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Iron(III) reduction and phosphorous solubilization in humid tropical forest soils

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Abstract—Phosphorus is one of the nutrients most commonly limiting net primary production in soils of humid tropical forests, mainly because insoluble Al and Fe phosphates and strong sorption to Fe(III) (hydr)oxides remove P from the bioavailable pool. Recent field studies have suggested, however, that this loss may be balanced by organic P accumulation under a wet moisture regime (>3350 mm annual precipitation). It has been hypothesized that, as the moisture regime changes from dry to mesic to wet, periods of anoxic soil conditions increase in intensity and duration, depleting Fe(III) (hydr)oxides and releasing sorbed P, but also slowing organic matter turnover, thus shifting the repository of soil P from minerals to humus. Almost no quantitative information is available concerning the coupled biogeochemical behavior of Fe and P in highly weathered forest soils that would allow examination of this hypothesis. In this paper, we report a laboratory incubation study of the effects of biotic Fe(III) (hydr)oxide reduction on P solubilization in a humid tropical forest soil (Ultisol) under a wet moisture regime (3000–4000 mm annual rainfall). The objectives of our study were: (1) to quantify Fe(III) reduction and P solubilization processes in a highly weathered forest soil expected to typify the hypothesized mineral dissolution-organic matter accumulation balance; (2) to examine the influence of electron shuttling on these processes using anthraquinone-2,6-disulfonate (AQDS), a well-known surrogate for the semiquinone electron shuttles in humic substances, as an experimental probe; and (3) to characterize the chemical forms of Fe(II) and P produced under anoxic conditions, both with and without AQDS. Two series of short-term incubation experiments were carried out, one without AQDS and another with an initial AQDS concentration of 150 μM. We measured pH, pE, and the production of Fe(II), total Fe [Fe(II) + Fe(III)], inorganic P, total P (inorganic P + organic P), and biogenic gases (CO_2, H_2 and CH_4). The same positive correlation was found between soluble P release and soluble Fe(II) production throughout incubation, implying that reduction of Fe(III) solubilized P. The Fe(II) produced was mainly particulate, evidently due to the formation of Fe(II) solid phases. Thermodynamic calculations indicated that precipitation of siderite and, in the presence of AQDS, vivianite was favored under the anoxic conditions that developed rapidly in the soil suspensions. Inorganic soluble P released during incubation was very small, indicating that the soluble P produced was mainly in organic form, which is consistent with the hypothesis that P accumulates in soil humus. Our net CO_2 production, H_2 consumption, and Fe(II) production data all suggested that reductive dissolution of Fe(III) (hydr)oxides was a terminal electron-accepting process coupled both to H_2 consumption and organic C oxidation by the native population of microorganisms in the soil. Addition of AQDS accelerated the production of Fe(II) and the release of soluble P, while hastening the decline in H_2 gas levels and suppressing CH_4 production. However, throughout incubation, the same quantitative relationships between soluble Fe(II) and P, and between pE and pH, were found, irrespective of AQDS addition. Thus we conclude that, in our soil incubation experiments, added AQDS functioned with the native microbial population solely as an electron shuttle catalyzing Fe(III) reduction. Whether humic substances in the soil also can act as electron shuttles in this way is a matter for future investigation.

1. INTRODUCTION

In soils of humid tropical forests, phosphorus is one of the primary nutrients limiting productivity (Vitousek and Sanford, 1986). Long-term P bioavailability in these soils has been thought traditionally to be determined primarily by their advanced state of mineral weathering (Walker and Syers, 1976). Weathering of primary minerals supplies soluble P that is subsequently incorporated into biomass and soil organic matter, or is lost from the bioavailable pool (defined operationally as either soluble or weakly sorbed inorganic and organic P; Cross and Schlesinger, 1995), partly through leaching and partly through the formation of insoluble Al and Fe phosphates (Sanchez, 1976) or sorption to Al and Fe(III) (hydr)oxides (Patrick and Khalid, 1974; de Mello et al., 1998; Reddy et al., 1999). By the time primary minerals have been depleted, most soil P is to be found in recalcitrant inorganic fractions.

These well-known conclusions about the effects of mineral weathering on P bioavailability tacitly assume oxic soil environments. Recent work by Miller et al. (2001) suggests that P biogeochemistry in highly weathered forest soils is more complex than traditionally portrayed and will be influenced significantly if intense anoxic conditions of sufficiently long duration occur. Miller et al. (2001) used sequential fractionation to study P biogeochemistry in acidic, highly weathered Inceptisols and Andisols along a precipitation gradient where other soil formation state factors (elevation, temperature, parent material, time) remained relatively constant. Their results led them to hypothesize that a balance between Fe(III) (hydr)oxide dissolution and organic matter accumulation mediated by varying soil redox conditions might control the P distribution along dry-mesic-wet rainfall gradients. Dry-mesic soils (<2400 mm annual precipitation) are predominantly oxic (average electrode potential

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(E_h) > + 400 mV), with low organic matter content and P retained primarily in sorbed forms (Miller et al., 2001). Mesic soils (2400–3350 mm annual precipitation) are characterized by short periods of anoxic conditions. Fluctuating anaerobiosis that induces reductive dissolution of Fe(III) (hydr)oxide minerals exposes the associated mineral-bound P to leaching losses, thus lowering the total P content significantly. Organic matter turnover is not slowed, however, because the periodic reducing conditions that occur are not of sufficient duration and intensity. Mesic soils thus have low total P, most of it being in recalcitrant inorganic forms (sorbed and occluded). In wet soils (>3350 mm annual precipitation), prolonged anoxic conditions develop (average $E_h < -100$ mV; Miller et al., 2001; Schuur et al., 2001). Reductive dissolution of Fe(III) (hydr)oxides depletes them from the soil, but loss of the P released can be impeded by its incorporation into organic matter that now accumulates because reducing conditions are of sufficient duration and intensity to retard turnover. Chadwick and Chorover (2001) have referred to the strong negative correlation between average annual rainfall and Fe(III) (hydr)oxide content as defining a "pedogenic threshold that can impact ecosystem processes such as phosphate retention." To this, following Miller et al. (2001), we would add the balancing impact of the strong positive correlation that exists between average annual rainfall and organic matter accumulation.

