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# Short-term Response of Soil Iron to Nitrate Addition

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The inhibition of soil Fe(III) reduction by fertilizer  $\text{NO}_3^-$  applications is complex and not completely understood. This inhibition is important to study because of the potential impact on soil physicochemical properties. We investigated the effect of adding  $\text{NO}_3^-$  to a moderately well-drained agricultural soil (Sadler silt loam) under Fe(III)-reducing (anoxic) conditions. Stirred-batch experiments were conducted where  $\text{NO}_3^-$  was added (0.05 and 1 mM) to anoxic slurries and changes in dissolved Fe(II) and Fe(III), oxalate-extractable Fe(II), and dissolved  $\text{NO}_3^-$  were monitored as a function of time. Addition of 1 mM  $\text{NO}_3^-$  inhibited Fe(II) production sharply with reaction time, from 10% after 1 h to 85% after 24 h. The duration of inhibition in Fe(II) production was closely related to the presence of available  $\text{NO}_3^-$ , suggesting preferential use of  $\text{NO}_3^-$  by nitrate reductase enzyme. Active nitrate reductase was confirmed by the fivefold decline in  $\text{NO}_3^-$  reduction rates in the presence of tungstate ( $\text{WO}_4^{2-}$ ), a well-known inhibitor of nitrate reductase. In addition,  $\text{NO}_3^-$ -dependent Fe(II) oxidation was observed to contribute to the inhibition in Fe(II) production. This finding was attributed to a combination of chemical reoxidation of Fe(II) by  $\text{NO}_2^-$  and  $\text{NO}_3^-$ -dependent Fe(II) oxidation by autotrophic bacteria. These two processes became more important at a greater initial oxalate-Fe(II)/ $\text{NO}_3^-$  concentration ratio. The inhibitory effects in Fe(II) production were short-term in the sense that once  $\text{NO}_3^-$  was depleted, Fe(II) production resumed. These results underscore the complexity of the coupled N-Fe redox system in soils.

Abbreviations: AAS, atomic absorption spectrometry; HIV, hydroxy-interlayered vermiculite.

In oxic soil environments, Fe(III) is present in Fe(III) (hydr)oxide minerals or phyllosilicate clays. The importance of microbial Fe(III) reduction to Fe(II) is in part due to its impact on soil physicochemical properties (Stucki et al., 1996; Favre et al., 2002) and its role in organic C oxidation (Lovley, 2000). The reduction of Fe(III) can be inhibited by the presence of  $\text{NO}_3^-$ . This inhibition alters the speciation of transition metals such as Zn that adsorb to Fe(III) (hydr)oxide surfaces (Cooper et al., 2000). Four hypotheses have been proposed to explain the inhibitory mechanism of  $\text{NO}_3^-$  on Fe(III) reduction. The first hypothesis is based on thermodynamic arguments. The classical model of terminal electron-accepting processes that occur during microbial degradation of organic C predicts a sequence of oxidant consumption to follow the order of  $\text{O}_2$  reduction,  $\text{NO}_3^-$  reduction,  $\text{MnO}_2$  reduction, Fe(III) (hydr)oxide reduction,  $\text{SO}_4^{2-}$  reduction, and  $\text{CH}_4$  production (McBride, 1994). Nitrate, due to its position on the redox ladder, increases the redox potential ( $E_H$ ) beyond where Fe(III) reduction occurs ( $E_H = 200\text{--}400$  mV), thereby preventing Fe(II) release (Ponnamperuma and Castro, 1964; Ponnamperuma, 1972). This view appears to be an oversimplification. It assumes that the more thermodynamically favorable reduction of  $\text{NO}_3^-$  excludes Fe(III) reduction, despite favorable thermodynamics in the latter reaction and variable reactivities of Fe(III) (hydr)oxide minerals (Roden, 2003). Furthermore, Munch and Ottow (1980) have pointed out that the activity of

microbial reductases are more important than  $E_H$  in governing Fe(III) reduction.

The second hypothesis proposed by Ottow (1970) claimed that nitrate reductases, enzymes produced by bacteria to catalyze heterotrophic reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$ , preferentially transfer electrons to  $\text{NO}_3^-$  rather than Fe(III). Sorensen (1982) suggested that nitrate reductase inhibited Fe(III) reduction because treatment of sediment slurries with 0.2 mM  $\text{NO}_3^-$  halted Fe(II) accumulation. After the  $\text{NO}_3^-$  supply was depleted, Fe(III) reduction resumed. Munch and Ottow (1980) pointed out that the presence of nitrate reductase is what leads to suppressed Fe(II) formation by  $\text{NO}_3^-$  under anoxic conditions. Nitrate reductases contain Mo in their active site that cycles between +4 ( $d^2$ ) and +6 ( $d^0$ ) oxidation states during reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  (Hille, 1996). Addition of  $\text{WO}_4^{2-}$  inactivates the enzyme by substitution for Mo (Scott et al., 1979; Smith, 1983). It may be possible to verify this hypothesis by adding  $\text{WO}_4^{2-}$  to soil slurries. To our knowledge, the effect of  $\text{WO}_4^{2-}$  on Fe(III) reduction is not known.

