Supplementary material

Redistribution of cadmium in soil aggregates under continuous carbon and nitrogen inputs: Insights from sequential extraction and modeling

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Text S1 Tessier Sequential Extraction Procedure

(i) F1-Cd: Exchangeable. The sediment was extracted at room temperature for 1 h with 8 mL of either magnesium chloride solution (1 M $MgCl_2$, pH 7.0) with continuous agitation.

(ii) F2-Cd: Bound to Carbonates. The residue from (i) was leached at room temperature with 8 mL of 1 M NaOAc adjusted to pH 5.0 with acetic acid (HOAc). Continuous agitation was maintained and the time necessary for complete extraction was evaluated.

(iii) F3-Cd: Bound to Fe- Oxides. The residue from (ii) was extracted with 20 mL of either 0.3 M Na_2S2O_4 + 0.175 M Na-citrate + 0.025 M H-citrate, or 0.04 M NH_2OH-HCl in 25% (v/v) HOAc. The latter experiments were performed at 96 ± 3 °C with occasional agitation and the time needed for complete dissolution of the free iron oxides was evaluated.

(iv) F4-Cd: Bound to Organic Matter. To the residue from (iii) were added 3 mL of 0.02 M HNO_3 and 5 mL of 30% H_2O_2 adjusted to pH 2 with HNO_3, and the mixture was heated to 85 ± 2 °C for 2 h with occasional agitation. A second 3-mL aliquot of 30% H_2O_2 (pH 2 with $HN0_3$) was then added and the sample was heated again to 85 ± 2 °C for 3 h with intermittent agitation. After cooling, 5 mL of 3.2 M NH_4OAc in 20% (v/v) HNO_3 was added and the sample was diluted to 20 mL and agitated continuously for 30 min. The addition of NH_4OAc is designed to prevent adsorption of extracted metals onto the oxidized sediment.

(v) F5-Cd: Residual. The residue from (iv) was digested with a HF-$HC1O_4$ mixture for total metal analysis.

