SI Materials and Methods

Soil sampling

Soil samples were collected from a NW-facing slope (12-15%) located at the West Virginia University Agronomy Farm in Morgantown, WV (39° 39' N, 79° 54' W). Historically the site received infrequent applications of lime or fertilizer, and did not support grazing livestock at the time of sampling. Soils formed primarily from parent material of the Allegheny Formation and Pottsville Group, mainly from interbedded shale, siltstone, sandstone, and some limestone. Dekalb (loamy-skeletal, mixed, mesic Typic Dystrochrepts) and Gilpin (Fine-loamy, mixed, mesic Typic Hapludults) are the dominant residual soils in the area and are generally more acid than the surrounding soils. Some of the local soils have a seasonal high water table. After roots had been separated, sample portions used for soil characterizations were air-dried, then ground and passed through a 2 mm sieve. However, in order to prevent microbial cell lysis, as well as other chemical changes in the soil (e.g., the oxidation of Fe(II) to Fe(III)), the soil portion used for incubations was not air-dried prior to the experiment.

Soil chemical and physical characterizations

The following soil chemical and physical characterizations were conducted on the air-dried composite soil (unless noted otherwise) prior to the anaerobic incubation. Soil pH was measured in a 1:1 soil to water ratio using a combination pH electrode (Mettler-Toledo, Inc., Columbus, OH, USA). Soil particle-size distribution was determined by pipette analysis after dispersion with sodium hexametaphosphate. Bulk density and porosity at the field site were determined based upon six core samples taken to a depth of 20 cm after the sod had been removed.
Total OM% (TOM) was determined by loss of mass on ignition. Total residual OM% (TROM) was determined by the same method, after samples had been treated with 35% H$_2$O$_2$ to remove the more labile OM fraction. Total labile OM% (TLOM (H$_2$O$_2$ reactive OM)) was calculated as the difference between TOM and TROM. Total organic C% (TOC) was determined by dry combustion using a TruSpec CHN analyzer (Leco, Corp., St. Joseph, Michigan, USA). Total residual OC% (TROC) was determined by using H$_2$O$_2$ treated soil samples in the same analysis as TOC. Total labile organic C% (TLOC) was calculated as the difference between TOC and TROC.

Soil Fe and P forms were characterized following the methods of Peretyazhko and Sposito (2005) (see main text for reference). Citrate-bicarbonate-dithionite (CBD) extraction was used to operationally measure total free Fe oxides. The solubilized Fe(II) was determined by a colorimetric reaction with 1,10-phenanthroline at 510 nm using a Cary 50 UV Spectrophotometer (Varian, Inc., Palo Alto, CA, USA). Total P in extracts was determined using an Optima 2100 DV Inductively Coupled Plasma-Atomic Emission Spectrophotometer (ICP-AES) (PerkinElmer, Waltham, Massachusetts, USA). The extracted P is reported here as an estimate of P chemically associated with reducible Fe(III) oxides in the soil.

Amorphous Fe oxide concentration was operationally determined using a citrate-ascorbate (CA) extraction. Reduced Fe and extracted P in solution were determined as described above. Total concentrations of Fe and P in the soil were determined by a microwave digest with concentrated HNO$_3$ using a Mars 5 microwave (CEM, Corp., Matthews, NC, USA). Total Fe and P from digested filtrates were determined by ICP.

Microbe reducible Fe(III) in field moist soil was estimated as follows: One of two separate groups of 0.2 g moist soil samples was extracted for 1 h, under standard conditions, in
either 5 ml of 0.5 M HCl or 0.25 M hydroxylamine hydrochloride (NH$_2$OH·HCl) in 0.25 M HCl. After 1 h, the solutions were passed through a 25 mm 0.2 µm filter (Millipore Corp., Billerica, MA, USA), and 0.1 ml of each filtrate was added to 5 ml of 1 g l$^{-1}$ ferrozine (3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p’-disulfonic acid, disodium salt hydrate) in 50 mM HEPES (N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid) buffer adjusted to pH 7 with 1 M NaOH. Standards were made using ferrous ethylenediammonium sulfate tetrahydrate (see Lovley and Phillips (1986) reference in main text). Absorbance at 562 nm of the resulting solutions was determined as described above. No inherent Fe(II) was detected in 0.5 M HCl extracts.

**Humic acid extraction and characterization**

The method used for HA extraction was the one outlined by the International Humic Substance Society (IHSS) and included: acidification of soil with 1 and 0.1 M HCl; extraction with 1 and 0.1 M NaOH under N$_2$ gas; precipitation of HA with 6 M HCl; re-dissolution with 0.1 M KOH with addition of solid KCl (0.3 M K$^+$) under N$_2$; precipitation of HA with 6 M HCl; and removal of inorganic materials by 0.1 M HCl/0.3 M HF treatment followed by dialysis against DDI water. The HA slurry from the dialysis tubing was freeze-dried and kept in a sealed, dark glass vial under laboratory conditions. The yield was 7.37 g HA kg$^{-1}$ dry soil; 0.74% of the dry soil was operationally defined as HA.

