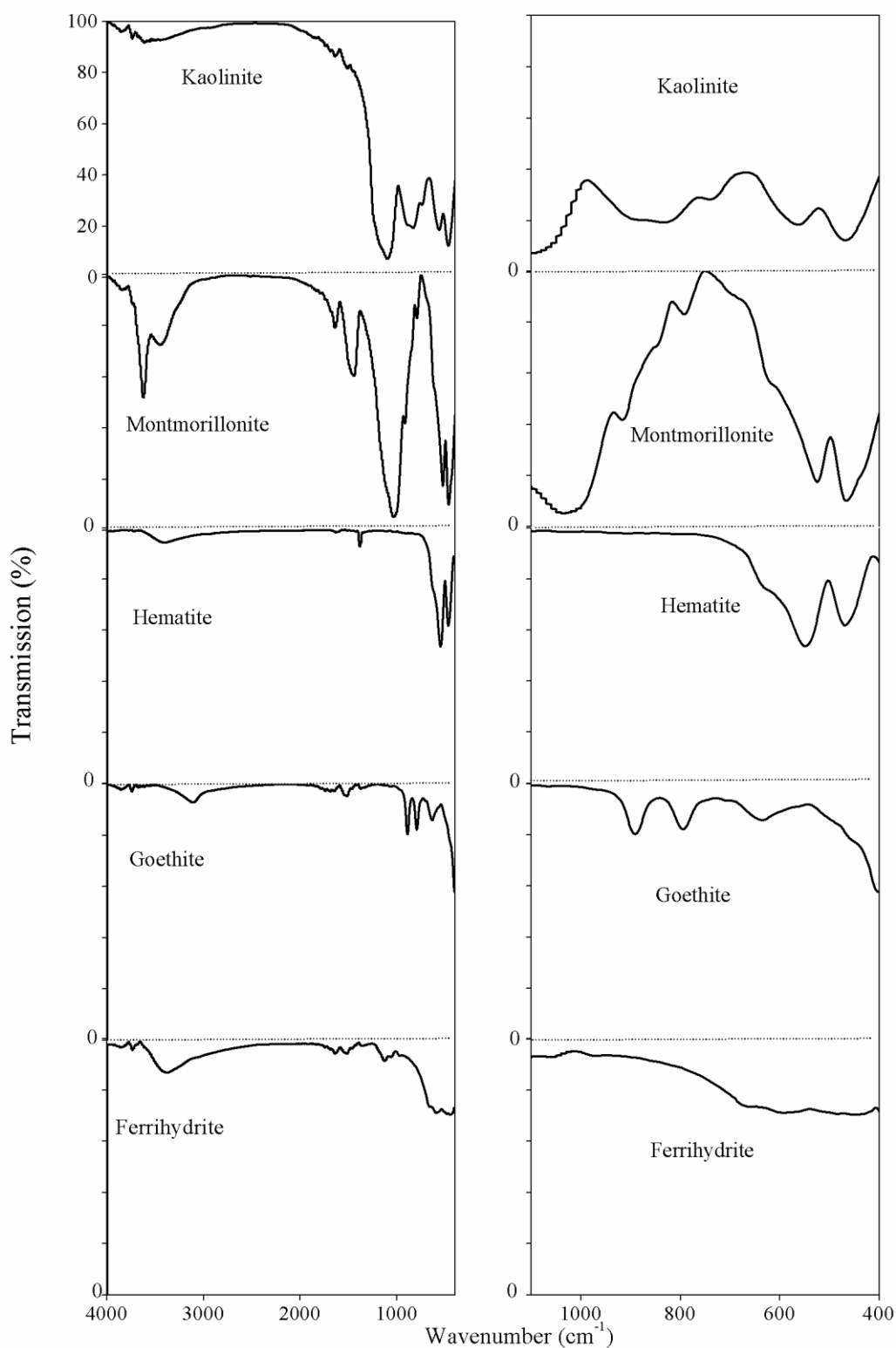
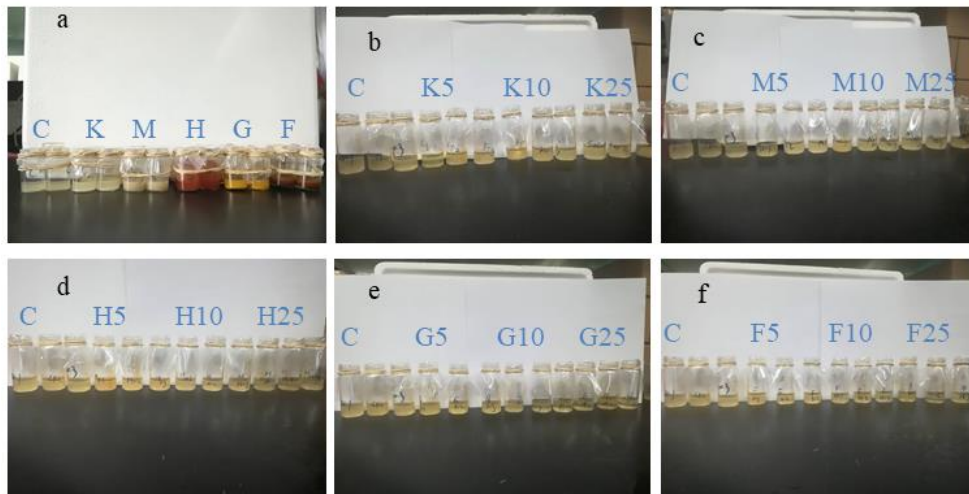