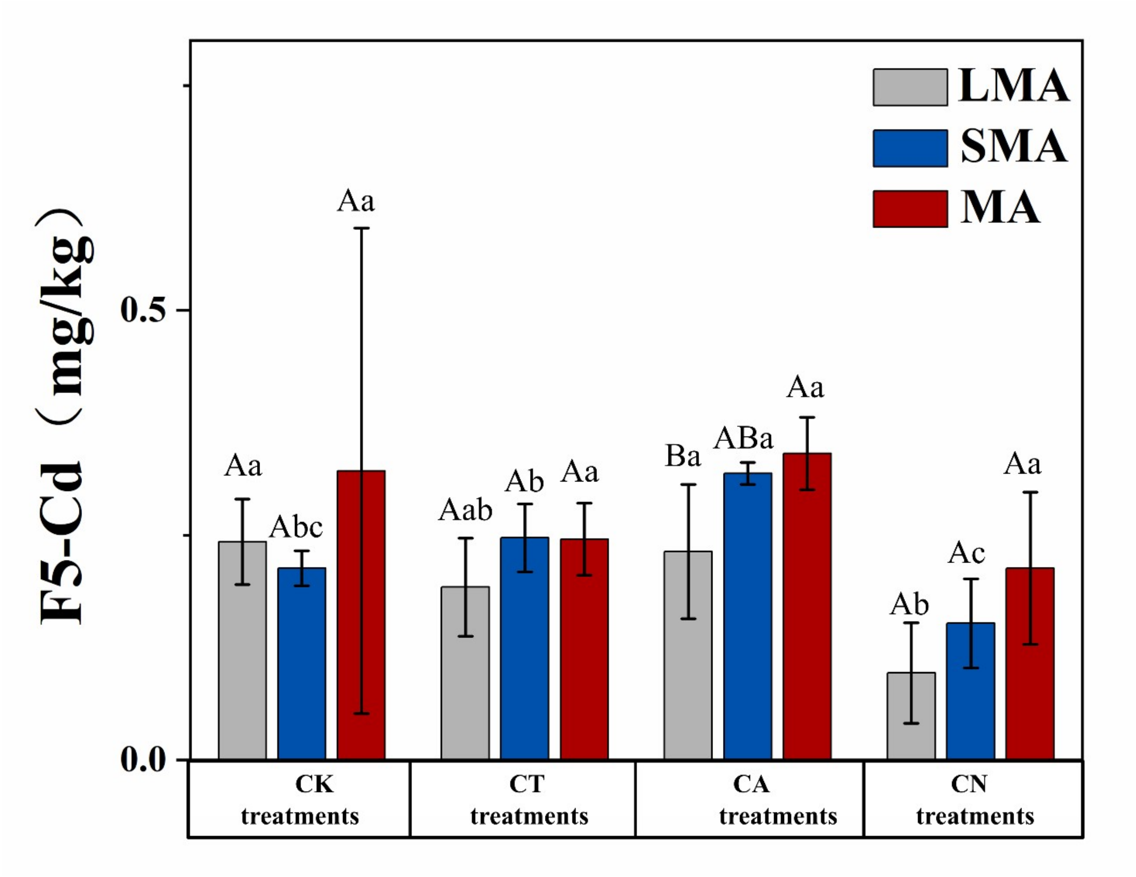

Fig. S1 The variation of F5-Cd fractions in soil aggregates. CK, control; CT, glucose treatment; CA, glucose + ammonium treatment; CN, glucose + nitrate treatment; a, LMA (>2 mm) aggregate; b, SMA (0.25-2 mm) aggregate; c, MA (< 0.25 mm) aggregate; F5-Cd, residual Cd. Upper-case letters indicate differences between particle sizes while lower-case letters indicate differences between N treatments ($P < 0.05$).

