Supporting Information.

Title: Arsenic distribution and speciation near rice roots influenced by iron plaques and redox conditions of the soil matrix

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Summary of the numbers in supporting information

- The number of pages:15
- The number of figures:9
- The number of table:3

Preparation of thin sections

The frozen soil blocks were prepared as described in the main document. They were freeze-dried and embedded in epoxy resin (Sundhoma TC241, DH Material, Tokyo, Japan) under vacuum. The resin-embedded soil blocks were hardened at ambient temperature for 10 days. The outer portions (1 cm) of the hardened soil blocks that potentially ingressed air before freeze-drying were discarded. One of soil block surface was polished by a grinder and cut into 500 μm thick sections using a diamond saw, then polished until the thickness of the section reached 80 μm. The thickness of the thin section was determined with an electronic microgauge (magnescale LU10, Sony, Tokyo, Japan).

Experimental setup for analyses of microscale distribution and As and Fe speciation

The microscale As and Fe distributions in soil thin sections were determined by μXRF with an excitation energy of 12.5 keV (at beamline 4A of the Photon Factory in KEK and at BL10.3.2 at the Advanced Light Source (ALS)). An incident X-ray beam was focused to a spot size of 5 μm in the vertical and horizontal directions. At ALS BL10.3.2, the distribution patterns of As(III) and As(V) were evaluated separately by mapping at three energies, 11855 eV (pre-edge region), 11875 eV (As(V) is mainly excited) and 11991 eV (both As(III) and As(V) are excited). The composition of As(III) and As(V) in each pixel was determined by least squares combination fitting (LCF) of the three energies to the normalized intensities of the As(V) and As(III) standards ($NaAsO_2$ for As(III) and Na_2HAsO_4 for As(V), respectively) by using the ALS BL10.3.2 software and a chemical speciation map was created.

Arsenic K-edge μ-X-ray absorption near edge structure (μXANES) spectra were collected from spots of interest in the thin section, based on the μXRF map near the soil-root interfaces and on the soil particles. Energy calibration was performed based on the Na_2HAsO_4 white line peak at 11875 eV for As. Normalization and LCF were performed with Athena software[1]. Reference components for LCF was sodium arsenite ($NaAsO_2$) for As(III) and arsenate sorbed on ferrihydrite for As(V). The sodium arsenite was purchased from WAKO chemicals, Tokyo, Japan whereas arsenate sorbed on ferrihydrite was synthesized as follows. Sodium arsenate (Na_2HAsO_4) solution was mixed with synthesized 2-line ferrihydrite suspended in 0.01M $NaNO_3$ solution and reacted for 24 h at pH 5. The suspension was centrifuged, washed by 0.01 M $NaNO_3$ solution and then freeze-dried. The adsorption amount of arsenate was 18 mmol kg^{-1}.

The Fe mineral phases in the selected points on Fe mottles were identified from micro-X-ray diffraction (μXRD) patterns at an incident energy of 17 keV (= 0.07293 nm) with a CCD camera at ALS BL10.3.2. Iron K-edge μXANES spectra were collected 5 μm below the spots where As K-edge μXANES were obtained.

Reference

1) Ravel, B.; Newvile, M. ATHENA, ARTEMIS, HEPHAESTUS: data analysis for Xray absorption spectroscopy using IFEFFIT. J. Synchrotron Radiat. 2005, 12, 537-541.

Table S1. Soil properties and arsenic concentrations.

	Soil A	Soil B
Cation exchange capacity (cmolc kg^{-1})	31	26
Exchangeable Ca (cmolc kg^{-1})	19	14
Exchangeable Mg (cmolc kg^{-1})	6.0	4.7
Exchangeable K (cmolc kg^{-1})	2.4	1.0
Acid oxalate extractable Fe (g kg^{-1})	15	13
Acid oxalate extractable Al (g kg^{-1})	2.9	1.1
DCB extractable Fe (g kg^{-1})	22	14
Total Carbon (g kg^{-1})	27	17
Particle size distribution (%)		
sand	33	47
silt	30	25
clay	38	28
Soil texture	LiC	LiC
Arsenic concentration (mmol kg^{-1})		
Total	0.9	0.49

*DCB: dithionite bicarbonate citrate extraction

Table S2. Concentrations of acid-oxalate extractable Fe and As in the soils and roots with and without Fe-plaque staining.

