Supplementary Information (SI) for

Iron minerals inhibit the growth of bacteria via a free-radical mechanism

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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 Kα 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 acidswas 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 ± SE (n = 3). A standard amino acid mixture of cysteic acid and methionine sulfone (20 μL mL^{-1}) was diluted to 100 μmol L^{-1} for amino acid quantification and calibration. An external standard was used to calculate the concentration of each amino acid.
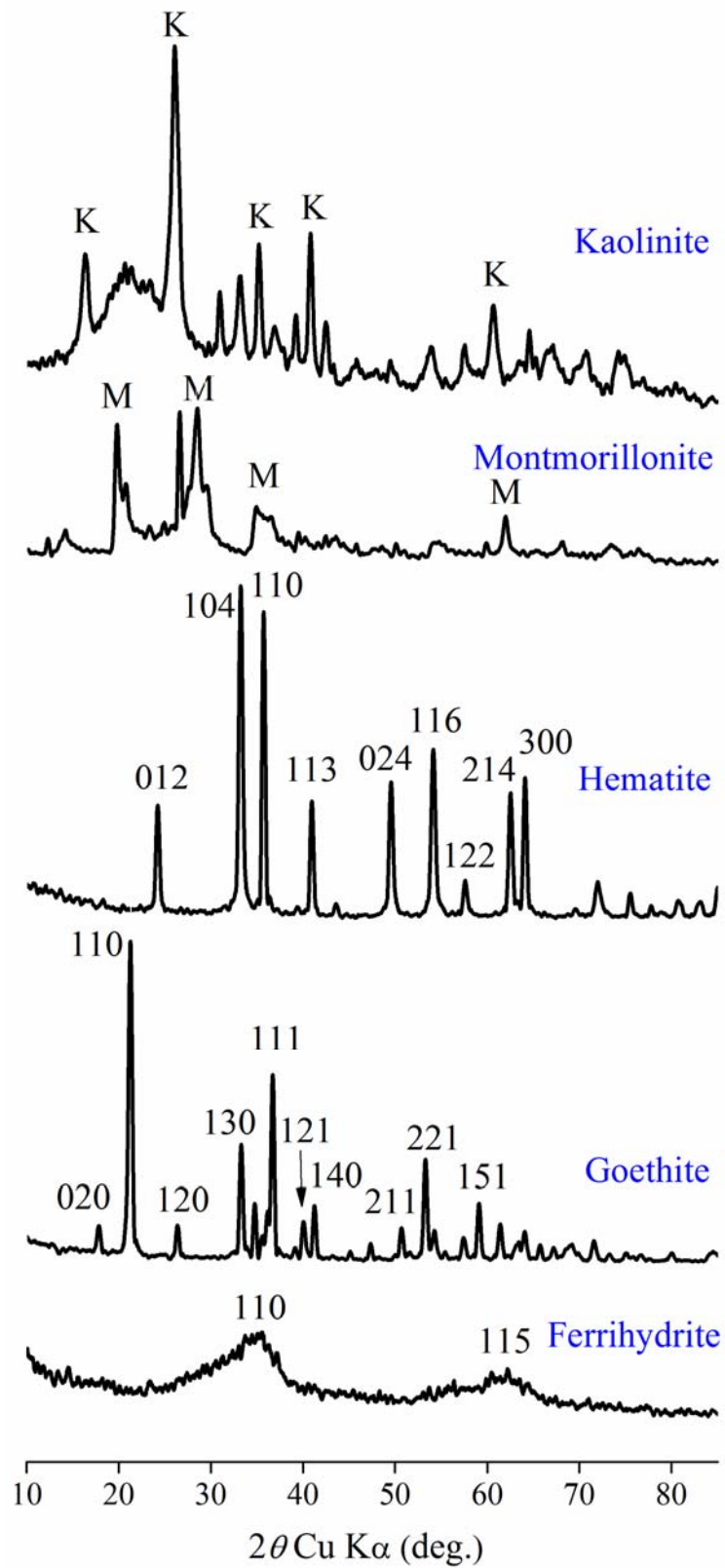
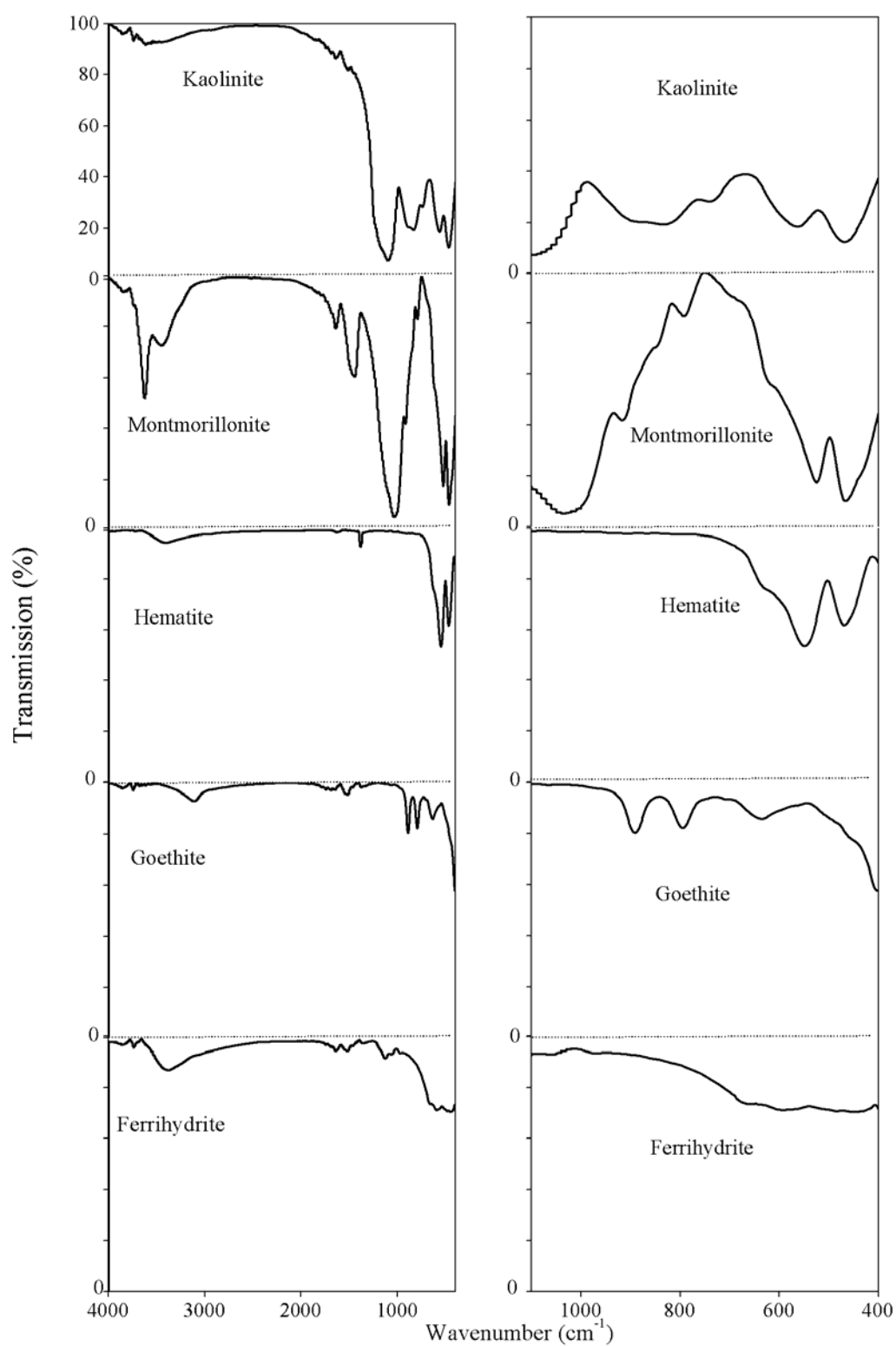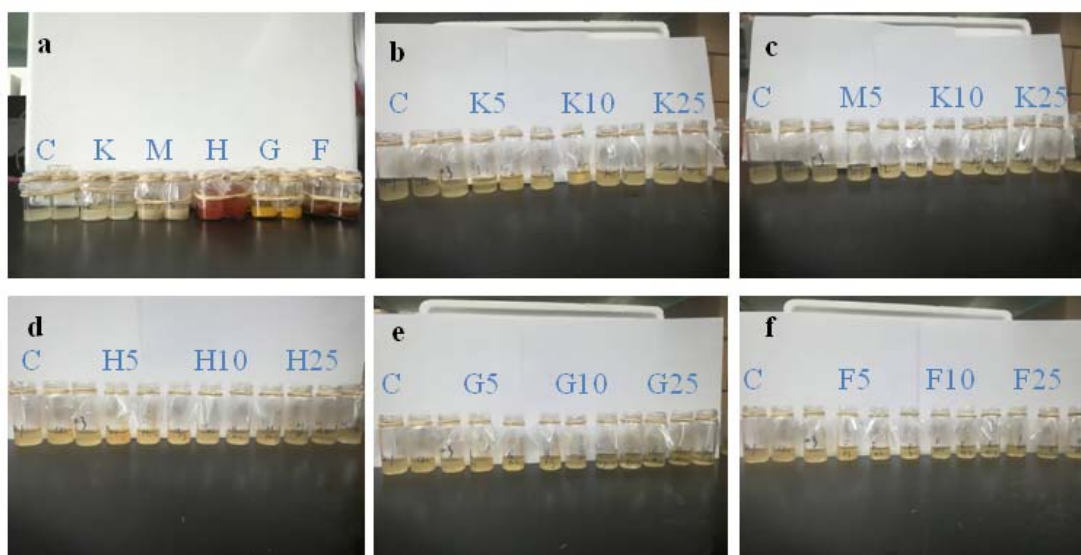**Figure S1.** Powder X-ray diffraction (XRD) pattern of the minerals used in this study.

