

Supplemental Materials: Temporal response of bacterial community associated Fe(III) reduction to initial pH shift of paddy soils

Supplementary legends

Text 1. Illustration for selection of pH regulator

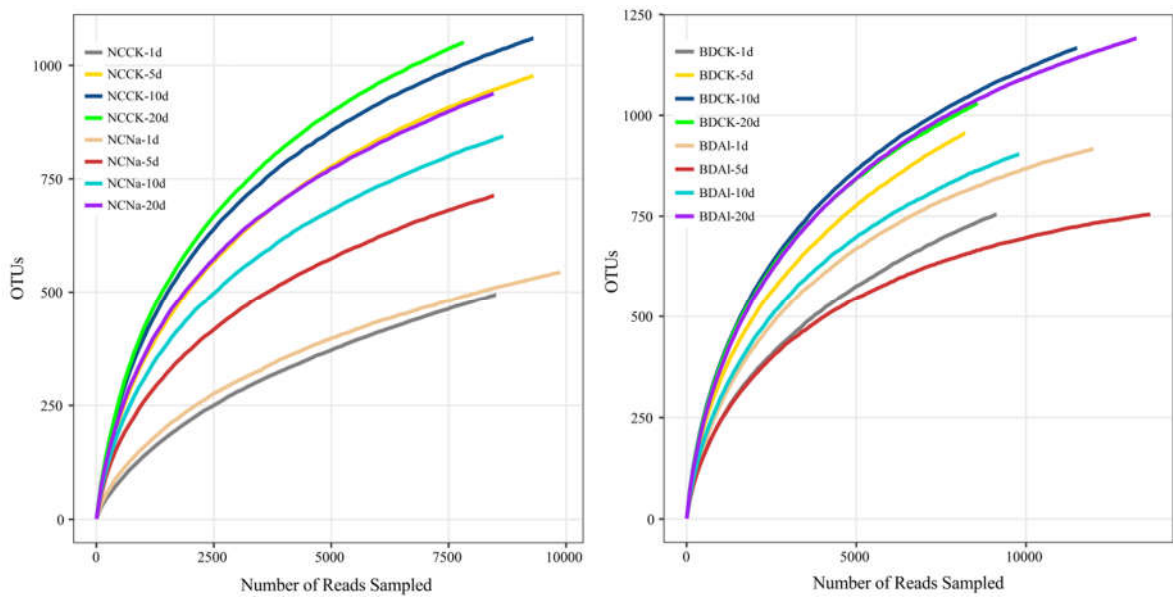


Figure S1 The rarefaction curves of tested samples. (NCCK, the original NC soil without pH adjustment; NCNa, NC soil with pH adjustment by sodium carbonate to alkaline level; BDCK, the original BD soil without pH adjustment; BDAI, BD soil with pH adjustment by aluminum sulfate to acidic level. Numbers following the treatments represented the flooding time, the same below).

Table S1. Soil physicochemical characteristics of tested paddy soil

Soils	Texture	Classification	pH	Amorphous Fe (mg g ⁻¹)	Free Fe (mg g ⁻¹)	Organic Matter (mg g ⁻¹)	Available Phosphorus (mg kg ⁻¹)	Available Potassium (mg kg ⁻¹)	Nitrate Nitrogen (mg kg ⁻¹)	Ammoniacal Nitrogen (mg kg ⁻¹)
NC	Clay loam	Fe-accumuli-Stagnic Anthrosol	5.17±0.02	3.62±0.04	13.88±0.16	47.89±5.07	5.77±0.16	114.43±2.72	5.26±0.04	37.67±0.47
BD	Silty clay	Hapli-Stagnic Anthropol	7.89±0.05	3.55±0.02	14.49±0.05	33.48±2.23	13.89±1.44	302.41±3.14	5.92±0.14	12.13±0.15

Note: NC, paddy soil taken from the drained post-harvest paddy fields in Nanchang county, Jiangxi Province (28°19'48" N, 115°33'36" E); BD, paddy soil taken from the drained post-harvest paddy fields in Baodi district, Tianjin Municipality (39°38'24" N, 117°33'36"E).