Figure S2. FTIR spectra of the minerals used in this study.



50 **Figure S3.** Photographs of the pre-cultivation media after 12 h growth (a) and the
sub-cultivation media after 8 h (b-f). (b) control vs K. (c) control vs M. (d) control vs
H. (e) control vs G. (f) control vs F. K, kaolinite; M, montmorillonite; H, hematite; G,
goethite; F, ferrihydrite; C, Control (i.e., no mineral). The number 5, 10, and 25
represents the concentration of mineral (Unit: mg mL^{-1}). All experiments were
55 performed in triplicate.

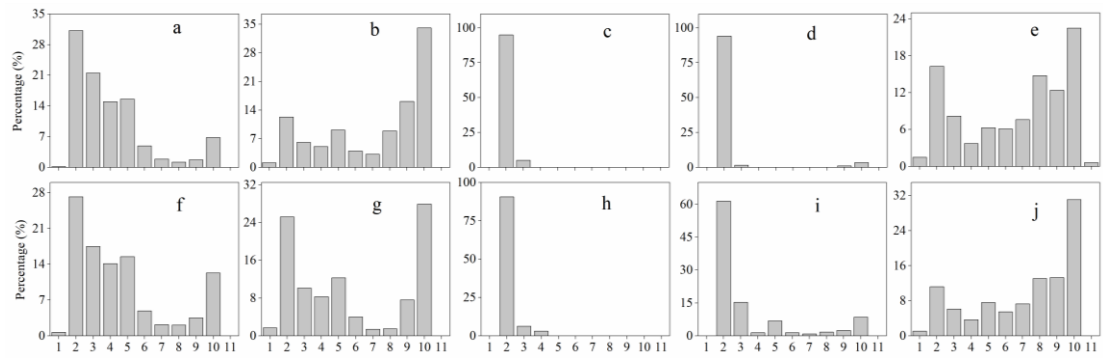
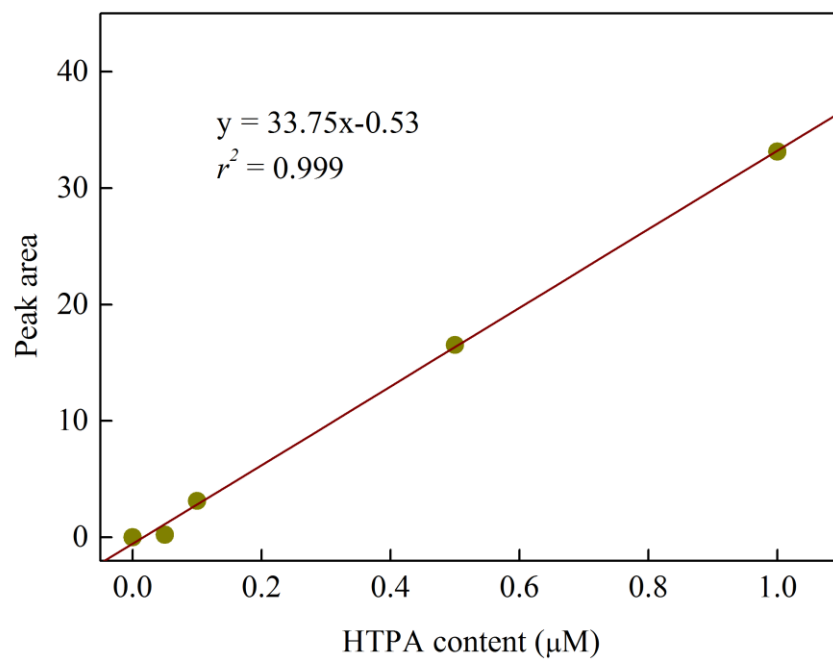