In natural soil environments, Fe(III) (hydr)oxide reduction is mainly biotic (Lovley and Phillips, 1986; Roden and Wetzel, 1996; Frenzel et al., 1999; Ratering and Schnell, 2000) and has been attributed to the activity of dissimilatory iron-reducing bacteria (DIRB) that couple the oxidation of H_2 gas or organic C to Fe(III) reduction (Lovley, 1997). These bacteria can reduce Fe(III) either by direct contact between the organism and the oxide surface or by indirect mechanisms not involving contact. These latter mechanisms include "electron shuttling" and soluble Fe(III) complexation with subsequent reduction (Lovley et al., 1996; Royer et al., 2002). When soil organic C content is significant, humic substances have been proposed to enhance Fe(III) reduction by serving as "electron shuttles" (i.e., biologically reducible kinetic intermediates) in extracellular electron-transfer processes between bacterial cells and Fe(III) (hydr)oxides (Lovley et al., 1996; Benz et al., 1998; Coates et al., 2002). Important candidates for the electron-accepting groups in humic substances are semiquinone moieties (Scott et al., 1998), complexed Fe(III) (Struyk and Sposito, 2001), and conjugated aromatic moieties (Chen et al., 2003).

Anthraquinone-2,6-disulfonate (AQDS), which contains quinone moieties that are believed to act in a manner similar to the semiquinones in humic substances, is a model compound that has been used frequently as a probe to study Fe(III) (hydr)oxide reductive dissolution by electron shuttling (Lovley et al., 1996; Coates et al., 1998; Cervantes et al., 2003). Microorganisms transfer two electrons to AQDS, generating anthrahydroquinone-2,6-disulfonate (AHDS), which can then shuttle electrons to Fe(III), thus regenerating AQDS (Lovley et al., 1996; Fredrickson et al., 1998). Fermentative bacteria, nitrate- and sulfate-reducing bacteria, DIRB, and methanogens all are able to transfer electrons to AQDS (Bond and Lovley, 2002; Cervantes et al., 2002). Anthraquinone-2,6-disulfonate has been shown in laboratory studies to increase the reductive dissolution of synthetic or natural, amorphous (Fredrickson et al., 1998) or crystalline Fe(III) (hydr)oxides (goethite, hematite; Zachara et al., 1998). However, there appear to be no published studies of Fe(III) (hydr)oxide reduction using AQDS as a probe with the native microbial communities in natural soils.

The Fe(II) produced by reductive dissolution undergoes complex secondary chemical transformations. Along with aqueous Fe(II) complex formation and Fe^{2+} adsorption on oxide surfaces and bacterial cells, Fe(II) secondary minerals precipitate (siderite ($FeCO_3$), vivianite ($Fe_3(PO_4)_2 \cdot 8H_2O$) and magnetite (Fe_3O_4); Zachara et al., 1998; Urrutia et al., 1999; Benner et al., 2002; Royer et al., 2002; Kirk, 2004). Formation of these Fe(II) secondary phases is influenced by the presence of soluble P, atmospheric composition, pH, temperature, time, and the species of bacteria present (Zachara et al., 1998; Roh et al., 2003).

Almost no quantitative information is available concerning the details of the coupled biogeochemical behavior of Fe and P in highly weathered forest soils that could be useful in examining the hypothesis of Miller et al. (2001). In this paper, we report a laboratory incubation study of the effects of biotic Fe(III) (hydr)oxide reduction on P solubilization in a humid tropical forest soil (Ultisol) under a wet moisture regime (3000–4000 mm annual rainfall). The objectives of our study were: (1) to quantify Fe(III) reduction and P solubilization processes in a highly weathered forest soil expected to typify the mineral dissolution-organic matter accumulation balance hypothesized by Miller et al. (2001); (2) to examine the influence of electron shuttling on these processes using AQDS as an experimental probe; and (3) to characterize the chemical forms of Fe(II) and P produced, both with and without AQDS. Our overall goal was to determine more precisely the mechanistic relationships between Fe redox transformations and P solubilization in humid tropical forest soils.

2. MATERIALS AND METHODS

2.1. Soil Sampling and Characterization

Soils were collected along a toposequence in Puerto Rico at the Bisley Research Watersheds, Luquillo Experimental Forest (LEF), part of the NSF-sponsored Long Term Ecological Research Network. The LEF is an area of high relief, with elevations ranging from 200 to 1300 m (Frizano et al., 2002). Soils in the LEF are derived from the Rio Blanco stock, a volcanic formation with Tertiary quartz-diorite intrusions (Beinroth, 1982; Frizano et al., 2002). Rocks in the Rio Blanco stock are medium- to coarse-grained and dominated by phenocrysts of quartz and plagioclase feldspars, with lesser amounts of biotite, hornblende, K-feldspar, and accessory magnetite, sphene, apatite, and zircon (White et al., 1998). Topographic zones in the study area are identified as ridge, slope or valley, based on the geomorphologic descriptions of Scatena (1989). Soils along the toposequence we sampled are classified as Ultisols in the U. S. Soil Taxonomy, with average soil atmosphere O_2 partial pressures decreasing significantly from ridge to valley, where they drop to values below 3 kPa at the 10–35 cm depth (Silver et al., 1999), mainly because of water input from runoff (Scatena, 1989). Silver et al. (1999) found that, relative to the ridge soils, the valley soils have higher methane partial pressures in the soil atmosphere at the 10 cm depth (0.1 and 3 Pa at the ridge and valley sites, respectively). Therefore, periodic depletion of O_2 and consequent development of anoxic conditions take place in these soils.

Soil samples (0–20 cm depth interval) for our study were collected at a valley site and stored in plastic bags during transport to the laboratory, where they were air-dried under aerobic conditions at 22 ± 3°C and passed through a 2-mm sieve, following standard procedure for soil incubation experiments performed under anoxic conditions

Table 1. Selected geochemical properties of valley soil.[a]

Characteristic	Value
pH[b]	5.01 ± 0.14
Total organic matter[c] (%)	9.24
Total organic C (%)	5.36
Residual organic matter[d] (%)	1.64 ± 0.04
Fe_c (g kg^{-1})	1.82 ± 0.04
Fe_{ca} (g kg^{-1})	17.6 ± 1.0
Fe_{dcb} (g kg^{-1})	27.53 ± 0.59
Fe_{tot} (g kg^{-1})	33.7 ± 0.1
P_c (mg kg^{-1})	49 ± 5
P_{ca} (mg kg^{-1})	128 ± 3
P_{dcb} (mg kg^{-1})	205 ± 4
P_{tot} (mg kg^{-1})	279 ± 1

Error bars are standard deviations of six replicate measurements.

[a] c = citrate extraction; ca = citrate-ascorbate extraction; dcb = dithionite-citrate-bicarbonate extraction; tot = nitric acid/hydrogen peroxide microwave digestion.

[b] Initial pH of the soil suspension (day 0 of incubation).

[c] Before H_2O_2 treatment.

[d] After H_2O_2 treatment.