The third hypothesis involves concomitant reduction of  $\text{NO}_3^-$  and Fe(III) coupled to chemical reoxidation of Fe(II) by  $\text{NO}_2^-$ . In laboratory incubations of field soils, aquifer materials, and pure cultures, reduction of  $\text{NO}_3^-$  by nitrate reductase occurs simultaneously with Fe(III) reduction (Komatsu et al., 1978; Obuekwe et al., 1981; DiChristina, 1992; Broholm et al., 2000; Cooper et al., 2003). Under the scenario of concomitant Fe(III) and  $\text{NO}_3^-$  reduction, intermediates generated such as  $\text{NO}_2^-$  can transiently accumulate and chemically oxidize Fe(II) to Fe(III), leading to no net Fe(III) reduction (Lovley, 2000). This process has been proposed to occur in agricultural soil slurries and pure cultures (Komatsu et al., 1978; Obuekwe et al., 1981). However, DiChristina (1992) observed that neither  $\text{NO}_3^-$  nor  $\text{NO}_2^-$  behaved as chemical oxidants of Fe(II), but the inhibition was due to  $\text{NO}_2^-$  blocking electron transport to

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Fe(III) in pure cultures of *Shewanella putrefaciens*. The latter study was performed in the absence of mineral surfaces and Fe(III) salts were used as a source of Fe. In soil environments, it is conceivable that Fe(II) generated from Fe(III) reduction coordinates to mineral surfaces in an inner sphere complex or reprecipitates as a secondary Fe(II) mineral phase (Fredrickson et al., 1998; Zachara et al., 2002). The Fe(II) in these coordination environments, where O is the ligating atom, could be more reactive toward  $\text{NO}_2^-$  because the reduction potential is lowered for the Fe(III)–Fe(II) half-reaction (Luther et al., 1992). In fact, Sorensen and Thorling (1991) found that Fe(II) adsorbed on lepidocrocite [ $\gamma\text{-FeOOH}_{(s)}$ ] reduced  $\text{NO}_2^-$  much more rapidly than dissolved, hexaquo Fe(II).

The fourth hypothesis involves autotrophic bacteria that can reduce  $\text{NO}_3^-$  to  $\text{N}_2$  with concomitant Fe(II) oxidation. These bacteria have been identified in European freshwater sediments (Straub et al., 1996), activated sludge treatment plants (Nielsen and Nielsen, 1998), urban lake sediments (Senn and Hemond, 2002), and anoxic rice paddy soil slurries (Klüber and Conrad, 1998). Dissolved Fe(II) and solid Fe(II) in minerals such as siderite [ $\text{FeCO}_{3(s)}$ ] and magnetite [ $\text{Fe}_3\text{O}_{4(s)}$ ] are capable of being used by bacteria during  $\text{NO}_3^-$  reduction (Straub et al., 1996; Weber et al., 2001). In addition, Fe(II) associated with phyllosilicate minerals has emerged as a suitable electron donor for autotrophs during  $\text{NO}_3^-$  reduction (Shelobolina et al., 2003).

Most of these studies evaluating the inhibitory effect of  $\text{NO}_3^-$  on Fe(III) reduction used pure cultures and synthetically prepared Fe(III) minerals or Fe(III) salts. This is due to the complexities associated with soil, where the inhibitory effect may be controlled in complex ways by these various processes that may compete with one another. The present study was undertaken to analyze the effect of  $\text{NO}_3^-$  addition on Fe(II) production in field soil subjected to anoxic [Fe(III)-reducing] conditions using stirred-batch experiments in the laboratory. Specifically, the objectives were to determine whether the effect of  $\text{NO}_3^-$  was due to: (i) competitive inhibition by nitrate reductase; (ii) concomitant reduction of Fe(III) and  $\text{NO}_3^-$  reduction linked to chemical reoxidation of biogenic Fe(II) by  $\text{NO}_2^-$ ; or (iii)  $\text{NO}_3^-$ -dependent Fe(II)-oxidizing autotrophic bacteria. Changes in different pools of Fe [dissolved Fe(II), oxalate-extractable Fe(II), and dissolved Fe(III)] and dissolved  $\text{NO}_3^-$  were monitored as a function of time and compared with controls (no  $\text{NO}_3^-$ ). This will provide insight into the relative importance of the hypothetical pathways enumerated above. If the nitrate reductase competitive model is the main mechanism for inhibiting Fe(II) production, then addition of  $\text{WO}_4^{2-}$  should curtail the inhibition. Tungstate-treated soil slurries would also allow evaluation of the contribution of autotrophic Fe(II) oxidation coupled to  $\text{NO}_3^-$  reduction. This work will provide a better understanding of what happens when  $\text{NO}_3^-$  is surface applied as N fertilizer under Fe(III)-reducing conditions.

## MATERIALS AND METHODS

### Soil Collection

A Sadler silt loam (fine-silty, mixed, mesic Glossic Fragiudalf) was collected from the surface (0–5 cm) Ap horizon of a field site in Christian County, Kentucky. The soil developed

in loess over sandstone parent materials and is moderately well drained (Karathanasis, 1987). Soil samples were taken with a cylindrical probe 2.0 cm in diameter from multiple soil cores (8–10) and placed in polyethylene bags. Preliminary extractions using oxalate and ferrozine revealed that Fe(II) was present at the time of sampling. Thus, the soil samples were immediately quick-frozen in the field using liquid  $\text{N}_2$  to preserve the Fe(II) oxidation state and placed on dry ice during transport back to the laboratory. Samples were thawed, homogenized, carefully sieved to <2 mm, and stored under Ar in an  $\text{O}_2$ -free glove box (Coy Laboratory Products, Grass Lake, MI).

### Soil Characterization

Part of the soil was removed from the anoxic chamber for characterization. Soil was separated into the following soil particle size separates: sand (2000–50  $\mu\text{m}$ ), silt (50–2  $\mu\text{m}$ ), and clay (<2  $\mu\text{m}$ ). Fractionation procedures followed those of Jackson (1974), using  $\text{NaHCO}_3$  to disperse the soil and omitting chemical pretreatment steps. Chemical extractions were performed in triplicate on the whole soil and particle size fractions to quantify poorly crystalline Fe(III) (hydr)oxides (acid ammonium oxalate extraction, Phillips and Lovley, 1987), and total reducible Fe(III) (hydr)oxides (dithionite–citrate–bicarbonate extraction, Mehra and Jackson, 1960). Total Fe was measured in the extracts using atomic absorption spectrometry (AAS) (Shimadzu AA-6800, Kyoto, Japan). In addition, total Mn and Al were measured in the same extracts using AAS. Soil organic matter content was determined by loss-on-ignition (Nelson and Sommers, 1996) and total soil N by the Kjeldahl method (Bremner, 1996). Soil pH was determined in a 1:1 soil/solution ratio using deionized water and a pH meter (Metrohm 744, Herisau, Switzerland) with a combination glass electrode. Water extracts were collected to determine native concentrations of important anions ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{PO}_4^{3-}$ ) and cations (total Fe and total Mn). Results of chemical analyses were calculated on a dry-weight basis.