Figure S4. The particle size distribution (% in volume) of both the applied raw minerals and the changes after 24 h of cultivation. 1-11 represent the particle size of < 60 0.1, 0.1-0.5, 0.5-1, 1-2, 2-5, 5-10, 10-20, 20-50, 50-100, 100-500 μm , respectively. (a) kaolinite; (b) montmorillonite; (c) hematite; (d) goethite; (e) ferrihydrite; (f) kaolinite + bacteria; (g) montmorillonite + bacteria; (h) hematite + bacteria; (i) goethite + bacteria; (j) ferrihydrite + bacteria.



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Figure S5. Standard curve of specific fluorescent dihydroxyterephthalic acid (HTPA).

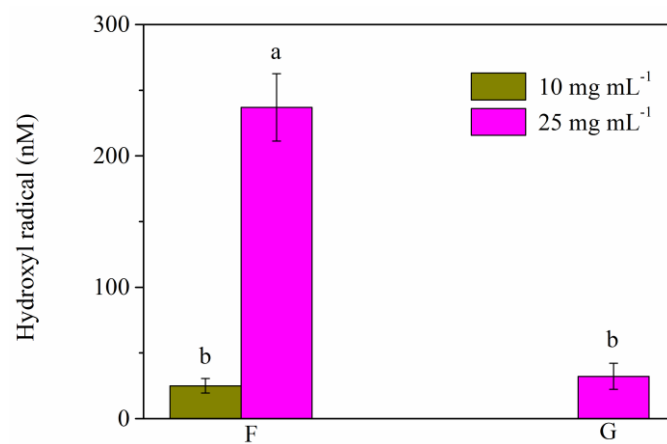


Figure S6. Generation of hydroxyl radical (HO[•]) after 2 h growth of *Pseudomonas brassicacearum* J12 with different concentrations (i.e., 10 and 25 mg ml⁻¹) of minerals. F, ferrihydrite; G, goethite. Values are the mean ± SE (n = 3).