Although Benz et al. (1998) treated in-lab extracted HA (fractionated from sediments of Lake Constance in Germany) and commercial HA (Aldrich) with 1 M HCl, they were unable to completely remove residual Fe. Consequently, the HA extracted in the present study was analyzed for total Fe and P (ICP), as well as NH$_2$OH-HCl-reducible Fe(III) and acid-extractable Fe(II). Infrared (FT-IR), $^{13}$C-NMR, and CHNS analyses were used to compare other important
chemical properties between the extracted HA (HA Ex) and the IHSS Elliott Soil HA standard (HA St) (University of MN, St. Paul, MN, USA).

Fourier transformed infrared (FT-IR) spectra were recorded in absorbance mode with a Spectrum One spectrophotometer (PerkinElmer, Waltham, Massachusetts, USA). Samples were prepared as pellets containing 0.5 mg freeze-dried HA and 300 mg potassium bromide (Drosos et al., 2009). Liquid state normal broadband $^{13}$C-NMR analysis of HA samples was performed on a 600 Inova spectrometer (Varian, Inc., Palo Alto, CA, USA) at 150 MHz (Albers et al., 2008). Samples were prepared by dissolving 150 mg of freeze-dried HA in 1.5 ml of 3 M sodium deuterioxide in deuterium oxide (3 M NaOD in D$_2$O), shaken for 24 h and then centrifuged at 11 000 g for 15 min. The supernatant (700 µl) was transferred to 5 mm, high precision (900 MHz) NMR tubes (Wilmad-LabGlass, Buena, NJ, USA). Chemical shifts of the spectra were referred to D$_2$O, and the deuterium signal of the solvent was used as an internal lock. Acquisition of spectra was stopped at 90 000 transients and a line broadening function (LB = 50 Hz) was applied before Fourier transformations. Percentages of carbon (C %), hydrogen (H %), nitrogen (N %), and sulfur (S %) were determined using a Flash 1112 series elemental analyzer (CE Instruments, Hindley Green, UK). Oxygen content (O %) was estimated by mass difference (Drosos et al., 2009).

**Gas evacuation with argon**

After all the soil treatments had been prepared, to initiate the experiment, each serum bottle was sealed with an autoclaved butyl rubber stopper (Geo-Microbial Technologies, Inc., Ochelata, OK, USA), a 20 mm crimped aluminum cap (Wheaton, Inc., Millville, NJ, USA), then stoppers were pierced with a 3.8 cm 18 gauge needle attached to a one-way stopcock (Baxter Healthcare Corp., Deerfield, IL, USA). Each sample was then gas evacuated under vacuum and
filled with argon (Ar) three times using an apparatus that consisted of a vacuum pump, an Ar gas tank, and a manifold of copper tubing to which serum bottles were connected.

**Incubation biogeochemical parameters**

For measurement of carbon dioxide (CO$_2$), headspace gas was collected by first filling a 20 ml syringe (with one-way stopcock) with 10 ml Ar, injecting it into the headspace of an incubated serum bottle, and after being thoroughly mixed, 10 ml of headspace gas was withdrawn and injected into a new autoclaved 125 ml serum bottle at atmospheric conditions. An additional 20 ml of Ar was then injected into each incubated bottle so that an equal volume of solution could be withdrawn for subsequent Fe and P analyses without creating a vacuum. Carbon dioxide in gas sample bottles was analyzed as described in the manuscript.

For characterization of Fe and P in solution, incubated samples were centrifuged at 680 g for 15 to 20 min. A centrifuge carriage capable of holding 250 ml centrifuge bottles was modified to accommodate the Wheaton 125 ml serum bottles used for the incubation. Centrifugation was used primarily to avoid subsequent filter clogging problems. A syringe was used to withdraw 20 ml of solution from the centrifuged serum bottles, which was then passed through a 0.2 µm filter. For Fe(II) determination, 0.1 to 4 ml of sample was directly added to 5 ml of ferrozine, and the absorbance at 562 nm was determined as described above. A separate 5 ml sample was diluted to 7 ml with DDI water, and then acidified with 1 M HCl to achieve a 0.5 M HCl solution. Total Fe and P in this acidified sample were determined by ICP. Inorganic P was determined by the ammonium molybdate method (Murphy and Riley, 1962; Kuo, 1996). Filtered solution (5 ml) was diluted to 20 ml with DDI water, mixed with 8 ml ammonium molybdate reagent, then diluted to 50 ml with DDI water. Absorbance at 880 nm was determined as described above.
Eh measurements were collected with a platinum electrode and AgCl reference electrode that had both been built in the lab specifically for the incubation. After incubated serum bottles had been analyzed for Fe and P, Eh (mV) was determined by using an auto-range digital multimeter (RadioShack, Fort Worth, TX, USA) connected to a platinum electrode and a AgCl reference electrode. This was accomplished by removing the aluminum seal and stopper from a selected serum bottle, and then quickly immersing both electrodes into the incubated solution. Probe function was checked with standards of quinhydrone mixed in pH 4 and pH 7 buffers. The pH of incubated samples was directly measured in an aliquot of solution that had been transferred to a separate container.