X-ray diffraction patterns were measured using a Siemens D-500 diffractometer with Cu  $\text{K}\alpha$  radiation and a current of 30 milliamps (Bruker AXS, Madison, WI). Intensities were recorded with a 0.05° step width and 1-s counting time per step. The powder method was used for the silt fraction and the scan range was 2 to 90° 2 $\theta$ . Oriented slides were prepared using clay subsamples saturated with Mg, K, and Mg glycerol solvated and scanned from 2 to 30° 2 $\theta$ . Cation exchange capacity was determined for the <2- $\mu\text{m}$  fraction using Ca–Mg exchange.

A subsample of the <2- $\mu\text{m}$  size fraction was examined using a Hitachi S-3200 scanning electron microscope equipped with a Noran Voyager energy dispersive x-ray spectrometer (Thermo Electron Corp., Waltham, MA) to better understand the chemical nature of Fe in the untreated clay fraction. The sample was prepared by sprinkling it on a C-covered sample stub attached to an Al holder that was sputter coated with Au–Pd.

### Anoxic Stirred-Batch Experiments

Anoxic conditions were chosen to eliminate  $\text{O}_2$  as a potential electron acceptor of Fe(II). All sample handling was conducted under an Ar atmosphere in a glove box to ensure anoxic conditions. Deionized water was purged for 3 h with high-purity Ar gas and transferred to the glove box for use in stirred-batch

experiments and preparation of solutions. Dissolved  $\text{O}_2$  concentrations of Ar-deoxygenated water were measured periodically with a Clark-type polarographic electrode (Warner Instruments, Hamden, CT). Values ranged from 0.5 to 2  $\mu\text{mol L}^{-1}$ , verifying anoxic conditions.

The <2-mm size fraction of the Sadler soil was used in five treatments (Table 1) of anoxic stirred-batch experiments. Experiments were prepared by adding 2 g of Sadler soil to a borosilicate glass serum bottle, after which 20 mL of Ar-deoxygenated water was added. The bottles were sealed with a rubber septum and allowed to prehydrate overnight. The reduction of  $\text{NO}_3^-$  was initiated by adding an aliquot of 1 M  $\text{NO}_3^-$  stock solution (prepared from a  $\text{NaNO}_3$  salt) to a stirred soil suspension to achieve initial  $\text{NO}_3^-$  concentrations of 1000 and 50  $\mu\text{mol L}^{-1}$ , corresponding to approximately 12 and 0.5  $\text{mmol kg}^{-1}$  air-dried soil. These treatments are referred to as *high N* and *low N* (Table 1). Stirring was achieved using isotherm magnetic stir plates (Fisher Scientific, Hampton, NH) set to 350 rpm and experiments were conducted at room temperature (23–25°C). Suspension aliquots (1–2 mL) were withdrawn at 0.02, 1, 2, 4, 6, 8, and 24 h for high-N treatments and 0.02, 0.08, 0.17, 0.25, 0.5, 1, 1.5, 2, and 4 h for the low-N treatment. Sampling times for soil oxalate Fe(II) extractions were 0.02, 1, 4, and 24 h. Redox potential and pH were measured in soil slurries at 0, 4, and 24 h of reaction time.

We also studied the effect of adding  $\text{WO}_4^{2-}$  to inhibit production of active nitrate reductase (Scott et al., 1979; Smith, 1983). The purpose of inhibiting nitrate reductase was twofold: to evaluate contributions of the nitrate reductase competitive pathway and of the autotrophic pathway to inhibiting Fe(II) production. This treatment (high N–W) was identical to high N except Ar-deoxygenated water was added to the soil with 10 mM  $\text{Na}_2\text{WO}_4$  (Table 1) and allowed to prehydrate overnight. Sampling times for high N–W were modified slightly because of slower  $\text{NO}_3^-$  reduction kinetics (see results below). To account for abiotic reactions, anoxic soil slurries were sterilized by autoclaving at 120°C (~138 kPa) for 30 min as described elsewhere (Tratnyek and Wolfe, 1993). Bottles were returned to the anoxic chamber and allowed to cool to room temperature before addition of 1000  $\mu\text{mol L}^{-1}$   $\text{NO}_3^-$ . The sterile treatment (high N–sterile) was otherwise treated identically to high N.

Control experiments (no added  $\text{NO}_3^-$ , Table 1) were reacted simultaneously with each series of treatments (high N, low N, high N–W, high N–sterile) to examine treatment-induced changes in Fe(II) and other soil properties (pH and  $E_H$ ). When making comparisons among treatment controls, the controls were referred to as high-N control and high-N–W control, for example.

Additional experiments were conducted under anoxic conditions to include a range of initial  $\text{NO}_3^-$  concentrations (50, 100, 500, and 1000  $\mu\text{mol L}^{-1}$ ). Initial rates of  $\text{NO}_3^-$  removal

were followed and fit to the Michaelis–Menten expression using nonlinear regression:

$$v = \frac{V_{\max} [\text{NO}_3^-]}{K_m + [\text{NO}_3^-]} \quad [1]$$

where  $v$  is the initial rate of  $\text{NO}_3^-$  removal ( $\text{mmol L}^{-1} \text{min}^{-1}$ ),  $V_{\max}$  is the estimated maximum rate ( $\text{mmol L}^{-1} \text{min}^{-1}$ ), and  $K_m$  is the affinity constant for the substrate ( $\mu\text{mol L}^{-1}$ ). The  $K_m$  value corresponds to the  $\text{NO}_3^-$  concentration at which the rate reaches half the maximum rate. Thus, a smaller  $K_m$  represents a stronger affinity between enzyme and substrate.