Table S2. Potential Fe(III)-reducing bacteria in paddy soils with different initial pH according to references and sequences published in NCBI

OTU	Reference sequences (GenBank accession No.)	Sequence identity
OTU2402	<i>Geobacter bemidjiensis</i> strain Bem (NR_042769)	99%
OTU437	<i>Geobacter bemidjiensis</i> strain Bem (NR_025895)	98%
OTU2547	<i>Geobacter picheringii</i> strain G13 (NR_043576)	98%
OTU745	<i>Geobacter picheringii</i> strain G13 (NR_026077)	97%
OTU2752	<i>Desulfuromonas carbonis</i> strain ICBM (KJ776405)	97%
OTU2540	<i>Desulfuromonas</i> sp. enrichment culture clone TF (KF242419)	98%
OTU965	<i>Anaeromyxobacter</i> sp. Fac12 (AJ504438)	97%
OTU2781	<i>Anaeromyxobacter</i> sp. clone 90D-58 (AJ504437)	97%
OTU1221	<i>Anaeromyxobacter dehalogenans</i> strain JpR-4-2 (EF067314)	97%
OTU2628	<i>Anaeromyxobacter</i> sp. Fw109-5 (CP000769)	97%
OTU511	<i>Anaeromyxobacter</i> sp. Fw109-5 (CP000769)	98%
OTU1063	<i>Rhizomicrobium palustre</i> strain A48 (NR_112186)	98%
OTU1271	<i>Pseudomonas</i> sp. RPS18 (MN841948)	97%
OTU2893	<i>Pseudomonas</i> sp. RPS33 (MN841963)	97%
OTU2001	<i>Pseudomonas</i> sp. RPS33 (MN841963)	99%
OTU1756	<i>Citrobacter</i> sp. LAR-1 (KC211014)	98%
OTU124	<i>Rhodoferrax_ferrireducens</i> T118 (NR_114646)	98%
OTU2711	<i>Rhodoferrax_ferrireducens</i> T118 (NR_114646)	97%
OTU1929	<i>Rhodoferrax_ferrireducens</i> T118 (NR_114646)	97%
OTU177	<i>Massilia</i> sp. RPS4 (MN841936)	98%
OTU558	<i>Massilia</i> sp. RPS16 (MN841947)	99%
OTU1357	<i>Bacillus</i> sp. PeC11 (AM177061)	98%
OTU1700	<i>Bacillus</i> sp. RPS23 (MN841953)	98%
OTU1109	<i>Bacillus</i> sp. RPS28 (MN841958)	98%
OTU186	<i>Bacillus infernus</i> strain TH-23 (NR_027227)	97%
OTU742	<i>Bacillus</i> sp. RPS27 (MN841957)	97%
OTU2736	<i>Bacillus subterraneus</i> strain COOI3B (NR_115305)	97%
OTU2979	<i>Bacterium</i> _HN1-10-1 (JN117981)	98%
OTU1965	<i>Bacillus</i> sp. RPS34 (MN841964)	97%
OTU1490	<i>Bacillus</i> sp. RPS34 (MN841964)	97%
OTU1338	<i>Bacillus</i> sp. RPS34 (MN841964)	100%
OTU916	<i>Bacillus</i> sp. RPS14 (MN841945)	97%
OTU488	<i>Bacillus</i> sp. RPS14 (MN841945)	97%
OTU651	<i>Bacillus</i> sp. RPS14 (MN841945)	99%
OTU1526	<i>Bacterium</i> _HN2-10-21 (JN118088)	97%
OTU2671	<i>Enterococcus gallinarum</i> (AF277567)	97%
OTU872	<i>Lysinibacillus</i> sp. strain A66 (MN853551)	98%
OTU2020	<i>Solibacillus</i> sp. strain A20 (MN853550)	99%
OTU322	<i>Paenibacillus</i> sp. RPS42 (MN841972)	97%
OTU1550	<i>Paenibacillus</i> sp. strain Cam9 (HE861860)	98%