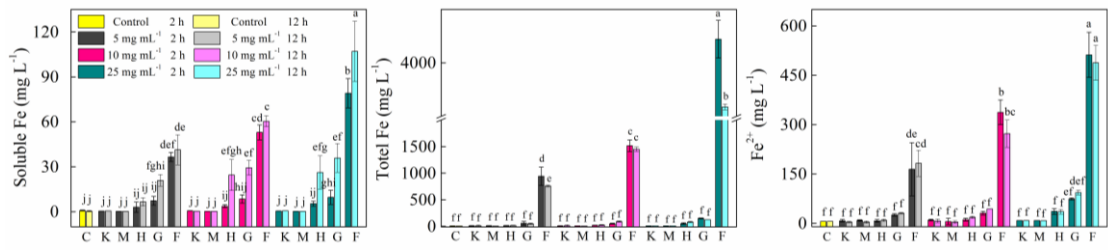


Figure S7. Statistical analysis for (a) soluble Fe, (b) total Fe, and (c) Fe(II).

75 Significance was determined using one-way ANOVA followed by Tukey's HSD post hoc test. Values in (a-c) are the mean \pm SE (n = 3).

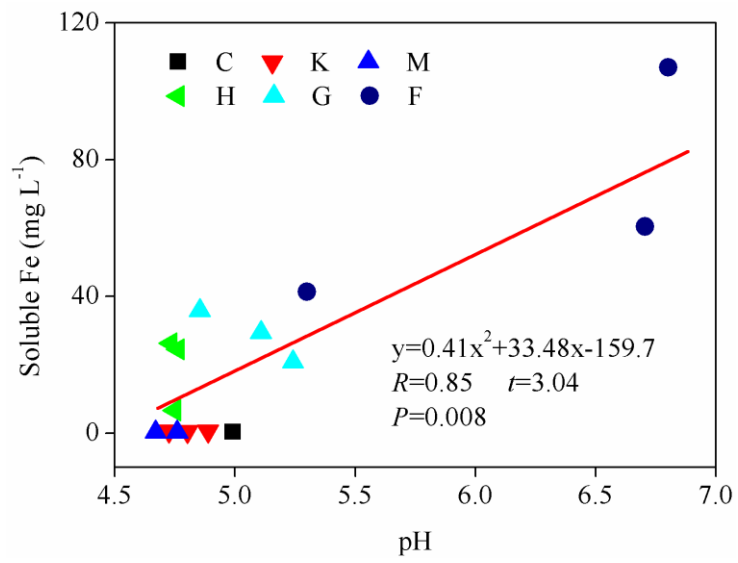


Figure S8. Correlation between pH and the soluble Fe.

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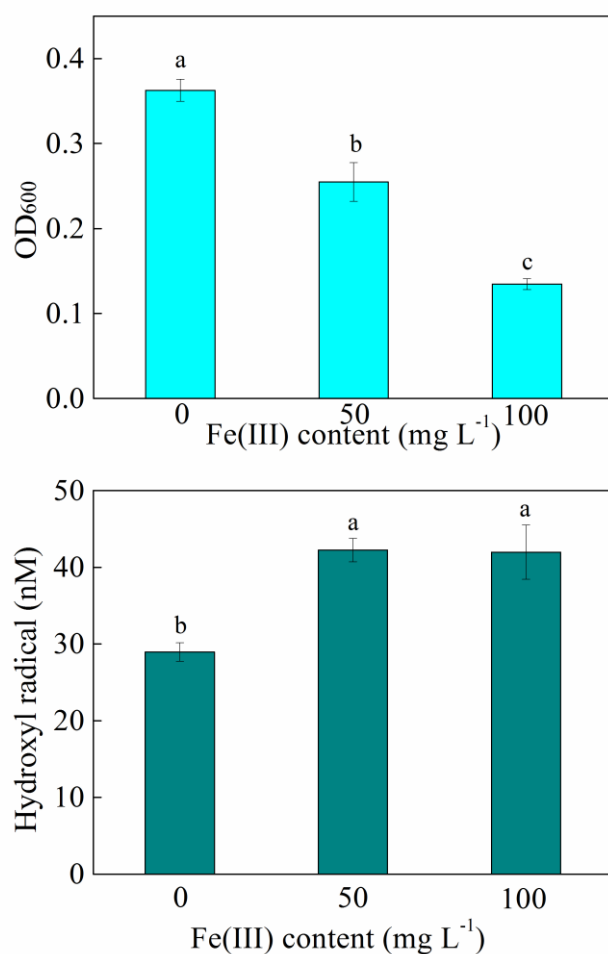