## Chemical Analysis of Stirred-Batch Samples

Slurry aliquots were passed through a 0.2- $\mu\text{m}$  membrane filter. Filtrates were immediately mixed with ferrozine [3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4', 4''-disulfonic acid monosodium salt] buffered to pH 5.5 with MES [2-(*N*-morpholino)ethanesulfonic acid monohydrate] to complex dissolved Fe(II). Absorbance spectra of the Fe(II)–ferrozine complex were recorded at 562 nm (Stookey, 1970) in Suprasil quartz cuvettes (Heraeus Optics, Buford, GA) with a 1-cm optical pathlength at 24°C on a double-beam Shimadzu UV-3101PC spectrophotometer (Kyoto, Japan) against appropriate standards. The estimated method detection limit is 0.5  $\mu\text{mol L}^{-1}$  in a 1-cm cell. Total Fe concentrations in ferrozine solutions were measured using AAS. The difference between total Fe and Fe(II) was used to estimate dissolved Fe(III). Total Mn concentrations in the filtrates were measured using AAS.

Nitrate and  $\text{NO}_2^-$  in filtrates were quantified by ion chromatography with conductivity detection using a Metrohm 792 Basic IC with a MetroSep A column and MetroSep RP guard disc holder and a 3.2  $\text{mmol L}^{-1}$   $\text{Na}_2\text{CO}_3$ /1  $\text{mmol L}^{-1}$   $\text{HCO}_3^-$  eluent (Metrohm, Houston, TX). Typical retention times for  $\text{NO}_2^-$  and  $\text{NO}_3^-$  were 6 and 9 min. In some cases, orthophosphate and  $\text{SO}_4^{2-}$  were quantified at 11.6 and 13.1 min, respectively. The indophenol blue method was used for quantification of  $\text{NH}_4^+$  on the remaining sample filtrates uncomplexed with ferrozine (Weatherburn, 1967). The redox potential was measured with a Metrohm combination Pt electrode with an Ag–AgCl reference electrode and corrected to the standard H electrode by adding 199 mV (Patrick et al., 1996). Total alkalinity in water extracts of control bottles was determined by titration with standardized 0.05 M HCl.

The acid ammonium oxalate extraction (0.2 M at pH 3) described by Phillips and Lovley (1987) was used to extract solid-phase Fe(II). The method targets reduced Fe(II) phases such as magnetite [ $\text{Fe}_3\text{O}_{4(s)}$ ] and siderite [ $\text{FeCO}_{3(s)}$ ] and poorly crystalline Fe(III) minerals (Van Bodegom et al., 2003). An oxalate extraction would

also remove adsorbed Fe(II). Soil slurry aliquots, between 0.1 and 0.5 mL, were pipetted into 20-mL glass serum bottles and combined with oxalate. Bottles were sealed with butyl rubber stoppers and Al crimp caps and shaken for 24 h in the dark. Bottles

**Table 1. Description of the treatments imposed for anoxic stirred-batch experiments.**

Objective	Treatment designation	Description
Follow $\text{NO}_3^-$ reduction and Fe(II) consumption	high N	1 mM $\text{NO}_3^-$ + anoxic soil
Effect of lowering $\text{NO}_3^-$ conc.	low N	0.005 mM $\text{NO}_3^-$ + anoxic soil
Inhibit $\text{NO}_3^-$ reductase	high N–W	1 mM $\text{NO}_3^-$ + anoxic soil + 10 mM $\text{WO}_4^{2-}$
Account for abiotic reactions	high N–sterile	1 mM $\text{NO}_3^-$ + autoclaved anoxic soil
Measure Fe(II) in absence of $\text{NO}_3^-$	control	no added $\text{NO}_3^-$



were decapped and suspensions were filtered through 0.2- $\mu\text{m}$  membrane filters. An aliquot of filtrate was combined with ferrozine and pH 5.5 MES buffer and Fe(II) was quantified as described above. Because the percentage change in Fe(III) during the experiments was small compared with the total Fe(III) already present in the soil (see below), it was decided to express the values as Fe(II) to avoid uncertainty in the data. The inhibition percentage of Fe(II) production was calculated as described in DiChristina (1992):

$$\text{inhibition percentage} = \left\{ 1 - \left[ \frac{\text{Fe(II)}_{\text{treatment}}}{\text{Fe(II)}_{\text{control}}} \right] \right\} 100 \quad [2]$$

The sum of dissolved Fe(II) and oxalate-extractable Fe(II) concentrations for each treatment and its control represented the values for  $\text{Fe(II)}_{\text{treatment}}$  and  $\text{Fe(II)}_{\text{control}}$ , respectively.

Geochemical modeling was used to assess possible Fe(II) solid phases such as siderite and magnetite that may form by computing saturation indices [SI =  $\log(\text{ion activity product/solubility product constant})$ ] with MINEQL+ (Schecher, 1998). Precipitation is favored when SI > 0, indicating that solutions are supersaturated with respect to the mineral. SI values < 0 indicate that solutions are undersaturated with respect to precipitation of the mineral.

## RESULTS

### Untreated Soil Characteristics

The Sadler soil contains 22% sand, 67% silt, and 11% clay. The soil had an initial pH of 7.1 and total organic C and N values of 13 and 1.2 g  $\text{kg}^{-1}$ . Initial  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  concentrations in water extracts were  $11 \pm 4$  and  $17 \pm 5 \mu\text{M}$ , respectively. The whole soil, silt, and clay fractions contained oxalate-extractable Fe/Na dithionite–citrate–bicarbonate (DCB)-extractable Fe ratios of 0.33, 0.32, and 0.47 (Table 2). This suggests the presence of crystalline Fe(III) oxides (Schwertmann and Cornell, 1991); however, these extractants will also chemically reduce structural Fe(III) from phyllosilicate minerals (Roth et al., 1969; Komadel et al., 1990). Quartz was the predominant mineral identified in the silt fraction, with a trace of feldspar, based on x-ray diffraction (XRD) analysis. The clay fraction, which accounted for only 11% of the dry weight of the soil, contributed 36 and 56% of the total oxalate- and DCB-extractable Fe (Table 2). Thus, the chemistry and mineralogy of this fraction was studied further.