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

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 performed in triplicate.
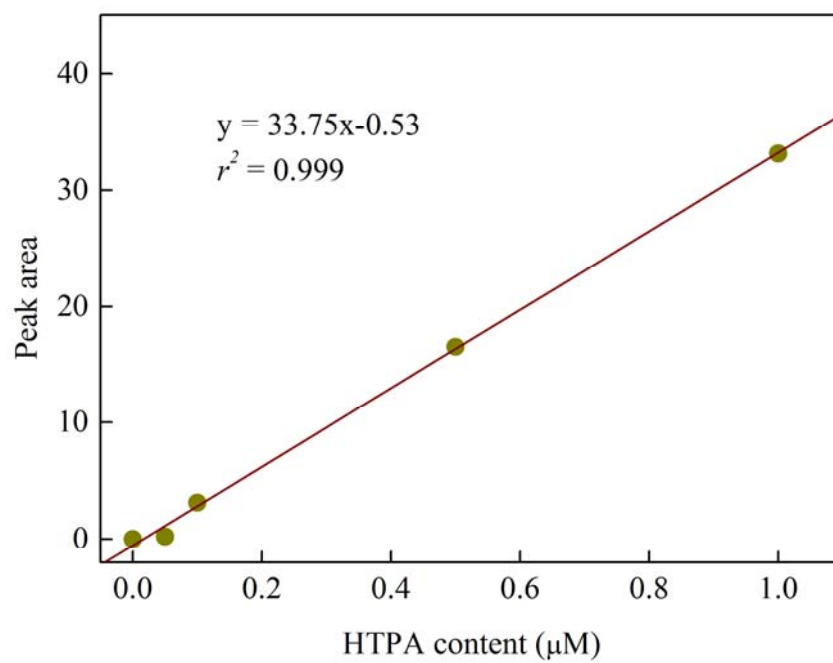
$y = 33.75x - 0.53$