(Yao et al., 1999). Preliminary incubation experiments comparing air-dried with undried soil samples (Electronic Annex) demonstrated no effect of previous air-drying on the production of soluble Fe and P or on the pH changes observed during incubation. Mayer and Conrad (1990) reported the same lack of effect of prior aerobic air-drying on methane production in their incubation study of rice soils under anoxic conditions.

Table 1 summarizes some geochemical properties of the valley soil that are pertinent to our study. Chemical extractions were performed to quantify poorly crystalline Fe(III) (hydr)oxides (citrate-ascorbate extraction (ca); Reyes and Torrent, 1997); organically bound Fe(III) (citrate extraction (c); Reyes and Torrent, 1997); and total reducible Fe(III) (hydr)oxides (dithionite-citrate-bicarbonate extraction (dcb); Loeppert and Inskeep, 1996). A nitric acid/hydrogen peroxide microwave digestion was performed to determine total concentrations of Fe and P (Sah and Miller, 1992). Total Fe was analyzed by atomic absorption spectrometry (AAS) and total P by inductively coupled plasma atomic emission spectrometry (ICP-AES). Organic matter content was determined by the Walkley-Black method (Nelson and Sommers, 1982). Organic matter content and total elemental concentrations reported in Table 1 were measured at the UC-DANR Analytical Laboratory (University of California at Davis).

Typical of Ultisols, the valley soil is acidic, clayey, highly leached, and well weathered (Scatena, 1989). Comparison of the extractable and total Fe (Table 1) shows that Fe in the soil is mainly found in Fe(III) (hydr)oxide form, much of which is poorly crystalline. Phosphorus measured by ICP-AES represents the sum of both inorganic and organic forms (Miller et al., 2001). Citrate-extracted P is associated with the most soluble fraction of poorly crystalline Fe(III) (hydr)oxides (de Mello et al., 1998) and possibly with organic P. Comparison of the extractable and total P indicates that P in the valley soil is mainly associated with Fe(III) (hydr)oxides, which is characteristic of Ultisols (van Wambeke, 1992).

Table 2 summarizes the mineralogical composition of the valley soil (<180, <50, and <2 μm size-fractions) as characterized by X-ray diffraction (XRD) after dcb extraction and H_2O_2 treatment to remove organic C (Jackson, 1969). X-ray diffraction patterns were recorded using a Siemens diffractometer (Model D-500 with Cu Kα radiation). Intensities were measured with a 0.02° step size and 2 s counting time per step. For the soil aggregates <180 μm and <50 μm the powder method was used, whereas for the clay fraction the slide method was used (Moore and Reynolds, 1989). Clay minerals in the valley soil are dominated by kaolinite and chlorite (or hydroxy-interlayer vermiculite), which is typical for Ultisols (Buol et al., 1980). Weatherable minerals in Ultisols may occur in the sand and silt fractions (Buol et al., 1980; van Wambeke, 1992). We detected the presence of primary silicates (feldspar) and carbonates (dolomite) in the <180 and <50 μm fractions. The low Fe content of the valley soil (3.4% by mass) generally prevented XRD detection of crystalline Fe(III) (hydr)oxides, although we did find trace amounts of hematite in the <180 μm fraction.

Table 2. Mineralogical composition of valley soil determined by XRD.

<180 μm	<50 μm	<2 μm
Kaolinite, quartz, hematite (trace), feldspar, dolomite	Kaolinite, quartz, feldspar, dolomite	Chlorite, kaolinite, quartz

2.2. Incubation Experiments

2.2.1. Incubation apparatus

To decrease the influence of soil heterogeneity, incubation experiments were performed in an apparatus from which soil suspension was collected at selected time points (Sposito et al., 1991). Its principal components are a 1-L glass beaker (Kimax, Kimble), rubber stopper (size #15, Fisher), magnetic stirring bar, sampling tube, needle (size 21G2) with valve for gas sampling, pH electrode (VWR, Symphony electrode), and Orion combination redox electrode (Orion, Model 97-78). The sampling tube, home-built in Berkeley, has a three-way stopcock to permit sampling under vacuum and the introduction of Ar gas into the suspension. Argon gas was used to remove O_2 at the beginning of the incubation experiments and to replace soil suspension collected under vacuum with an equal gas volume.

2.2.2. Anoxic incubation experiments

The <2 mm size fraction of the valley soil was used in two series of soil incubations, one without AQDS (anthraquinone-2,6-disulfonate, Fluka) and another with an initial AQDS concentration of 150 μM (7.5 mmol kg^{-1} air-dried soil). Three replicates were prepared by adding 18 g air-dried valley soil to a 1-L beaker, after which the beaker was closed by a rubber stopper and sealed with silicone grease. Then an aliquot of 5 mmol L^{-1} AQDS stock solution and freshly prepared, deoxygenated Milli-Q water were added through the sampling tube under vacuum to achieve a 900 mL total solution volume. After preparation of the soil suspensions, Ar gas was passed through for 30 min to remove O_2. The laboratory temperature varied between 22 and 25°C during the incubation experiments. Sampling times were at 0, 3, 5, 7, 9, 11, 13, and 14 d. Before soil suspension withdrawal, gas samples were collected from the apparatus head space. Then soil suspensions were collected into 125-mL amber serum bottles and sealed in the laboratory. Argon gas was then passed through them immediately for several minutes to remove O_2 from the head space. The bottles were opened in a glove box (Coy Laboratory Products, Model B) under an atmosphere of 95% CO_2, 5% H_2 for further subsampling.

2.3. Analytical Techniques

Ferrozine (Aldrich) solution (1 g L^{-1}) was prepared in 50 mmol L^{-1} HEPES buffer (Sigma) and titrated to pH 7 with 1 mol L^{-1} NaOH (Fisher). Hydrochloric acid (HCl, 6 mol L^{-1}, Fisher) was used for a 0.5 mol L^{-1} HCl extraction (Fredrickson et al., 1998). Phosphorous and iron ICP standard solutions of 10 000 mg L^{-1} were used (Spex, CertiPrep). All solutions were prepared with high purity 18 MΩ cm^{-1} water (Milli-Q, Millipore). The AQDS stock solution was prepared at a concentration of 5 mmol L^{-1} with fresh solution made before each incubation experiment. Deionized, boiled, Ar-deoxygenated Milli-Q water was used for preparing the AQDS solutions and soil suspensions. Ultra high purity grade Ar gas was used in the incubation experiments.

Both pH and pE in the soil suspensions were monitored daily with a Beckman Φ71 pH meter. The pH electrodes were calibrated using pH 4 and 7 buffers (Fisher). Platinum electrodes (model 97-78, Orion) were calibrated with redox buffers comprising 0.1 mol L^{-1} potassium

Table 3. Operational definitions of the chemical forms of Fe and P quantified.