Mineralogy of the clay (<2- $\mu\text{m}$ ) fraction was mixed and dominated by hydroxy-interlayered vermiculite (HIV) and kaolinite. The HIV was identified in the Mg-saturated sample by the 1.3-nm XRD peak, which failed to expand on glycerol treatment and collapsed to 1.0 nm in the K-saturated sample at 550°C (Fig. 1a). The presence of kaolinite was confirmed by the disappearance of the 0.7-nm peak at 550°C in the K-saturated sample. Quartz was present based on diagnostic d-spacings at 0.33 and 0.42 nm (not shown). The measured cation exchange capacity of the clay fraction was  $39 \pm 11 \text{ cmol}_c \text{ kg}^{-1}$ , consistent with low-charge kaolinite and steric block-

age of interlayer charge sites by the hydroxyl interlayer in HIV (Barnhisel and Bertsch, 1989). Representative scanning electron microscope (SEM) micrographs of the clay size fraction showed that Fe was associated with phyllosilicate minerals. Particles with platy morphology and diameters that were 2 to 3  $\mu\text{m}$  were common and typified by relative Si/Al ratios of 1.75 (Fig. 1b). These findings, in combination with the considerable oxalate-extractable Al (Table 2), are consistent with HIV containing Al interlayers. A peak at 6.4 keV in the energy dispersive spectra was identified as the  $\text{K}\alpha$  feature of Fe. Iron could be present as a structural cation in the octahedral sheet of HIV, in agreement with published average structural chemical formulas for HIV (Barnhisel and Bertsch, 1989). Discrete Fe(III) (oxy)hydroxides were not detected by XRD or SEM in the clay fraction. This suggests that they are either absent or below detection limits.

### Anoxic Control Experiments

The reduction of Fe(III) in high-N control soil slurries was evident based on Fe(II) accumulation in the dissolved and oxalate-extractable pools (Fig. 2a and 2b). The concentration of oxalate-extractable Fe(II) produced was only a small fraction of the total oxalate-extractable Fe ( $\text{Fe}_{\text{ox}}$  in Table 2), indicating that a majority of the Fe still remained as Fe(III). The measured redox potential ( $E_{\text{H}}$ ) in control bottles for both high N and high N–W verified that conditions were Fe(III) reducing (Table 3). The rise in soil pH is consistent with bioreduction of soil Fe(III) (Table 3) due to proton consumption (Munch and Ottow, 1980).

In general, Fe(II) production in the oxalate-extractable fraction was at least 10 times greater than the dissolved Fe(II) fraction (Fig. 2a,b). This suggests that as Fe(III) reduction proceeds, the released Fe(II) reprecipitates as solid Fe(II) minerals (Fredrickson et al., 1998; Zachara et al., 2002) or resorbs to the soil (Kukkadapu et al., 2001). Regarding the former scenario, thermodynamic modeling indicated that solutions were supersaturated with respect to siderite precipitation based on SI values > 1 in all treatments and controls (Table 3). Siderite is a common secondary product of crystalline and poorly crystalline Fe(III) (hydr)oxide reduction (Fredrickson et al., 1998; Lovley, 2000; Zachara et al., 2002). In our calculations, it was assumed that the carbonate required for  $\text{FeCO}_3(\text{s})$  precipitation originated from the alkalinity generated (as measured by titration in control bottles) during microbial decomposition of organic C coupled to dissimilatory Fe(III) reduction under anoxic conditions (Suess, 1979). As pointed out by Zachara

**Table 2. Chemical characterization of the Sadler silt loam soil and size fraction†.**

Fraction	Fe <sub>DCB</sub>	Mn <sub>DCB</sub>	Al <sub>DCB</sub>	Fe <sub>ox</sub>	Mn <sub>ox</sub>	Al <sub>ox</sub>
	mmol $\text{kg}^{-1}$					
Whole soil	157 (6.4)‡	15.0 (2)	52.3 (1)	53.2 (4)	12.2 (1.2)	ND§
Sand	20.9 (12.6)	2.95 (2.4)	8.2 (4.4)	9.9 (2.4)	1.28 (0.4)	6.4 (0.7)
Silt	25.7 (2.3)	1.81 (0.1)	21.6 (3.6)	38.2 (32.2)	2.35 (0.2)	11.4 (9.4)
Clay	58.7 (0.1)	3.43 (0.2)	21.7 (1.2)	26.5 (5.6)	10.2 (3.3)	74.0 (6.7)

† Fe<sub>DCB</sub>, Al<sub>DCB</sub>, and Mn<sub>DCB</sub> (dithionite–citrate–bicarbonate-extractable Fe, Al, and Mn, respectively) were extracted according to Mehra and Jackson (1960); Al<sub>ox</sub>, Fe<sub>ox</sub>, and Mn<sub>ox</sub> (oxalate-extractable Fe, Al, and Mn, respectively) extracted according to Phillips and Lovley (1987).

‡ Values in parentheses represent one standard deviation from the mean.

§ ND = not determined.

et al. (2002), siderite precipitation can occur biologically and abiologically, with the latter possibly occurring at a faster rate.

A factor that could potentially inhibit Fe(II) production is the presence of Mn(III,IV) oxide minerals (Table 2). Manganese(III,IV) oxide minerals that persist under the reducing conditions encountered here in a state of redox disequilibrium could react with Fe(II), producing Fe(III) (Hyacinthe et al., 2001). If this occurred, then an increase in dissolved Mn and a decrease in dissolved Fe(II) would be expected. Total dissolved Mn in control bottles remained constant (data not shown) and dissolved Fe(II) increased (Fig. 2a), ruling out this possibility.