		Fe	As	As/Fe ratio
		mmol kg^{-1}		
A	Soil	155	0.58	0.0038
	Root with Fe-plaque staining	986	15	0.0155
	Root without visible Fe-plaque	477	3.1	0.0065
B	Soil	189	0.26	0.0014
	Root with Fe-plaque staining	995	2.5	0.0025
	Root without visible Fe-plaque	291	0.72	0.0025

Table S3. Results of linear combination fitting of As K-edge μXANES

Reference compounds for linear combination fitting			As(III): $NaAsO_2$ As(V): arsenate sorbed on ferrihydrite		As(III): $NaAsO_2$ As(V): Na_2HAsO_4	
			As(III) %	R-factor[a]	As(III) %	R-factor[a]
Fig.1b	Fe-plaque	1	66	0.0073	70	0.0097
		2	71	0.010	74	0.011
		3	80	0.010	83	0.012
	soil matrix	4	86	0.034	86	0.042
		5	68	0.026	77	0.031
		6	70	0.015	80	0.018
		7	60	0.0095	72	0.017
		8	78	0.015	81	0.016
		9	69	0.014	72	0.027
Fig. 1e	Fe-plaque	10	83	0.030	76	0.027
		11	63	0.038	73	0.020
Fig. 1h	Fe-plaque	12	45	0.014	48	0.014
		13	45	0.026	30	0.014
		14	45	0.030	48	0.027
		15	51	0.031	48	0.030
	soil matrix	16	31	0.030	32	0.082
		17	30	0.059	39	0.026
Fig. 2b	Fe-plaque	18	18	0.0031	8	0.019
	soil matrix	19	40	0.0075	33	0.012

[a]R-factor = $\Sigma(\text{data-fit})^2 / \Sigma\ \text{data}^2$

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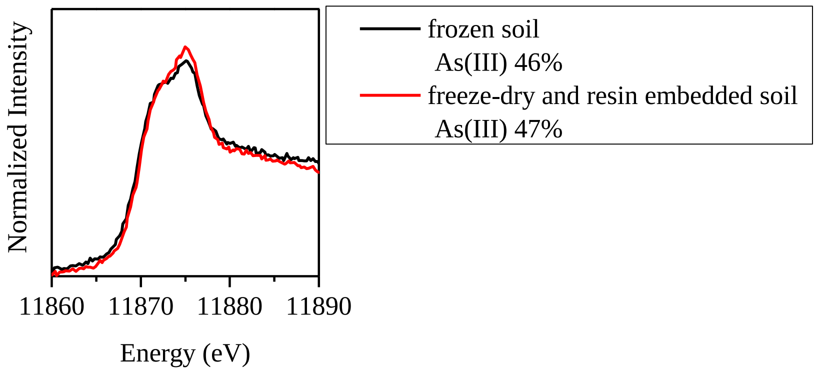

Figure S1. Effects of freeze-drying and resin embedding on As speciation in soil.