Figure S9. (a) Optical density at 600 nm (OD₆₀₀) of 8-h-old *Pseudomonas brassicacearum* J12 subcultures taken after 12 h growth. (b) Generation of hydroxyl radical (HO[•]) at 12 h as measured by dihydroxyterephthalic acid (HTPA) production, which are derived from terephthalic acid (TPA) trapping HO[•]. The concentrations of Fe(NO₃)₃ were 0, 50 and 100 mg L⁻¹.

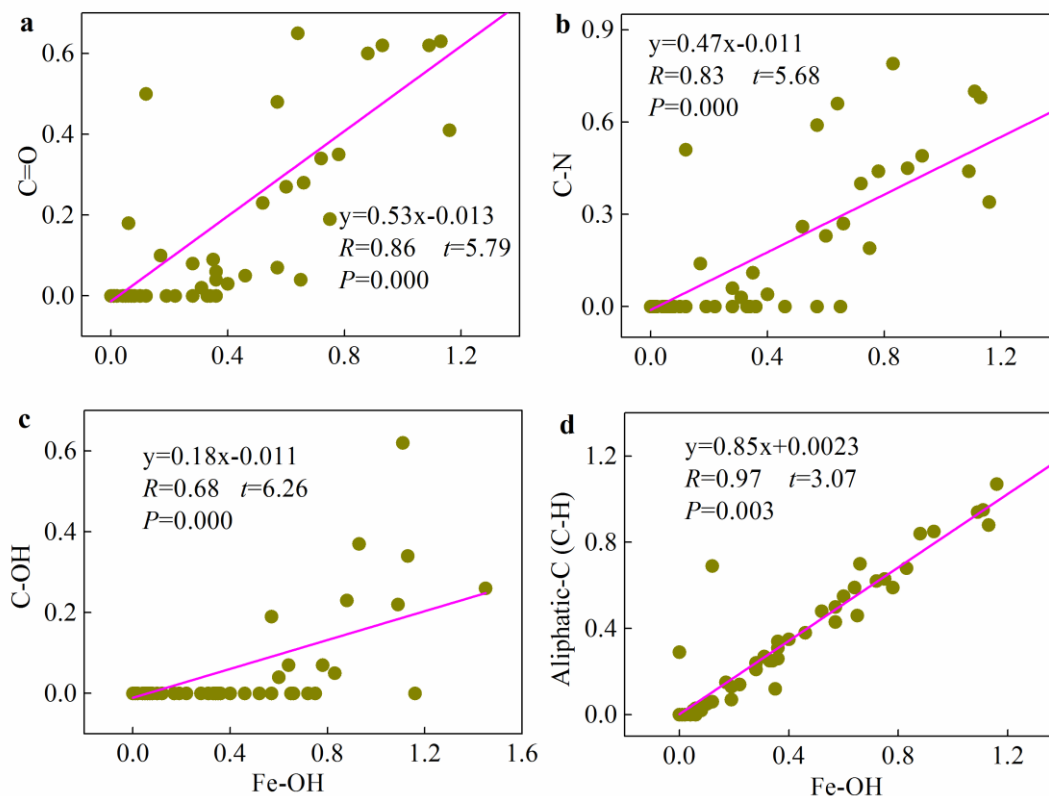


Figure S10. Correlation analysis between the distribution of Fe-OH (absorbance at 90 3344 cm^{-1}) and aliphatic C (C-H, absorbance at 2921 cm^{-1}) or aromatic C=O (absorbance at 1632 cm^{-1}) or C-OH (absorbance at 1513 cm^{-1}) or C-N (absorbance at 1030 cm^{-1}). $n = 47$.