Chemical form	Definition	Method
	Soluble Fe and P	
Soluble Fe(II)	Filterable Fe(II) in supernatant solution	Ferrozine assay (Stookey, 1970)
Soluble Fe-ICP	Filterable Fe(II) + Fe(III) in supernatant solution	ICP-AES
Soluble Fe(III)	Filterable Fe(III) in supernatant solution	Difference between soluble Fe-ICP and soluble Fe(II)
Soluble inorganic P	Filterable inorganic P in supernatant solution	Molybdate-ascorbic acid assay (Murphy and Riley, 1962)
Soluble P-ICP	Filterable inorganic P + organic P in supernatant solution	ICP-AES
Soluble organic P	Filterable organic P in supernatant solution	Difference between soluble P-ICP and soluble inorganic P (Miller et al., 2001)
	Extractable Fe and P	
Extractable Fe(II)	0.5 M HCl-extractable Fe(II) in soil suspension	Ferrozine assay (Stookey, 1970)
Extractable Fe-ICP	0.5 M HCl-extractable Fe(II) + Fe(III) in soil suspension	ICP-AES
Extractable inorganic P	0.5 M HCl-extractable inorganic P in soil suspension	Molybdate-ascorbic acid assay (Murphy and Riley, 1962)
Extractable P-ICP	0.5 M HCl-extractable inorganic P + organic P in soil suspension	ICP-AES
	Particulate Fe and P	
Particulate Fe(II)	Iron(II) in the particulate form	Difference between extractable Fe(II) and soluble Fe(II)
Particulate Fe-ICP	Particulate Fe(II) + Fe(III)	Difference between extractable Fe-ICP and soluble Fe-ICP
Particulate inorganic P	Inorganic P in particulate form	Difference between extractable inorganic P and soluble inorganic P
Particulate P-ICP	Particulate inorganic P + organic P	Difference between extractable P-ICP and soluble P-ICP

ferrocyanide (Sigma) and 0.05 mol L^{-1} potassium ferricyanide or 0.01 mol L^{-1} potassium ferrocyanide, 0.05 mol L^{-1} potassium ferricyanide, and 0.36 mol L^{-1} potassium fluoride (Sigma). The measured electrode potentials were respectively + 236 ± 5 mV (pE = 4.0 ± 0.1) and + 302 ± 3 mV (pE = 5.1 ± 0.1), as expected for the two buffers. Electrodes were calibrated in the incubation apparatus before sealing.

For biogenic gas measurements, ~25 mL were withdrawn with a syringe from the head space of the incubation apparatus and transferred to 25-mL sealed serum bottles (Wheaton). Air was pumped out of the bottles before sampling. Hydrogen gas was analyzed with a TA3000R process gas analyzer (~2 mL gas sample, Trace Analytical); CH_4 and CO_2 were analyzed by gas chromatography using SRI 8610 gas chromatograph (~3–4 mL gas sample, SRI instruments).

Operational definitions of the chemical forms of Fe and P quantified in our experiments are summarized in Table 3. For the soluble Fe(II) determination, soil suspension was withdrawn from the sealed serum bottle to decrease the risk of Fe(II) oxidation should O_2 be present in the glove box. An aliquot of the suspension was withdrawn with a syringe from the sealed 125-mL serum bottle and passed through a 0.22 μm filter (Millipore, Millex-GP) directly into the ferrozine reagent. Then the serum bottles were opened for further subsampling. Because of filter clogging problems, centrifugation was used in the experiments. Soil suspensions were centrifuged at 12,000 RCF (10,000 rpm) for 10 min outside of the glove box, then brought back to the anaerobic chamber. The supernatant solution was passed through a 0.22 μm filter and acidified with HCl to achieve a final HCl concentration of 0.5 M. This fraction was analyzed by ICP-AES to measure soluble Fe and P as well as soluble inorganic P (Table 3).

A 0.5 M HCl-extractable fraction was obtained by placing the remaining soil suspension directly into 6 M HCl. The suspensions were then removed from the glove box, shaken overnight, and passed through a 0.22 μm filter. The 0.5 M HCl extraction is deemed reliable for extracting Fe(II) generated in oxide suspensions and sediments (Lovley and Phillips, 1986; Fredrickson et al., 1998). Among the reduced-iron solid phases, only highly crystalline magnetite is resistant to dissolution in 0.5 M HCl (Fredrickson et al., 1998). Chao and Zhou (1983) demonstrated that 0.5 M HCl extracts up to 17 or 30% of natural or synthetic amorphous Fe(III) (hydr)oxides, respectively, but only ~0.5% of crystalline iron oxides.

Iron(II) in filtrates and HCl extracts was measured colorimetrically at 562 nm by ferrozine assay (Stookey, 1970). Organically bound Fe(II) and inorganic Fe(II) are indistinguishable by this method (Pullin and Cabaniss, 2003). For the Fe(II) determination, an aliquot of 2.5 mL (when Fe(II) < 10 mg L^{-1}) or 0.1 mL (when Fe(II) > 10 mg L^{-1}) of the filtered solution was added to 5 mL ferrozine reagent. Soluble and HCl-extractable inorganic P were measured colorimetrically at 712 nm by a molybdate-ascorbic acid procedure (Murphy and Riley, 1962). Murphy and Riley (1962) demonstrated that the presence of ascorbic acid and a short reaction to form an intense bluish-purple color (~30 min) suppress hydrolysis of organic phosphorus to phosphate. Iron and phosphorous in acidified filtrates and extracts were analyzed by ICP-AES (hereafter denoted Fe-ICP or P-ICP, Table 3). Soluble Fe-ICP may contain colloidal Fe together with dissolved Fe due to dissolution of colloidal particles after acidification with HCl (Viollier et al., 2000). Particulate Fe and P concentrations were defined to be the difference between 0.5 M HCl-extractable and soluble concentrations (Table 3). Concentrations of Fe and P are reported in either grams or milligrams per kilogram of dry soil (g kg^{-1}, mg kg^{-1}), except in Figure 7, where the Fe(II) concentration is expressed in μmol L^{-1}.