$r^2 = 0.999$

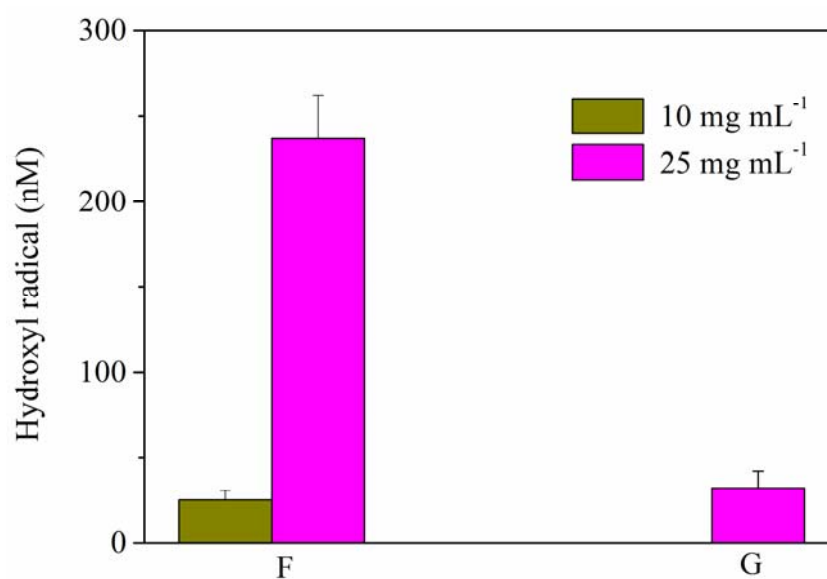**Figure S4.** Standard curve of specific fluorescent dihydroxyterephthalic acid (HTPA).

Figure S5. Generation of hydroxyl radical (HO$^\bullet$) 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).
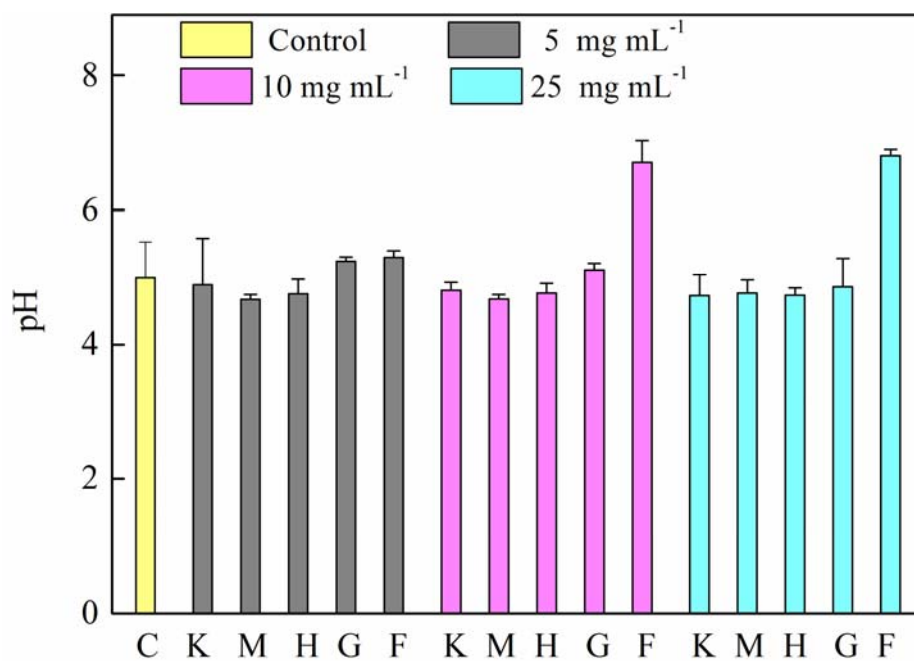
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Figure S6. Determination of pH after 12 h growth of *Pseudomonas brassicacearum* J12 with different minerals and with no minerals (control). K, kaolinite; M, montmorillonite; H, hematite; G, goethite; F, ferrihydrite; C, Control (i.e., no mineral). Values are the mean ± SE (n = 3).