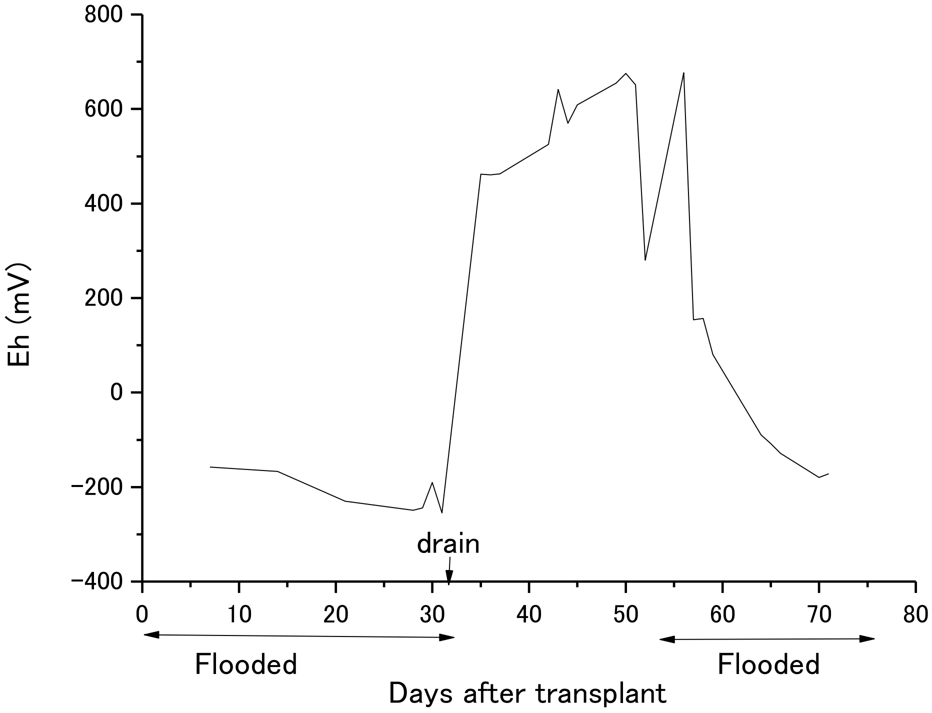

Figure S2. Eh changes in soil A.

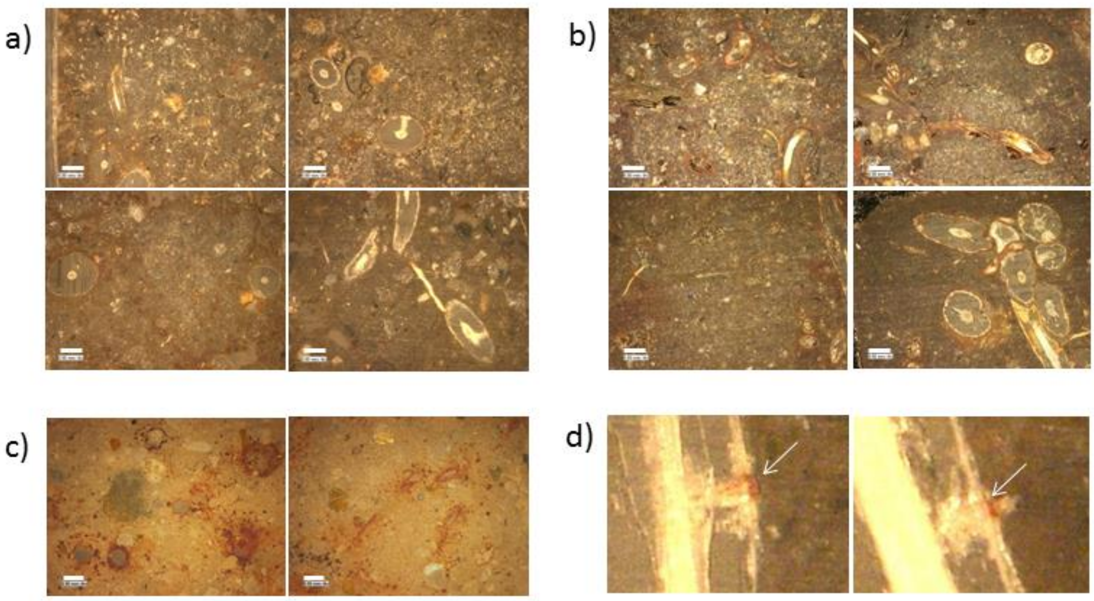

Figure S3. Photographs of the thin sections that were collected during the flooded period (a, d), 1 month after harvest (b) and 6 months after harvest (c). Arrows indicate the points of lateral root emergence with staining from iron hydroxide deposition. White bars indicate 500 μm._On the roots with minimal Fe-plaque, pigmentation from the Fe-plaque was found on the emergence point of the lateral roots but not on their apex (d). Oxygen that was transported through the aerenchyma potentially escaped from the emergence point of the lateral roots and caused Fe^{2+} oxidation.