2.4. Saturation Index (SI) Calculations

Saturation indices were calculated for Fe(II) solid phases using PHREEQC (Interactive version 2.8; Parkhurst and Appelo, 1999). Values of SI ≥ +0.5 were taken to indicate supersaturation and a tendency to precipitate, whereas SI ≤ −0.5 was taken to indicate undersaturation and tendency to dissolve. Siderite, vivianite and magnetite were considered as possible Fe(II) solids in the soil suspensions because these solid phases have been observed in studies of the biotic reductive dissolution of Fe(III) (hydr)oxides (Fredrickson et al., 1998; Zachara et al., 1998; Benner et al., 2002; Roh et al., 2003). Solubility product constants (K_{sp}) for vivianite and siderite were taken from Al-Borno and Tomson (1994) and Bruno et al. (1992), respectively. The solubility product constant of magnetite was calculated from the standard Gibbs energy of formation for magnetite (Fe_3O_4; Robie and Hemingway, 1995), water (H_2O; Robie and Hemingway, 1995), and aqueous Fe^{3+} and Fe^{2+} (Parker and Khodakovskii, 1995). The log K_{sp} values used in PHREEQC were:

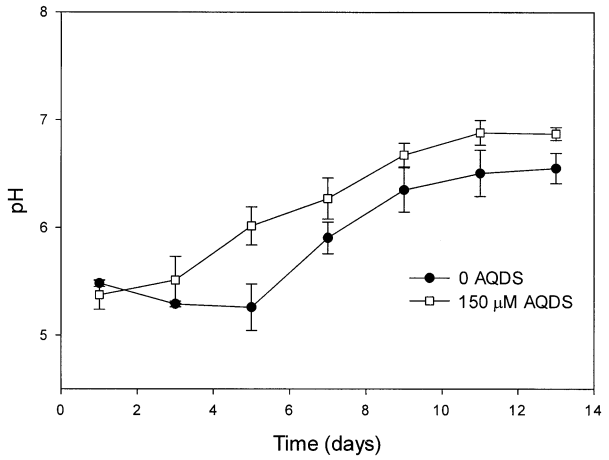

Fig. 1. Time course of pH in the incubated valley soil suspension with 0 and 150 μM AQDS added. Error bars show standard deviations (three replicates).

$$FeCO_{3(s)} = Fe^{2+} + CO_3^{2-} \quad \log K_{sp} = -10.8 \pm 0.2 \tag{4}$$

$$Fe(PO_4)_2 \cdot 8H_2O_{(s)} = 3Fe^{2+} + 2PO_4^{3-} + 8H_2O_{(l)} \quad \log K_{sp} = -35.767 \pm 0.076 \tag{5}$$

$$Fe_3O_{4(s)} + 8H^+ = 2Fe^{3+} + Fe^{2+} + 4H_2O_{(l)} \quad \log K_{sp} = +10.3 \pm 0.7 \tag{6}$$

We assumed that CO_2 measured in the gas phase was in equilibrium with aqueous CO_2. Our S. I. computations used measured soluble Fe(II) and inorganic P, solution pH, and electrode potential as input. Thermodynamic data for dissolved species (except as noted above) were taken from the WATEQ4F database (Ball and Nordstrom, 1991).

3. RESULTS AND DISCUSSION

3.1. Iron(II) Production

The soil suspensions rapidly become anoxic during incubation as the electrode potential (E_h) dropped from initial values near + 200 mV (pE = +3.4) to approximately −300 mV (pE = −5.1) by the third day, declining slowly afterward, irrespective of whether AQDS was present (data not shown). Correspondingly, pH in both suspensions tended to increase toward a plateau (Fig. 1). In the soil suspension without AQDS ("0 AQDS"), pH showed a lag phase of ~5 d, whereas in the presence of 150 μM AQDS, a pH increase was clearly observed by the fifth day of incubation.

Soluble Fe(II) in the 0 AQDS soil suspension also showed a lag phase of 5 d (Fig. 2a, lower curve), following a sigmoidal curve that appeared to be reaching a plateau after 2 weeks. In the 150 μM AQDS suspension, soluble Fe(II) did not show a lag phase, but did follow a sigmoidal curve that after 9 d rose to a plateau 50% higher than in the 0 AQDS suspension (Fig. 2b, lower curve). This accelerated Fe(II) production is evidence of native microorganisms in the valley soil that are able to reduce Fe(III) (hydr)oxides utilizing AQDS as an electron shuttle.

Irrespective of AQDS addition, soluble Fe-ICP always exceeded soluble Fe(II), while showing a similar time trend (Fig. 2, upper curves), suggesting that soluble (or filterable colloidal) Fe(III) was released during anoxic incubation. The source of this Fe(III) is possibly enhanced nonreductive Fe(III) (hydr)oxide dissolution promoted by organic ligands that were solubilized as E_h decreased and pH increased (Stevenson, 1994; Kirk, 2004).

Particulate Fe(II) production (difference between HCl-extractable Fe(II) and soluble Fe(II), Table 3), also accelerated by AQDS, was ~10 times greater than soluble Fe(II) production (Fig. 3), pointing to secondary precipitation of Fe(II) released by reductive dissolution (Fredrickson et al., 1998; Yao et al., 1999; Kirk, 2004). Saturation indices calculated for siderite and vivianite are summarized in Table 4. Given the assumption that $-0.5 \leq SI \leq 0.5$ can be interpreted as consistent with solid-phase formation, one may conclude that siderite precipitation was possible after ~12 d without AQDS and after 7 d in the presence of 150 μM AQDS. Comparison of these results with the data in Figure 1 suggests further that, with or without AQDS, siderite precipitation was favored when pH had in-

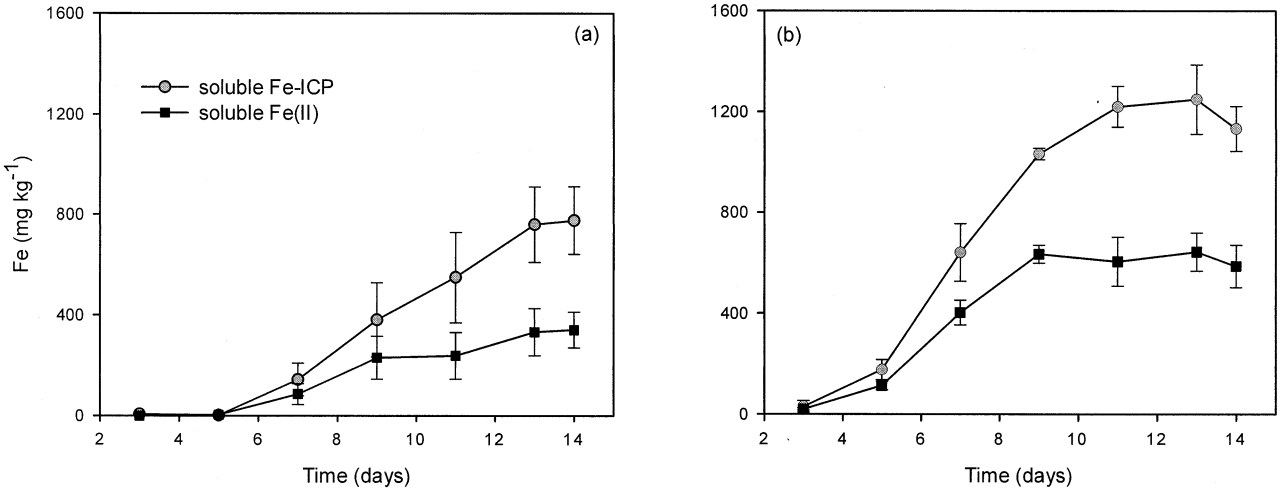

Fig. 2. Soluble Fe(II) and Fe-ICP produced in the valley soil as a function of incubation time. Soil suspensions with (a) 0 and (b) 150 μM AQDS added. Error bars show standard deviations (three replicates).