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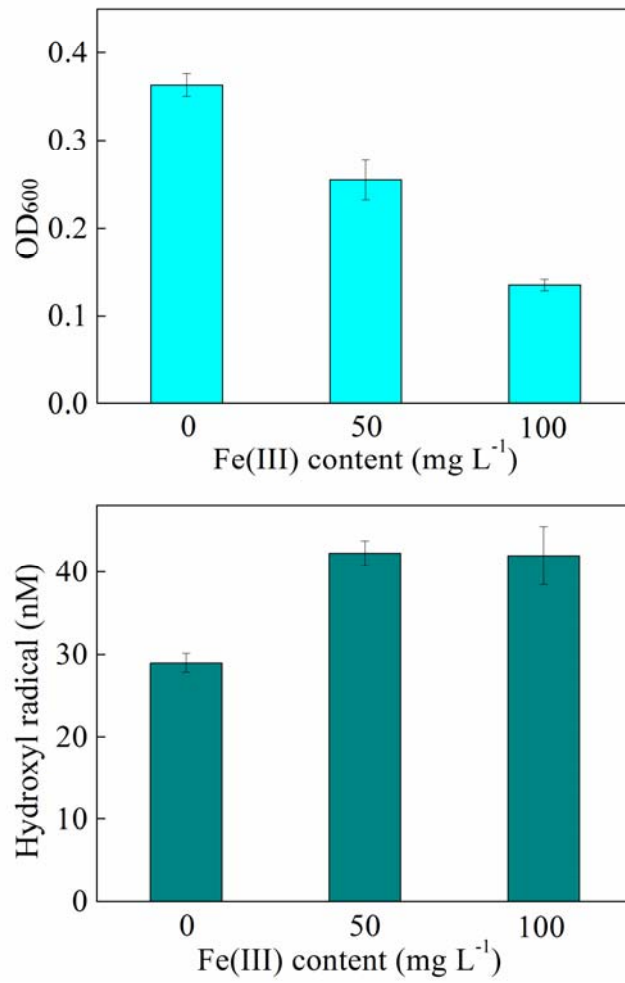**Figure S7.** (a) Optical density at 600 nm (OD_{600}) of 8-h-old *Pseudomonas brassicacearum* J12 subcultures taken after 12 h growth. (b) Generation of hydroxyl radical ($HO^\bullet$) at 12 h as measured by dihydroxyterephthalic acid (HTPA) production, which are derived from terephthalic acid (TPA) trapping $HO^\bullet$. The concentrations of

75   $Fe(NO_3)_3$ were 0, 50 and 100 mg L^{-1}.