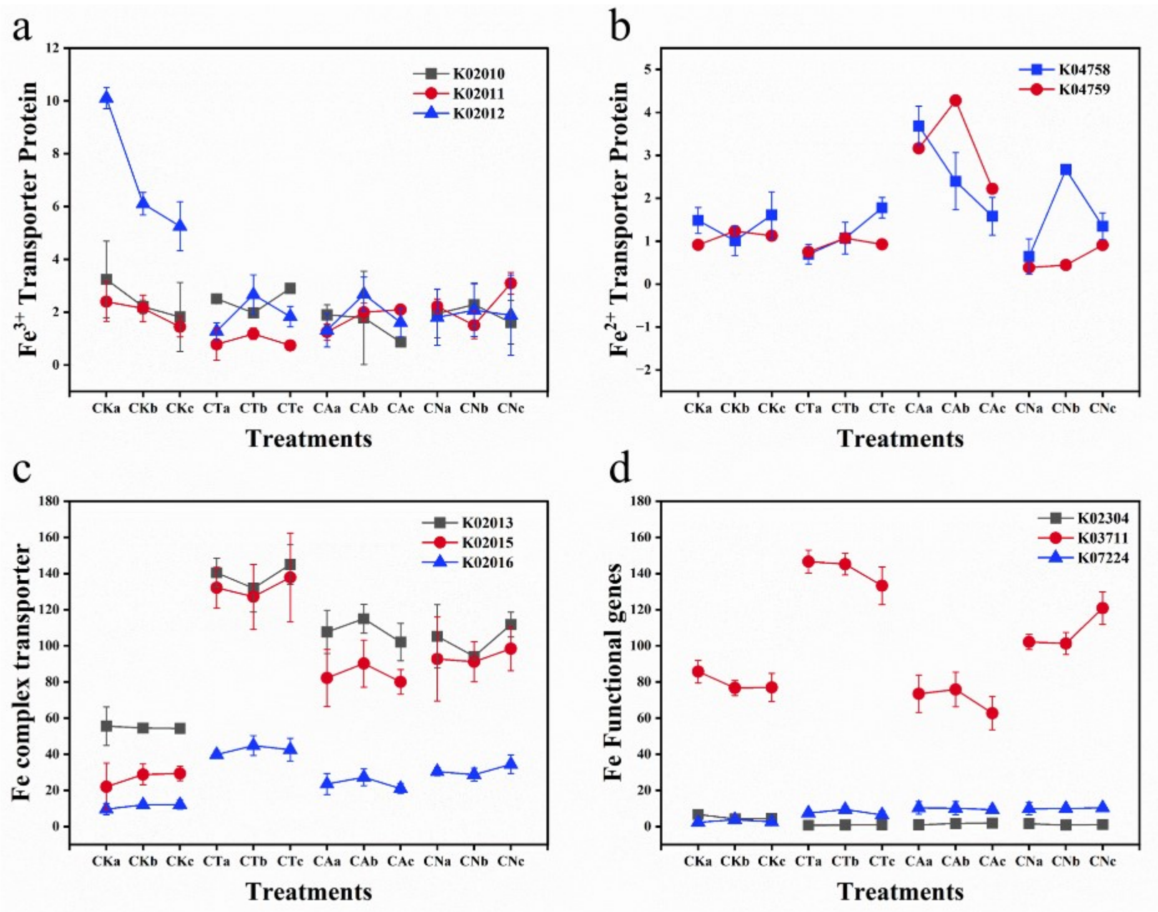

Fig. S2 Changes in Fe related genes. (a) Gene encoding for Fe^{3+} transport protein; (b) Gene encoding for Fe^{2+} transport protein; (c) Gene encoding for Fe complex transport protein; (d) Gene encoding for sirolium ferrochloride, Fe uptake regulatory factors, and components of the Fe absorption system. a, large macro-aggregates (LMA, > 2 mm); b, small macro-aggregates (SMA, 0.25-2 mm); c, micro-aggregates (MA, < 0.25 mm).

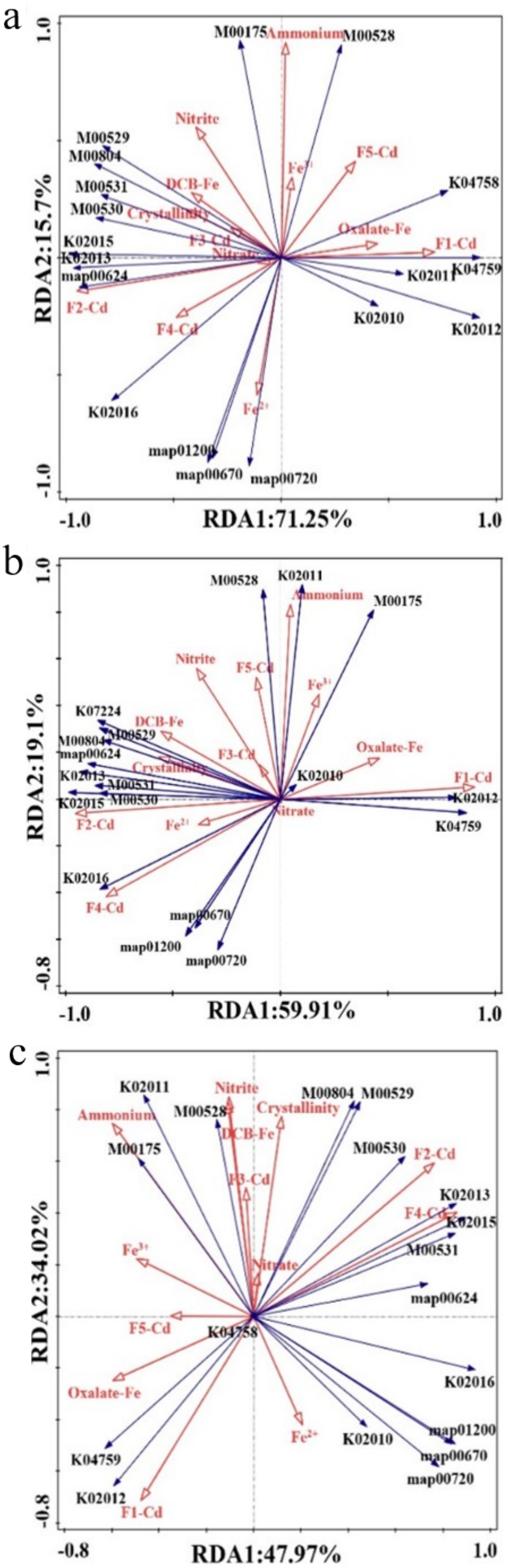

Fig. S3 Redundancy analysis (RDA) of genes and environmental factors in three soil aggregates. a and b, large macro-aggregates (LMA, >2 mm); c and d, small macro-aggregates (SMA, 0.25-2 mm); e and f, micro-aggregates (MA, < 0.25 mm).