Table S1 OD₆₀₀ of the mineral suspension (n = 3).

Mineral	Absorbance at 600 nm					
	Mineral suspension			Diluted 200 times		
	5 mg/mL	10 mg/mL	25 mg/mL	5 mg/mL	10 mg/mL	25 mg/mL
Kaolinite	1.625 ± 0.059	1.798 ± 0.047	2.046 ± 0.023	0.005 ± 0.001	0.019 ± 0.004	0.035 ± 0.003
Montmorillonite	1.196 ± 0.047	1.319 ± 0.017	1.802 ± 0.025	0.010 ± 0.005	0.005 ± 0.001	0.017 ± 0.003
Hematite	2.856 ± 0.085	2.977 ± 0.041	2.942 ± 0.062	0.004 ± 0.001	0.004 ± 0.001	0.004 ± 0.001
Goethite	2.228 ± 0.071	2.485 ± 0.052	2.703 ± 0.057	0.008 ± 0.001	0.023 ± 0.002	0.037 ± 0.002
Ferrihydrite	1.477 ± 0.149	1.999 ± 0.179	2.814 ± 0.034	0.002 ± 0.000	0.007 ± 0.003	0.015 ± 0.002

Table S2. One-sample Kolmogorov-Smirnov Test of data in **Figure 5**.

One-sample Kolmogorov-Smirnov Test						
Indices		OD600	HO'	soluble Fe	Total Fe	Fe(II)
N		16	16	16	16	16
Normal distribution parameters	Average	0.262	106.0	22.2	390.4	76.1
	SD	0.110	122.3	29.3	936.6	133.3
The most extreme differences	Absolute value	0.123	0.314	0.227	0.423	0.346
	Positive	0.110	0.314	0.207	0.423	0.346
	Negative	-0.123	-0.250	-0.227	-0.340	-0.294
Kolmogorov-Smirnov Z		0.493	1.255	0.907	1.693	1.384
Asymptotic significant (bilateral)		0.968	0.086	0.383	0.006	0.043

Table S3. The linear combination fitting (LCF) results of the Fe K-edge XANES spectra of ferrihydrite in **Figure 7**.

Position	Linear combination fitting results (%)					Parameters	
	Ferrihydrite	Goethite	Hematite	FeC ₂ O ₄	Fe ₂ (C ₂ O ₄) ₃	R-factor	Chi-square
A	81.6	0	0	17.3	6.1	0.00086	0.000226
B	44.2	18.9	13.4	25.9	0	0.00013	0.000212

Table S4. The concentration of free and total amino acids in cultures without or with mineral cultivation (12 h).

Cultures	FAA ($\mu\text{g mL}^{-1}$)	TAA ($\mu\text{g mL}^{-1}$)	FAA/TAA ratio
Raw culture	740.7 ± 3.74	6861 ± 7.05	0.108 ± 0.0004
M + bacteria	236.9 ± 59.33	6785 ± 34.31	0.035 ± 0.0086
F + bacteria	972.9 ± 2.65	6230 ± 363.5	0.156 ± 0.0096

100 Note: FAA, free amino acids. TAA, total amino acids. M, montmorillonite. F, ferrihydrite. Mineral concentration was 25 mg mL^{-1} .

Table S5. Eh of the suspension of minerals alone (25 mg/ml) and of bacteria-mineral mixture.

Treatment	Eh (mV)	
	Before incubation (alone)	After incubation (+ bacteria)
Control	190.6 ± 20.18	110.3 ± 16.21
Kaolinite	135.1 ± 34.27	90.8 ± 10.84
Montmorillonite	142.5 ± 15.62	117.1 ± 25.17
Hematite	306.5 ± 43.74	189.5 ± 21.65
Goethite	139.1 ± 11.17	199.4 ± 41.27
Ferrihydrite	103.3 ± 5.88	79.8 ± 12.17

105 **References**

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