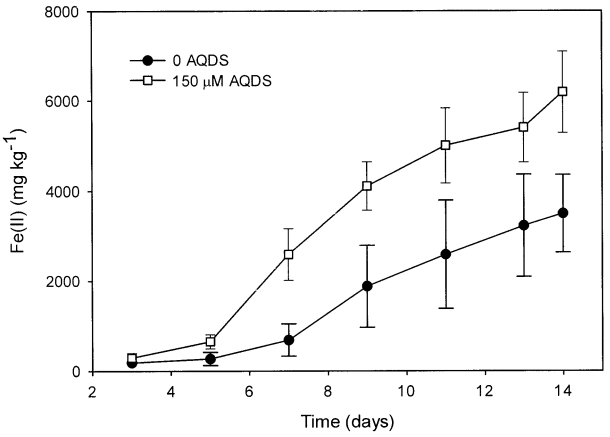

Fig. 3. Particulate Fe(II) produced in the valley soil as a function of incubation time with 0 and 150 μM AQDS. Error bars show standard deviations (three replicates).

creased above ~6.2. Because the addition of AQDS enhanced Fe(II) production (Fig. 2b), vivianite precipitation became thermodynamically favorable after 9 to 10 d in the soil suspension to which AQDS was added (Table 4). Magnetite (Fe_3O_4) precipitation was not favored in either soil suspension (data not shown), but the formation of less well-crystallized Fe(II, III) solid phases during incubation cannot be ruled out.

These results, and the additional possibility that soluble Fe^{2+} produced by reductive dissolution can be adsorbed, suggest three independent scenarios for the production of particulate Fe(II) in the soil suspensions. Precipitation of siderite and vivianite may be modeled by the reactions:

$$Fe(OH)_{3(s)} + CO_{2(g)} + H^+ + e^- = FeCO_{3(s)} + 2H_2O_{(l)} \quad (7a)$$

$$Fe(OH)_{3(s)} + 2/3\ H_2PO_4^- + 5/3\ H^+ + e^- = 1/3\ Fe_3(PO_4)_2 \cdot 8H_2O_{(s)} + 1/3\ H_2O_{(l)} \quad (7b)$$

where the presence of biogenic CO_2 and acidic pH are assumed. These two reactions imply a pE-pH relationship with a pH coefficient between -1.0 and -2.0 (or -3.0, if HPO_4^{2-} were the reactant instead of $H_2PO_4^-$ in Eqn. 7b), whereas Fe^{2+} adsorption would imply a pH coefficient as low as -3.0, depending on the number of surface protons Fe^{2+} displaced. Linear regression analysis of our pE-pH data for both soil suspensions throughout the entire incubation period yielded the statistical relationship:

$$pE = 10.02(\pm 2.97) - 2.46(\pm 0.52)pH \quad R^2 = 0.759^{**} \quad (8)$$

whose pH coefficient is consistent with the proposed scenarios for Fe(II) release in particulate form.

Our Fe release data indicate that the biotic dissolution of Fe(III) (hydroxides in the valley soil is a complex process involving both reductive and nonreductive mechanisms, as well as secondary precipitation (and possibly adsorption) of the soluble Fe^{2+} released. The consistency among our data on soluble Fe(II), pH, and E_h for both soil suspensions suggests that AQDS served only as a catalyst to enhance the rate of reductive dissolution of Fe(III) (hydr)oxides. It is not likely that AQDS was either toxic to the microorganisms in the soil (Shyu et al., 2002) or was biodegraded, since this latter process would lead to a pH decrease, not the pH increase we observed experimentally. Moreover, a simple calculation of the dissolved CO_2 that would be produced were AQDS to be oxidized completely led to an estimate of 2.1 mmol L^{-1} for 150 μM AQDS, which is 10 times as much as was actually observed in our incubation experiments (see below, section 3.3). No significant biodegradation of AQDS also was found by Coates et al. (2002) in their study of nitrate reduction using AH_2DS as an electron donor. Thus we conclude that, in our soil incubation experiments, added AQDS functioned with the native microbial population solely as an electron shuttle catalyzing Fe(III) reduction.

3.2. Phosphorus Release

Soluble P-ICP release was also accelerated by the addition of AQDS (Fig. 4), achieving a plateau after 12 d. Soluble inorganic P (Fig. 4, bottom curve) remained very low and essentially without change throughout incubation, regardless of the presence of AQDS. A positive power-law correlation was found between soluble P-ICP and soluble Fe(II) during incubation that also was statistically independent of whether AQDS were present (Fig. 5):

$$[\text{P-ICP}] = 0.7(\pm 0.3)[Fe(II)]^{0.4\pm0.1} \quad R^2 = 0.628^{**} \quad (9)$$

where both solute concentrations are in mg kg^{-1}. Eqn. 9, an empiric correlation which subsumes a complex suite of reactions, including complexation, secondary precipitation, and sorption, as well as bioassimilation, is consistent nonetheless

Table 4. Calculated saturation indices for siderite ($FeCO_3$) and vivianite ($Fe_3(PO_4)_2 \cdot 8H_2O$) in soil suspensions incubated with 0 or 150 μM AQDS during 14 days.[a]

	0 AQDS		150 μM AQDS	
Day	$FeCO_3$	$Fe_3(PO_4)_2 \cdot 8H_2O$	$FeCO_3$	$Fe_3(PO_4)_2 \cdot 8H_2O$
3	−5.96 ± 0.15	−13.81 ± 0.69	−4.26 ± 0.52	−9.23 ± 1.11
5	−4.95 ± 0.58	−12.31 ± 1.29	−2.04 ± 0.31	−4.92 ± 0.85
7	−2.46 ± 0.66	−5.80 ± 1.31	−1.04 ± 0.37	−2.42 ± 0.73
9	−1.11 ± 0.61	−2.96 ± 1.43	*−0.03 ± 0.23*	−0.59 ± 0.53
11	−0.76 ± 0.45	−2.30 ± 1.27	*0.32 ± 0.27*	*0.08 ± 0.54*
13	*−0.53 ± 0.45*	−1.69 ± 0.83	*0.43 ± 0.14*	*0.04 ± 0.26*
14	*−0.53 ± 0.35*	−1.64 ± 0.74	*0.30 ± 0.09*	*0.17 ± 0.23*

[a] Italic indicates formation of a solid phase is thermodynamically favorable (see text).