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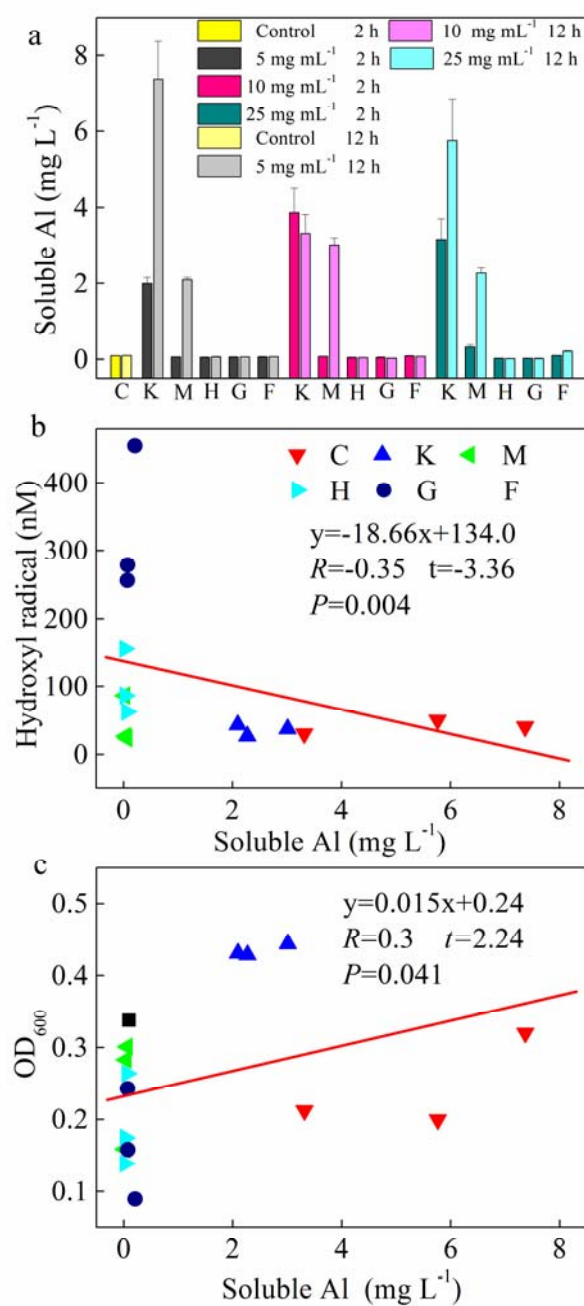
Figure S8. (a) Production of soluble Al after 2 h and 12 h cultivation. K, kaolinite; M, montmorillonite; H, hematite; G, goethite; F, ferrihydrite; C, control, no minerals. Values in (**a**) are the mean ± SE (n = 3). Correlation analysis between HO$^\bullet$ concentration and soluble Al is shown in (b), and correlation analysis between OD_{600} and soluble Al is shown in (c).
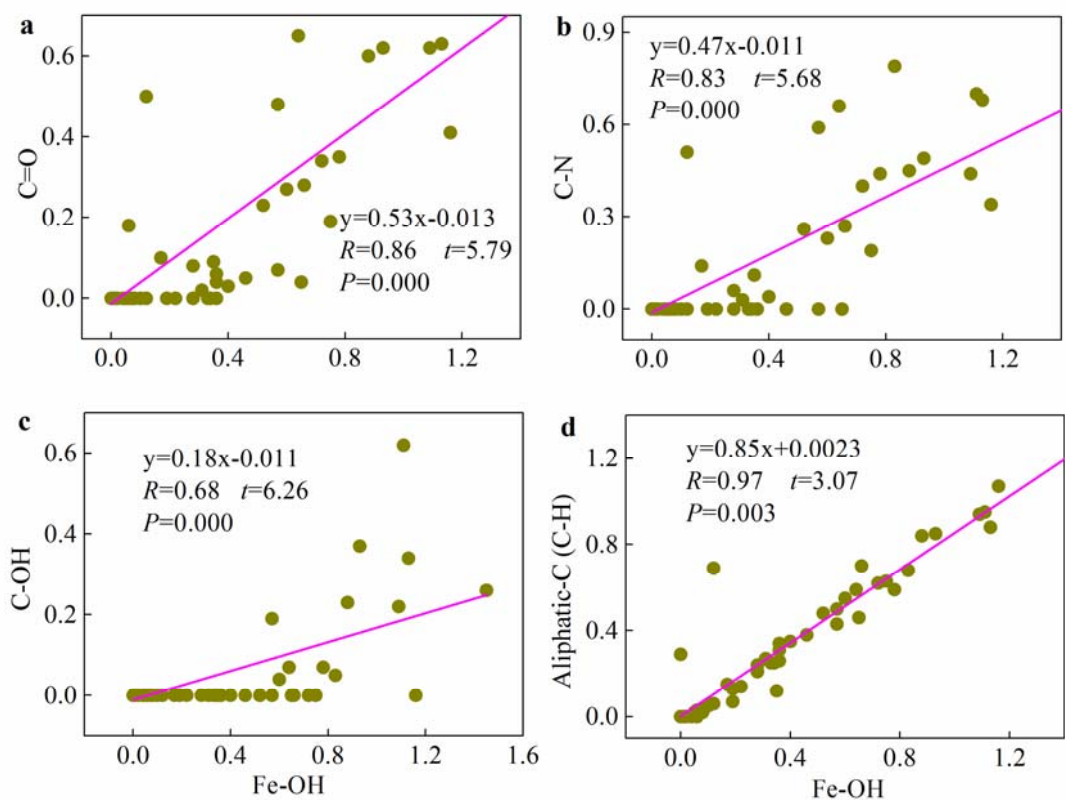
Figure S9. Correlation analysis between the distribution of Fe-OH (absorbance at 3344 cm^{-1}) and aliphatic C (C-H, absorbance at 2921 cm^{-1}) or aromatic C=O (absorbance at 1632 cm^{-1}) or C-OH (absorbance at 1513 cm^{-1}) or C-N (absorbance at 1030 cm^{-1}). n = 47.