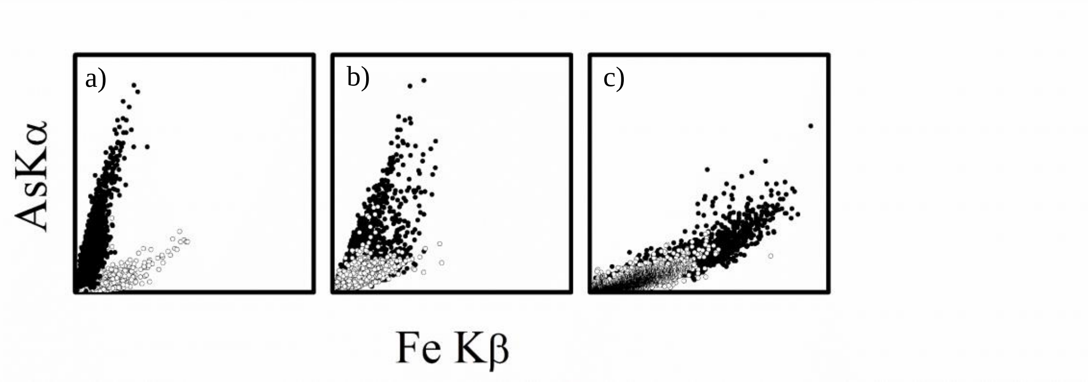

Figure S4. Relationships between the fluorescence intensities of Fe Kβ and As Kα associated with the micro X-ray fluorescence (μXRF) images shown in Fig. 1 in the main document. a) A root with a visible Fe-plaque coating collected from soil A (correspond to Fig. 1a-c), b) a root without a visible Fe-plaque coating was collected from soil A (correspond to Fig. 1d-f), c) a root with a visible Fe-plaque coating was collected from soil B (correspond to Fig. 1g-i). The closed and open circles correspond to spots on the soil-root interface and on the soil matrix, respectively.

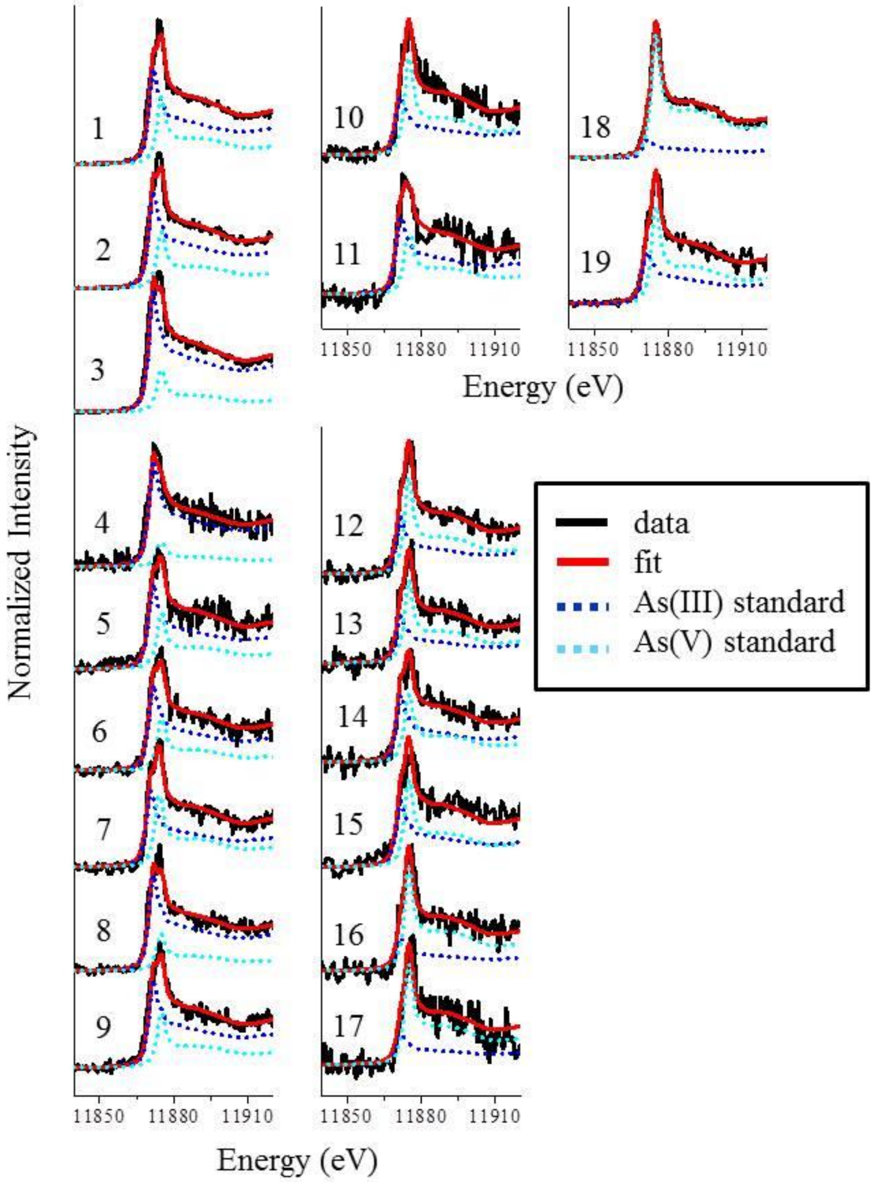

Figure S5. Arsenic K-edge μXANES. The numbers correspond to the points in Figure 1a (1-9), 1e (10, 11), 1h (12-17) and Figure 2b (18, 19) and Table S3. As(III) standard: $NaAsO_2$, As(V) standard: arsenate sorbed on ferrihydrite.