### Impact of Nitrate on Iron(II) Production

Results of the high-N experiments are shown in Fig. 2. The addition of  $1000 \mu\text{mol L}^{-1} \text{NO}_3^-$  ( $\sim 12.5 \text{ mmol kg}^{-1}$ ) inhibited Fe(II) production in both the oxalate-extractable and dissolved fractions (Fig. 2a and 2b). The inhibitory effects increased

sharply with time, from 10% after 1 h to 85% after 24 h (Table 4). Dissolved Fe(III) production was accelerated in the high-N treatments, generally greater than the control with an apparent plateau reached after  $\sim 6$  h (Fig. 2c). In contrast, there was a small but noticeable decrease in dissolved Fe(III) in the control bottles, corresponding to conditions that became more reducing during the experiments (Table 3).

The inhibition of Fe(II) production coincided with reduction of  $\text{NO}_3^-$ , which was reduced at a rate of  $0.68 (\pm 0.06) \text{ mmol kg}^{-1} \text{ h}^{-1}$  ( $R^2 = 0.99$ ,  $p < 0.001$ ; Fig. 2d). No  $\text{NO}_2^-$  was detected as an intermediate and only slight increases in  $\text{NH}_4^+$  were observed, accounting for only  $\sim 5\%$  of the reduced  $\text{NO}_3^-$  (data not shown).

The predominantly biological nature of  $\text{NO}_3^-$  reduction was shown by the sensitivity toward autoclaving (Fig. 2d, high N-sterile). Additional evidence in support of a biological reduction of  $\text{NO}_3^-$  was found in the saturation behavior shown by  $\text{NO}_3^-$ .

Nitrate reduction was described well by Michaelis-Menten kinetics ( $r^2 = 0.99$ ; Fig. 3). The affinity constant  $K_m$  value derived from the fit of Eq. [1] to the data in Fig. 3 was  $70 \mu\text{mol L}^{-1} \text{NO}_3^-$  and  $V_{\text{max}}$  was  $1.2 \mu\text{mol L}^{-1} \text{NO}_3^- \text{ min}^{-1}$ .

Results where the initial  $\text{NO}_3^-$  concentration was lowered (low N) are shown in Fig. 4. There was an immediate inhibition in Fe(II) production of 66% after 1 h where most of the  $\text{NO}_3^-$  was reduced (Fig. 4a and 4b, Table 4). The rate of  $\text{NO}_3^-$  reduction was  $0.3 (\pm 0.02) \text{ mmol kg}^{-1} \text{ h}^{-1}$ . After  $\text{NO}_3^-$  depletion, Fe(III) reduction resumed in the low-N slurries. This was shown by the drop in Fe(II) inhibition at 4 and 24 h (Table 4).

### Effect of Tungstate on Nitrate Reduction and Iron(II) Production

The response of  $\text{NO}_3^-$  reduction and Fe(II) production to added  $\text{WO}_4^{2-}$  in soil slurries is shown in Fig. 5. It appears that  $\text{WO}_4^{2-}$  elicited inhibitory effects on Fe(III) reduction based on three- to fourfold less oxalate-extractable Fe(II) in high-N-W control slurries than their high-N control counterparts at the outset of the reactions (compare Fig. 5b and Fig. 2b). As a result, introduction of  $\text{NO}_3^-$  in soil slurries treated with  $\text{WO}_4^{2-}$  (high N-W) resulted in a different inhibition pattern than the high-N treatment (Fig. 5a and 5b). The greatest inhibition in Fe(II) production was observed during the first 4 h of reaction, followed by only