OTU	Reference sequences (GenBank accession No.)	Sequence identity
OTU817	<i>Paenibacillus guangzhouensis</i> strain GSS02 ((NR_134114)	99%
OTU46	<i>Paenibacillus guangzhouensis</i> strain GSS02 (NR_134114)	97%
OTU1691	<i>Clostridium</i> sp. strain P74 (MN853540)	99%
OTU733	<i>Clostridium</i> sp. strain P74 (MN853540)	97%
OTU1005	<i>Clostridium</i> sp. strain P74 (MN853540)	97%
OTU1975	Bacterium_HN1-10-42 (JN118006)	97%
OTU372	<i>Clostridium</i> sp. RPS6 (MN841938)	97%
OTU1537	<i>Clostridium</i> sp. RPS6 (MN841938)	97%
OTU719	<i>Clostridium</i> sp. RPS6 (MN841938)	98%
OTU997	<i>Clostridium</i> sp. C71 (N223392)	99%
OTU857	<i>Fervidicella metallireducens</i> strain AeB (NR_104504)	97%
OTU1589	<i>Geosporobacter</i> sp. IRF9 (KF601937)	98%
OTU2692	<i>Anaerosolibacter carboinniphilus</i> strain IRF19 (KF601947)	100%
OTU2485	<i>Alkaliphilus peptidofragmentans</i> strain Z-7036 (EF382660)	99%
OTU884	<i>Desulfosporosinus lacus</i> strain STP12 (NR_042202)	98%
OTU903	<i>Desulfosporosinus lacus</i> strain STP12 (NR_042202)	98%
OTU357	<i>Desulfitobacterium metallireducens</i> strain 853-15 (NR_025125)	97%
OTU525	<i>Desulfitobacterium metallireducens</i> strain 853-15 (NR_025125)	99%
OTU2406	<i>Desulfitobacterium aromaticivorans</i> strain UKTL (NR_116427)	99%
OTU2437	<i>Desulfosporomusa polytropia</i> strain STP3 (NR_114691)	98%
OTU703	<i>Desulfosporomusa polytropia</i> strain STP3 (NR_114691)	97%
OTU2148	Iron-reducing bacterium enrichment culture clone HN51 (FJ269060)	100%
OTU2615	Iron-reducing bacterium enrichment culture clone FEA_2_C2 (FJ802294)	99%
OTU995	Iron-reducing bacterium enrichment culture clone HN3 (FJ269045)	97%
OTU1768	Iron-reducing bacterium enrichment culture clone HN3 (FJ269045)	100%
OTU1432	Iron-reducing bacterium enrichment culture clone HN-HFO75 (FJ269060)	99%
OTU1438	Iron-reducing bacterium enrichment culture clone HN-HFO91 (FJ269102)	98%
OTU2319	Iron-reducing bacterium enrichment culture clone HN66 (FJ269063)	100%
OTU901	<i>Pelosinus fermentans</i> strain R7 (NR_043577)	98%
OTU3013	<i>Azotobacter</i> sp. strain C2 (MN853543)	100%

Text 2. Selection of pH regulator

Dissolved aluminum and iron in soil were widely accepted to play critical role in the biogeochemical process of soil acidification and formation [1]. Carbonate was the driving force for soil alkaline as the hydrolysis of carbonate could result in the increase of soil pH [1,2]. Therefore, we used aluminum sulfate to decrease the soil initial pH and sodium carbonate to increase soil pH in the present study [3-5]. Other alternative regulator like phosphate buffer was not used because of its inhibition on the solubility of Fe-oxides [6]. HCl-NaOH buffer was also discarded to avoid the destruction on soil structure and organism [7]. However, there was still a certain amount of sulfate input when amended with aluminum sulfate, which was considered as alternative electron competitor with Fe(III) [8]. Studies have confirmed the ferrous sulfide (FeS) was the initial product of sulfate reduction and subsequently converted to either pyrite (FeS₂) or O-coordinated ferrous species [9,10]. In the present study, the acid-soluble total iron on the 40 d of incubation was decreased by 0.787 mg g⁻¹ with addition of Al₂(SO₄)₃, illustrating the formation of insoluble FeS or FeS₂ [11](Lefort Mucci, & Sundby, 2012; Kwon et al. 2014). We calculated that no more than 4.62% of the remarkable inhibition of pH shift on Fe(III) reduction with Al₂(SO₄)₃ was caused by SO₄²⁻ input, while the remaining 10.91% was attributed to soil pH decrease to acidic by Al³⁺. Whereas iron in FeS or FeS₂ was still in a ferrous state in spite of inhibition of sulfate on Fe(III) reduction. It implied that sulfate inhibition might be less significant than the theoretical estimates. Additionally, Wu (2013) amended BD soil with similar amount of SO₄²⁻ input to our study in the form of Na₂SO₄ and demonstrated similar inhibitory effect on Fe(III) reduction [12]. This indirectly indicated that the input of Na⁺ showed limited influence on Fe(III) reduction.

References

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