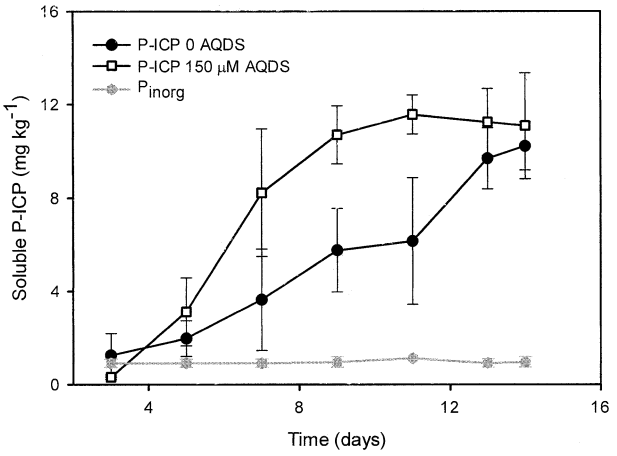

Fig. 4. Time course of soluble phosphorous accumulation during incubation in the valley soil suspensions. Error bars show standard deviations (three replicates).

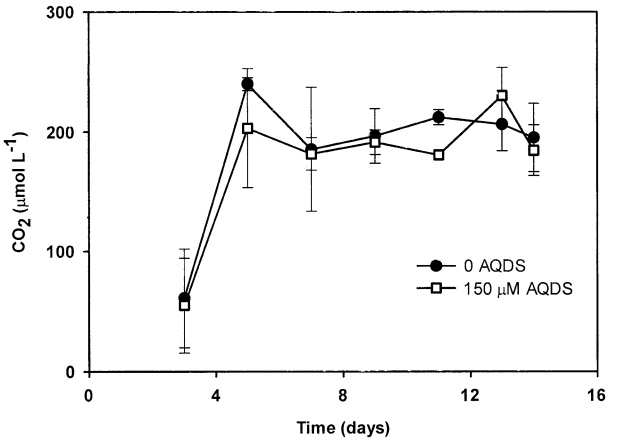

Fig. 6. Net CO_2 concentration produced by the valley soil with 0 and 150 μM AQDS as a function of incubation time. Error bars show standard deviations (three replicates).

with soluble P release linked to soluble Fe(II) production in the valley soil under anoxic conditions. Our observation of negligibly low soluble inorganic P suggests that soluble P-ICP represents mainly organic (and/or filterable colloidal) P in the valley soil.

In wetlands soils, for which P is also the most limiting nutrient, the principal biogeochemical processes associated with P solubilization are (Kirk, 2004): (1) reduction of Fe(III) minerals binding P in sorbed or occluded forms; (2) displacement of sorbed P by solubilized organic ligands; (3) complexation of solubilized metals (e.g., Fe and Al) by these organic ligands, thereby preventing the metals from precipitating P; (3) release of organic P (attributable to some extent to a lysing soil microbial biomass following soil rewetting after air drying; Turner and Haygarth, 2001). Phosphorus solubilized by these processes may be subsequently immobilized by sorption onto poorly crystalline Fe(II) minerals formed after reduction of Fe(III) or by precipitation of vivianite (Kirk, 2004).

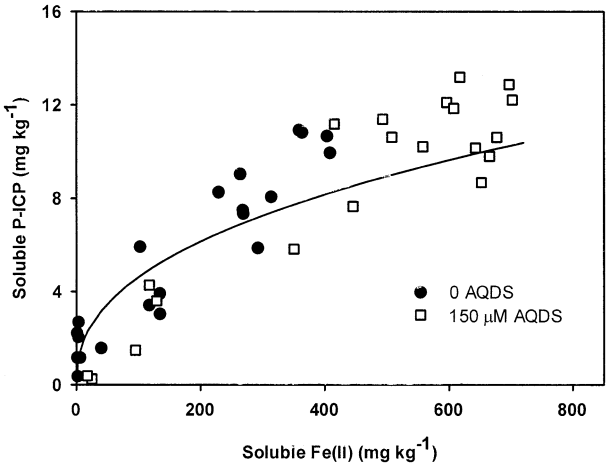

Fig. 5. Graph of soluble P-ICP concentration vs. soluble Fe(II) concentration measured in the valley soil suspensions during incubation. The power-law regression through the data are Eqn. 9.

The valley soil we studied was collected from an area receiving 3000–4000 mm annual precipitation (Silver et al., 1994) and, being located at the bottom of a toposequence, it received its most important water inputs from runoff. There is a doubling of organic matter content in going from the ridge to the valley soils (45 to 92 g kg^{-1}) that can be attributed to slow turnover, as well as to inputs from runoff and deposition from streams. Therefore, the release of mainly soluble organic P during the valley soil incubation (Fig. 4) can be related circumstantially to the significant organic P accumulation expected in a soil at the bottom of a toposequence under a wet moisture regime (Miller et al., 2001). Our data on extractable P (Table 1) further suggest that this organic P is associated with Fe(III) in the valley soil.

3.3. Biogenic Gas Production

Rising CO_2 concentration occurred during the first 5 d of incubation, increasing from 50 to ~200 μmol L^{-1} (Fig. 6), after which the concentration remained essentially constant within the precision of our measurements. Anaerobic oxidation of organic matter, which occurs when pE drops below −4 (before the third day of incubation in our experiments), is an important cause of this CO_2 production (Yao et al., 1999; Kirk, 2004). For example, laboratory studies with amorphous Fe(III) (hydr)oxides and fermenting bacteria (Benz et al., 1998) show that these latter microorganisms are able to reduce added humic acid, leading to a significant shift in the fermentation pattern toward more oxidized products. In our experiments, net CO_2 production was insensitive to AQDS addition (Fig. 6). In addition, the net CO_2 produced represents only ~0.2% of the total organic C stored in the valley soil (Table 1), suggesting that only a small fraction of it was available to microorganisms during our experiments, and/or that microbial C oxidation was significantly inhibited by prior soil sieving and air-drying, and/or that an effective CO_2 sink existed in the soil. Thermodynamic calculations (data not shown) based on Eqn. 7a were roughly consistent with the CO_2 concentrations observed, supporting the possibility that siderite formation may have been an