Experimental method for Figure S6

Soil blocks (5 × 5 mm, 10 mm in depth) that included roots were obtained at approximately 5 cm from the soil surface 1 week after panicle exertion. The soil blocks were immediately frozen in liquid nitrogen and immersed in cooled low-viscosity embedding substance before refreezing. The frozen thin sections were prepared as described above and then freeze-dried. The μXRF maps and the μXANES spectra were obtained at BL4A at the Photon Factory (KEK), as described in the text.

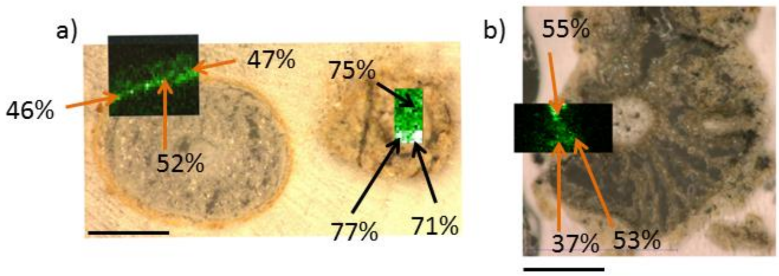

Figure S6. Light microscope and micro X-ray fluorescence (μXRF) images the rice roots without aerenchyma (a, left), the soil particle (a, right), and the root with aerenchyma (b) that were collected from the pot soil 1 week after heading while the soil was flooded. The numbers indicate the percentages of As(III) that were estimated by linear combination fitting of the As-K edge micro X-ray absorption near edge structure spectra. Black bars indicate 200 μm.

Experimental method for Figure S7

A root from the rice seedlings that were grown in a pot for 4 weeks under flooded conditions were carefully removed with tweezers and were immediately frozen in liquid nitrogen. The frozen root was immersed in cooled low-viscosity embedding substance (Cryo Mount I, Muto Pure Chemicals, Tokyo, Japan) before immediately refreezing. Transverse and cross-sections (50 µm in thickness) of the root were prepared with a cryomicrotome (CM1850, Leica, Wetzlar, Germany) and were frozen until analysis. Although this sample preparation method resulted in the loss of the spatial relationships between the root and soil, it preserved the elemental distribution and speciation of the elements in the roots. The µXRF maps of the frozen root sections were obtained by maintaining a frozen thin section under a stream of cryogenic N_2 gas (Cryojet XL, Oxford Instruments, UK) at BL37XU, SPring-8. An incident X-ray beam of 12.8 keV was focused to a vertical spot size of 1.02 µm and a horizontal spot size of 1.16 µm. The scan step sizes and the spectral acquisition times for each spot were 5 µm and 0.1 s for the longitudinal section and 10 µm and 0.3 s for the cross-section, respectively.

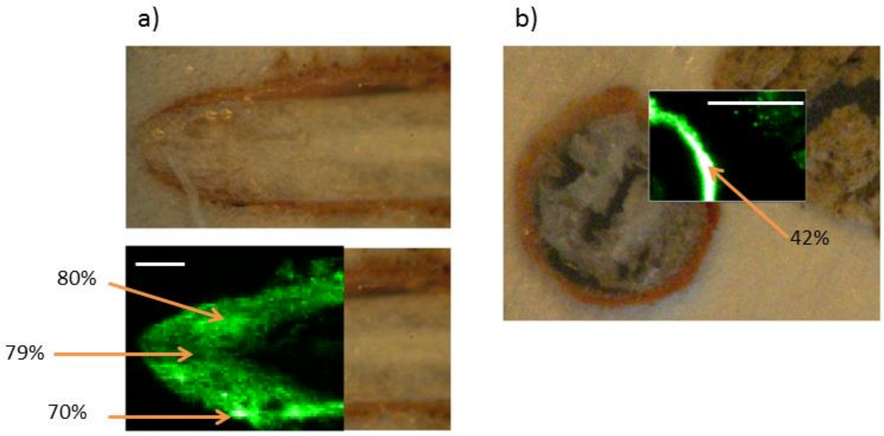

Figure S7. Light microscope and micro-X-ray fluorescence images around the rice roots that were collected 4 weeks after transplanting the seedlings. (a) the transverse section of the root tip and (b) the cross-section of the root at approximately 1 cm from the root tip. The numbers indicate the percentages of As(III) that were estimated from linear combination fitting of the As-K edge micro-X-ray absorption near edge structure spectra for the points indicated by the orange arrows. White bars indicate 500 µm.