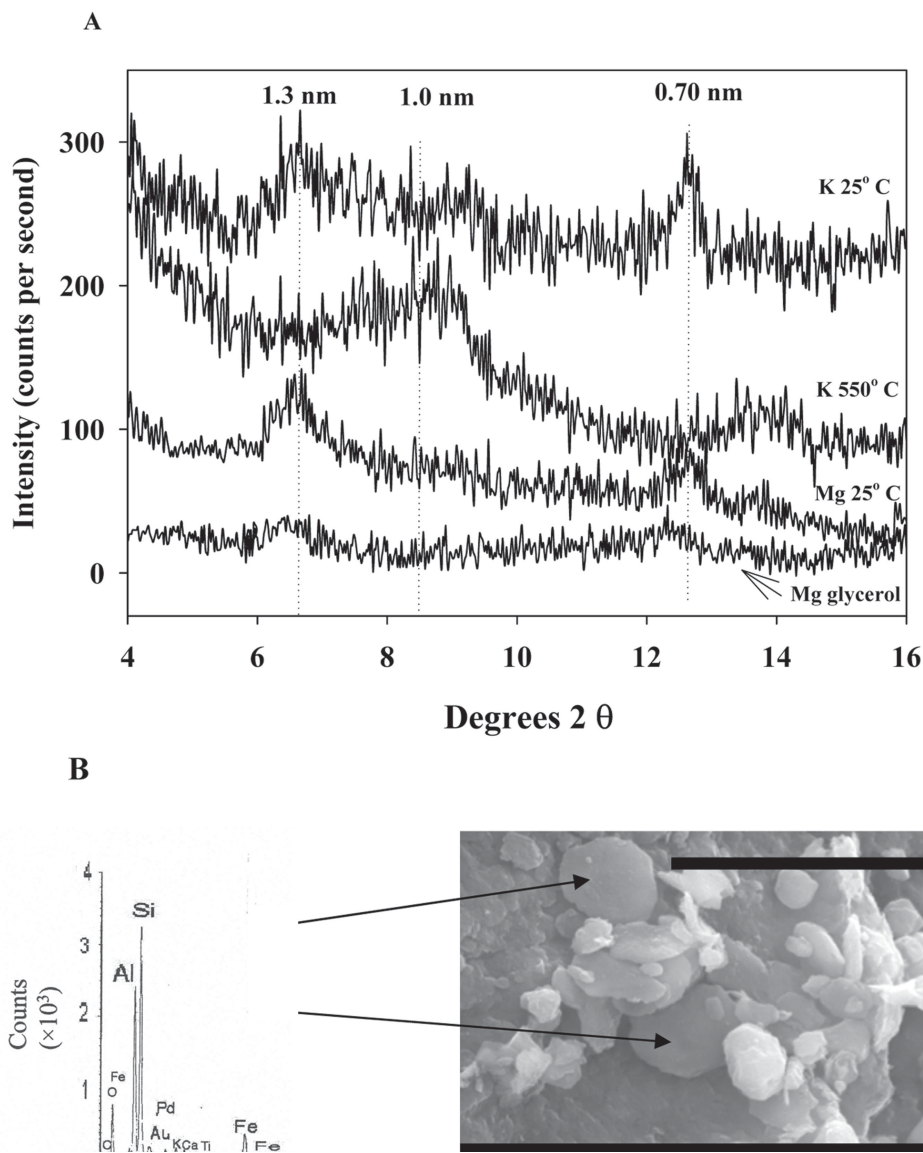


Fig. 1. (A) X-ray diffraction patterns of the clay fraction after Mg saturation at 25°C, Mg-glycerol solvation at 25°C, K saturation at 25°C, and K saturation at 550°C; (B) representative scanning electron micrograph of the clay fraction. The energy dispersive x-ray spectrum shown corresponds to the platy particle indicated by the arrows. The Au and Pd were a result of the coating process. Scale bar equals  $5 \mu\text{m}$  and no peaks were detected beyond 10 keV.

a slight inhibition at 24 h (Table 4). It is possible that this early inhibition of Fe(II) production arose from cells taking time to build new electron transport chains to use Fe(III) as an electron acceptor now that  $\text{NO}_3^-$  was blocked. No clear trend was evident in dissolved Fe(III) in response to  $\text{NO}_3^-$  addition (Fig. 5c).

The rate of  $\text{NO}_3^-$  reduction in high N-W (Fig. 5d) was  $0.14 (\pm 0.01) \text{ mmol kg}^{-1} \text{ h}^{-1}$  ( $R^2 = 0.99, p < 0.001$ ). This rate was approximately fivefold less than the high-N experiments and twofold less than the low-N treatment.

## DISCUSSION

### Inhibition of Soil Iron(II) Production by Nitrate Reductase

As already mentioned, several hypothetical pathways have been proposed to explain the mechanism of the suppression of Fe(II) production following addition of  $\text{NO}_3^-$ . Inhibition of Fe(II) production cannot be explained by an increase in  $E_H$  in high-N and high-N-W experiments (Table 4). Although the variability was high,  $E_H$  values never rose above  $-100 \text{ mV}$  during the course of the reaction for high-N and high-N-W soil slurries (Table 3). All values were well within the Fe(III)-reducing zone (Patrick et al., 1996) and did not increase to positive potentials ( $200\text{--}400 \text{ mV}$ ) as suggested elsewhere (Ponnamperuma and Castro, 1964; Ponnamperuma, 1972) to explain the inhibition of Fe(II) production by  $\text{NO}_3^-$ . Similarly, Klüber and Conrad (1998) reported no change in redox potential due to addition of  $1000 \mu\text{mol L}^{-1} \text{NO}_3^-$  to anoxic rice (*Oryza sativa* L.) paddy soil slurries. The pH values tended to increase with reaction time in high N, high N-W, and their

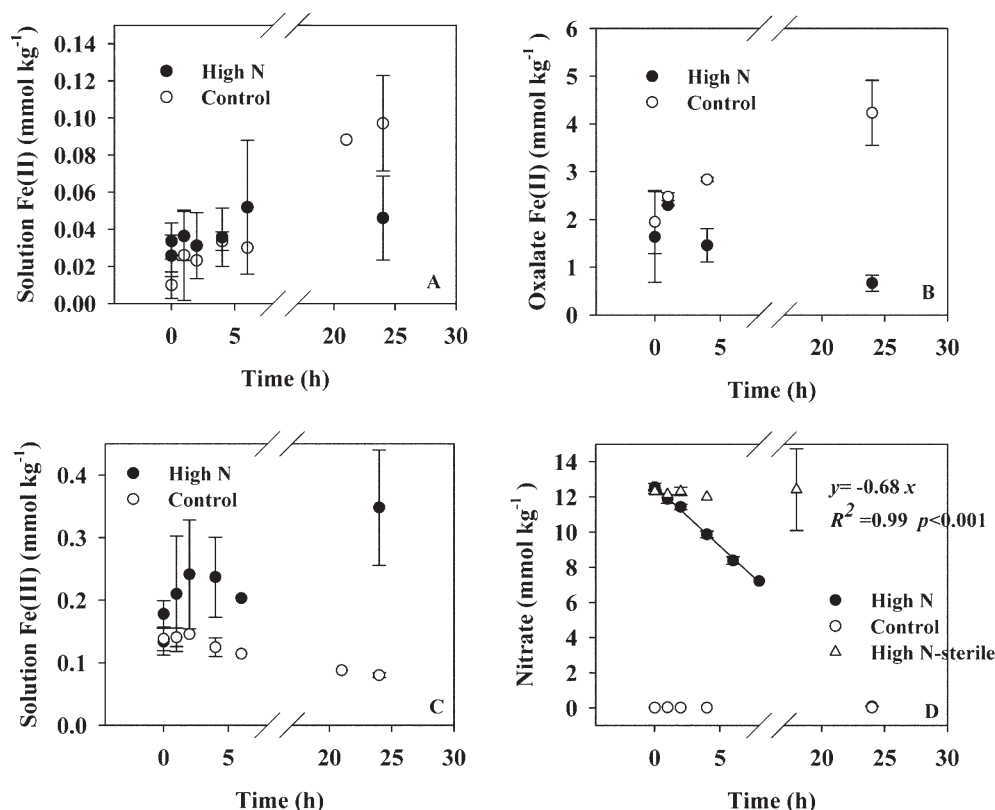


Fig. 2. Time course of (A) solution Fe(II), (B) oxalate-extractable Fe(II), (C) solution Fe(III), and (D) solution  $\text{NO}_3^-$  for the high-N treatment and high-N control soil slurries. The solution  $\text{NO}_3^-$  values for high-N-sterile are plotted in (D). Error bars represent the standard deviation from the mean for duplicate or triplicate runs.