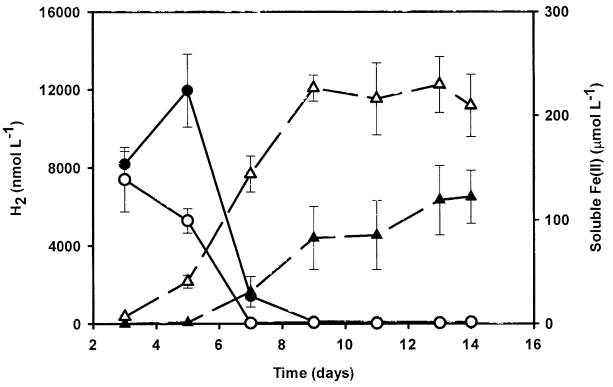

Fig. 7. Time course of H_2 gas (circles, left vertical axis) and soluble Fe(II) (triangles, right vertical axis) produced in the valley soil suspension with either 0 (filled symbols) or 150 μM (open symbols) AQDS. Error bars show standard deviations (three replicates).

important sink for CO_2. A similar finding was reported by Yao et al. (1999) for incubated rice paddy soils.

Hydrogen gas produced during fermentation is consumed by microorganisms using NO_3^-, Mn(IV), Fe(III), SO_4^{2-} or CO_2 as terminal electron acceptors, with the steady-state concentration being highest for methane production and lowest when Fe(III) reduction occurs (Chapelle, 2001; Kirk, 2004). In the case of Fe(III) reduction, the steady-state H_2 concentration can range from 0.2–0.8 nmol L^{-1} (Chapelle et al., 1997) up to 40–200 nmol L^{-1} (found for iron-reducing methanogenic strains in the presence of Fe(III) (hydr)oxide; Bond and Lovley, 2002), whereas for methane production the H_2 concentration is in the range 5–30 nmol L^{-1} to 1–4 μmol L^{-1}, as also confirmed by Yao et al. (1999) in their study of methanogenesis in 16 rice paddy soils. In our experiments, over the initial 7–9 d of incubation, the H_2 gas concentration decreased sharply to 20–40 nmol L^{-1} while an increase in soluble Fe(II) occurred (Fig. 7), indicating that Fe(III) reduction had become an important terminal electron-accepting process. Because a number of different microorganisms are capable of oxidizing H_2 coupled to the reduction of AQDS (Cervantes et al., 2000), it is not surprising that H_2 concentrations dropped more rapidly, and soluble Fe(II) increased more quickly, in the presence of AQDS (Fig. 7).

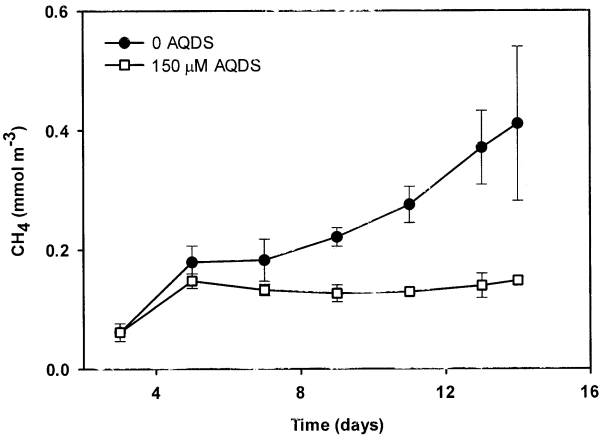

Fig. 8. Net CH_4 concentration produced by the valley soil with 0 and 150 μM AQDS as a function of incubation time. Error bars show standard deviations (three replicates).

As also shown by Yao et al. (1999), in the absence of AQDS, CH_4 production was initiated when CO_2 production had stabilized (Figs. 6 and 8). Introduction of AQDS suppressed CH_4 production (Fig. 8), in agreement with the results of Cervantes et al. (2000). However, an approximate electron-balance calculation indicated that this diversion of electrons from methane production could not account fully for the increase in Fe(II) production. For example, the decrease of electron equivalents calculated from the difference in CH_4 concentrations (2.1 $\pm$ 0.7 μmol L^{-1}) is much lower than the electron equivalents consumed in Fe(III) reduction (88 $\pm$ 28 μmol L^{-1}). Thus, production of Fe(II) was the result of H_2 consumption and organic C oxidation by a variety of different native microorganisms in the valley soil.

4. CONCLUSIONS

Our laboratory incubation experiments showed that anoxic conditions caused significant Fe(II) production in an Ultisol sampled from a riparian valley in a humid tropical forest in Puerto Rico. The Fe(II) produced over time scales of days to weeks accumulated mainly in particulate form, evidently due to adsorption processes and the formation of Fe(II) solid phases, with siderite and vivianite being likely candidates for the latter according to our thermodynamic calculations. Significant release of soluble P also occurred, mainly in organic form, an observation that is consistent with the accumulation of humus and organic P in highly weathered forest soils under wet moisture regimes (Miller et al., 2001).

A positive power-law correlation was found between soluble P release and soluble Fe(II) production, consistent with the idea that reduction of Fe(III) in the soil served to solubilize phosphorus, possibly through desorption processes mediated by subsequent bioassimilation, precipitation, and resorption. Similar Fe(III) redox-mediated P solubilization is observed in wetlands soils (Kirk, 2004) and has been reported also for As(V) solubilization in Bengal delta sediments subject to fluctuating oxic-anoxic conditions (Islam et al., 2004).

Hydrogen gas, a product of fermentation processes in anoxic soil, dropped sharply, while both soluble Fe(II) and CH_4 increased correspondingly, indicating the presence of a variety of H_2-consuming microorganisms in the valley soil. Carbon dioxide rose sharply only during the first few days of incubation, then fluctuated narrowly around a constant level that was consistent with the formation of siderite. Thus Fe(III) (hydr)oxide reductive dissolution served as a terminal electron accepting process coupled to both H_2 consumption and organic C oxidation in the soil.

Addition of AQDS, a synthetic electron shuttle compound, accelerated the production of Fe(II) and soluble P, while hastening the decline in H_2 gas levels and suppressing CH_4 production. These results indicate that microorganisms capable of utilizing "electron shuttle" compounds exist in the valley soil and, when electron transfer is suitably catalyzed. Our finding that the linear correlation relationship between pE and pH during soil incubation is the same irrespective of the presence of AQDS is strong evidence that this synthetic electron shuttle

served only a catalytic role. However, we cannot conclude that humic substances in the valley soil also serve as electron shuttles in Fe(III) (hydr)oxide reductive dissolution. Further investigation is required to study the catalytic role of humic substances in Fe(II) production.

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ELECTRONIC ANNEX

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.gca.2005.03.045.