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Table S1. The particle size distribution (% in volume) of both the applied raw minerals and the changes after 24 h of cultivation.

Samples [a]	<0.1 μm	0.1-0.5 μm	0.5-1 μm	1-2 μm	2-5 μm	5-10 μm	10-20 μm	20-50 μm	50-100 μm	100-500 μm	500-1000 μm
K	0.2	31.2	21.5	15.0	15.6	4.9	1.9	1.2	1.7	6.8	0.0
M	1.1	12.3	6.1	5.1	9.1	4.0	3.2	8.9	16.1	34.1	0.0
H	0.0	95.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
G	0.0	94.1	1.5	0.0	0.0	0.0	0.0	0.0	1.0	3.4	0.0
F	1.5	16.3	8.2	3.7	6.3	6.1	7.6	14.8	12.4	22.5	0.6
K+bacteria	0.7	27.2	17.5	14.1	15.5	4.9	2.2	2.1	3.5	12.3	0.0
M+bacteria	1.7	25.2	10.1	8.3	12.3	4.0	1.4	1.5	7.6	27.9	0.0
H+bacteria	0.0	90.6	6.3	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
G+bacteria	0.0	61.5	15.3	1.4	6.8	1.4	0.9	1.7	2.5	8.5	0.0
F+bacteria	1.1	11.2	6.1	3.7	7.6	5.5	7.3	13.1	13.3	31.1	0.0

90   [a] K, kaolinite; M, montmorillonite; H, hematite; G, goethite; F, ferrihydrite.

Table S2. One-sample Kolmogorov-Smirnov Test of data in **Figure 4**.

One-sample Kolmogorov-Smirnov Test							
Indices			**OD600**	**HO$^\bullet$**	**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

95   **Table S3.** The linear combination fitting (LCF) results of the Fe K-edge XANES spectra of ferrihydrite in **Figure 5**.

Position	Linear combination fitting results (%)					Parameters	
	Ferrihydrite	Goethite	Hematite	FeC_2O_4	$Fe_2(C_2O_4)_3$	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 ($\mu g\ mL^{-1}$)	TAA ($\mu g\ mL^{-1}$)	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

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

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References

Li, H., Hu, S., Polizzotto, M. L., Chang, X., Shen, Q., Ran, W., and Yu, G.: Fungal biomineralization of montmorillonite and goethite to short-range-ordered minerals, Geochim. Cosmochim. Acta, 191, 17-31, 2016.

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