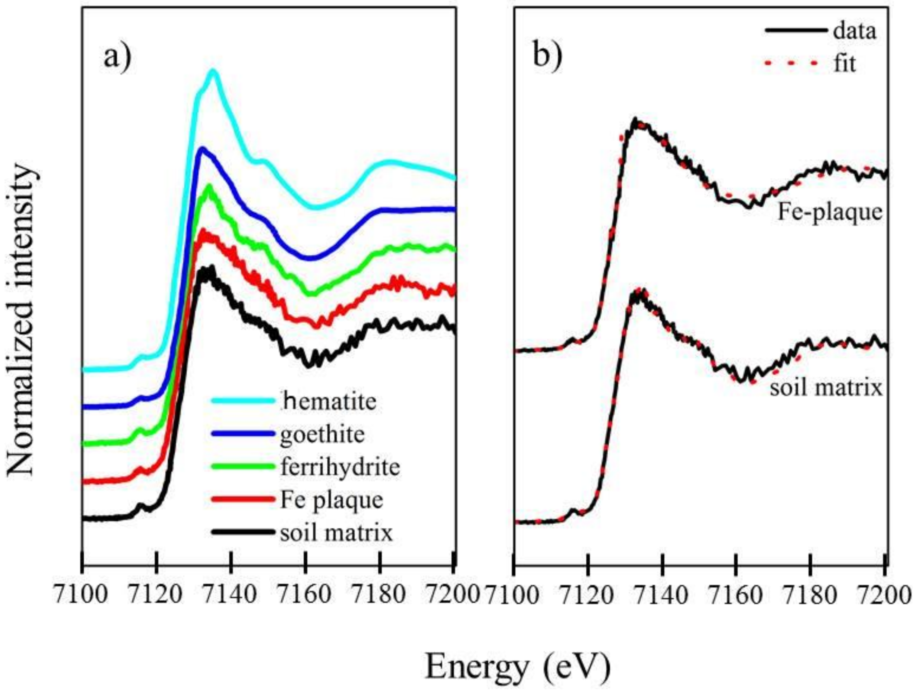

Figure S8. Fe K-edge μXANES on the Fe-plaque (near point 20) and soil matrix (near point 19) in the soil thin section presented in Fig. 2a-c in the main document (a). Linear combination fit by using ferrihydrite, goethite and hematite as reference compounds[1] showed that Fe minerals in the Fe-plaque and in soil matrix were composed of 100% of ferrihydrite (R-factor is 0.001 for Fe-plaque and 0.0008 for soil matrix, respectively).

Reference for linear combination fitting

1. Das Soumya.; Hendry, M. J.; Essilfie-Dughan, J., Transformation of two-line ferrihydrite to goethite and hematite as a function of pH and temperature. *Environ. Sci. Technol.* **2011**, 45, 268-275.

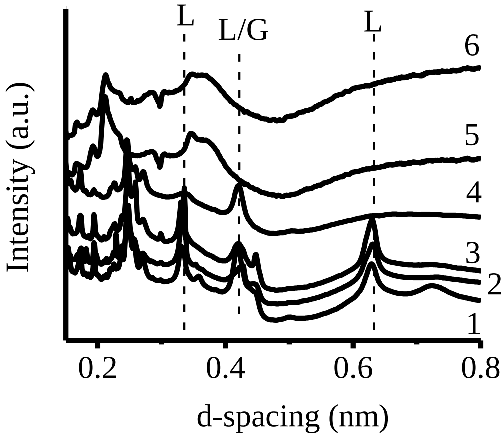

Figure S9. Micro-X-ray diffraction patterns of the Fe mottles that were collected at a depth of 24-27 cm from soil B. L: lepidocrocite, G: goethite. Numbers correspond to the points in Figure 3f.