Table S1 The variation of Fe, Fe associated Cd and mineral N in soil aggregates

	DCB-Fe (g/kg)	oxalate-Fe (g/kg)	HCl-Fe^{2+} (g/kg)	HCl-Fe^{3+} (g/kg)	Crystallinity	SOC (g/kg)	DOC (mg/kg)	NO_3^- (mg/kg)	NO_2^- (mg/kg)	NH_4^+ (mg/kg)
CKa	12.60±0.06	2.28±0.03	2.89±0.38	4.24±0.06	0.65±0.01	22.06±0.54	657.53±0.70	107.51±20.52	3.62±0.01	182.28±3.15
CKb	12.33±0.20	2.28±0.03	2.48±0.69	4.12±0.03	0.63±0.01	19.42±0.05	644.52±30.32	116.52±2.48	3.56±0.10	168.57±4.01
CKc	12.32±0.20	2.55±0.02	3.03±0.56	4.09±0.22	0.62±0.01	19.43±0.11	627.76±10.85	107.53±20.51	3.59±0.03	167.09±5.86
CTa	12.80±0.27	2.23±0.01	3.19±0.28	4.01±0.12	0.65±0.01	25.23±0.51	987.52±71.25	19.73±1.09	3.54±0.08	51.08±37.72
CTb	12.75±0.81	2.18±0.01	2.84±0.36	3.69±0.16	0.66±0.05	20.74±0.41	894.55±6.61	19.66±0.28	3.57±0.06	71.39±1.03
CTc	12.32±0.16	2.31±0.01	3.09±0.60	3.89±0.27	0.62±0.02	20.84±0.24	960.63±7.65	19.64±1.13	3.49±0.02	71.56±1.39
CAa	13.59±0.60	2.12±0.02	2.49±0.34	4.50±0.82	0.76±0.03	22.74±0.52	1005.37±83.80	134.90±68.23	5.46±0.37	467.26±4.08
CAb	13.68±0.03	2.15±0.01	2.57±0.14	4.57±0.67	0.78±0.01	21.68±0.22	899.12±13.77	116.98±49.70	5.74±0.09	429.29±0.51
CAc	13.62±0.09	2.40±0.05	2.66±0.20	4.28±0.22	0.71±0.03	21.04±0.05	946.21±25.26	98.88±41.29	5.57±0.30	423.98±5.96
CNa	13.97±0.51	1.78±0.01	2.83±0.32	4.42±0.10	0.72±0.03	23.20±0.43	908.35±4.88	344.09±5.91	5.21±0.18	166.25±3.95
CNb	14.02±0.01	1.63±0.01	2.62±0.15	4.12±0.10	0.74±0.01	21.08±0.21	833.11±4.14	313.07±0.01	5.31±0.05	140.90±0.10
CNc	13.21±0.48	1.86±0.01	2.79±0.20	3.53±0.31	0.70±0.01	20.27±0.52	822.75±18.73	299.16±2.39	5.31±0.06	130.89±0.09

a, large macro-aggregates (LMA, > 2 mm); b, small macro-aggregates (SMA, 0.25-2 mm); c, micro-aggregates (MA, < 0.25 mm).

Table S2 Fe-related genes and metabolic pathway predicted based on the KEGG database.

Fe-related genes	
KO	Gene
K02010	afuC, fbpC
K02011	afuB, fbpB
K02012	afuA, fbpA
K02013	ABC.FEV.A
K02015	ABC.FEV.P
K02016	ABC.FEV.S
K02304	MET8
K03711	fur, zur
K04758	feoA
K04759	feoB
K07224	efeO

Table S3 N-related metabolic module predicted based on the KEGG database.

N-related metabolic module	
KEGG Module	Name
M00175	Nitrogen fixation
K00528	Nitrification
K00529	Denitrification
K00530	Dissimilatory nitrate reduction
K00531	Assimilatory nitrate reduction
K00804	Complete nitrification

Table S4 C-related metabolic pathway predicted based on the KEGG database.

C-related metabolic pathway	
KEGG Pathway	Name
Map00624	Polycyclic aromatic hydrocarbon degradation
Map00670	One carbon pool by folate
Map00710	Carbon fixation in photosynthetic organisms
Map00720	Carbon fixation pathways in prokaryotes
Map01200	Carbon metabolism
Map04964	Proximal tubule bicarbonate reclamation