controls (Table 3). This suggests that changes in pH or  $E_H$  were not responsible for the inhibitory effects in Fe(II) production.

According to the nitrate reductase competitive model (second hypothesis),  $\text{NO}_3^-$  would be preferentially used as an electron acceptor. Once all the  $\text{NO}_3^-$  was reduced, then Fe(III) would be used as an electron acceptor and Fe(II) production would resume (Munch and Ottow, 1980; Sorensen, 1982). In the high-N experiments, the inhibition in Fe(II) production increased  $>70\%$  from 1 to 24 h as  $\text{NO}_3^-$  was being reduced (Fig. 2a, 2b, and 2d, Table 4). Iron(II) production resumed after 48 h subsequent to complete  $\text{NO}_3^-$  depletion (data not shown). At low initial  $\text{NO}_3^-$  concentrations (low N), the maximum in Fe(II) inhibition occurred

Table 3. The pH, reduction potential ( $E_H$ ), and calculated saturation indices (SI) $\dagger$  for predicted Fe(II) mineral solids in high-N and high-N-W treatments and their controls.

Treatment	pH			$E_H$			SI $\ddagger$			
	0 h	4 h	24 h	0 h	4 h	24 h	Siderite		Magnetite	
							0 h	24 h	0 h	24 h
— mV —										
High N	7.8(0.1)§	7.5(0.3)	8.2(0.1)	-113(25)	-154(5)	-151(50)	1.8	2.5	-59	-66
High N control	7.8(0.1)	7.6(0.1)	7.9(0.1)	-129(6)	-206(7)	-162(37)	1.7	1.9	-59	-61
High N-W	7.6(0.2)	7.7(0.03)	7.9(0.1)	-124(15)	-126(11)	-145(15)	2.1	2.7	-58	-60
High N-W control	7.7(0.2)	ND¶	8.1(0.1)	-160(36)	ND	-145(39)	1.9	2.6	-59	-66

$\dagger$  Determined using MINEQL+ (Schecher, 1998). Input data included alkalinity, dissolved Fe(II), dissolved Fe(III), pH, and  $E_H$ .

$\ddagger$  Log ion activity product/solubility product constant.

§ Values in parentheses represent one standard deviation from the mean.

¶ ND = not determined.



**Table 4. Inhibition percentage in Fe(II) production and contribution of Fe(II) oxidation to  $\text{NO}_3^-$  reduction in high-N, high-N-W, and low-N treatments.**

Treatment	Inhibition of Fe(II) production			Fe(II) oxidation by $\text{NO}_3^-$ †		
	1 h	4 h	24 h	1 h	4 h	24 h
High N	10(0.1)‡	62(2)	85(3)	8(0.1)	10(3)	6(1)
Low N	66(2)	53(2)	0§	87(11)	51(7)	0
High N-W	56(17)	72(10)	5(4)	0	16(6)	0

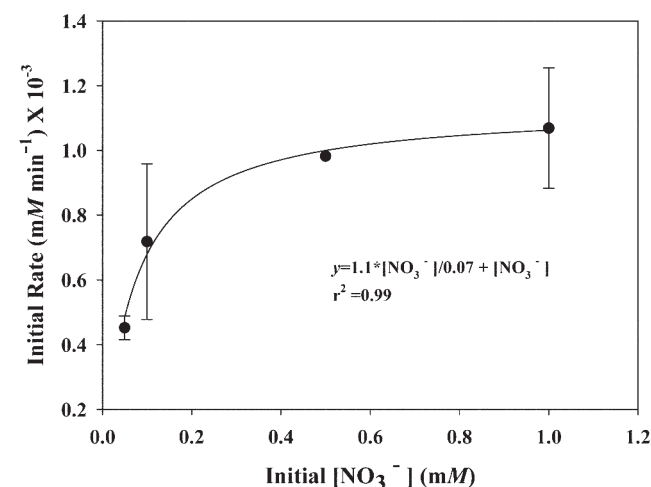
† Determined assuming  $\text{NO}_3^-$  was reduced to  $\text{N}_2$ , and by subtracting measured Fe(II) (oxalate-extractable + dissolved) for each treatment from the control.

‡ Values in parentheses represent one standard deviation from the mean.

§ Values of 0 represent points where Fe(II) production has resumed or where negligible  $\text{NO}_3^-$  has been reduced.

earlier than in the high-N treatment (Fig. 4a and 4b, Table 4). The production of Fe(II) clearly resumed after complete  $\text{NO}_3^-$  reduction in low-N treatments (Fig. 4a and 4b). These results agree with Sorensen (1982), who found that Fe(II) production commenced only after complete reduction of  $200 \mu\text{mol L}^{-1}$   $\text{NO}_3^-$  in anoxic slurries. Munch and Ottow (1980) argued that  $\text{NO}_3^-$  will inhibit Fe(II) production in soils due to the direct action of nitrate reductase.

It was expected that addition of  $\text{WO}_4^{2-}$  would curtail Fe(II) inhibition if the nitrate reductase competitive model is the main mechanism involved. Iron(II) inhibition continued in the high-N-W experiments (Table 4); however, this trend was confounded by the unanticipated effect of  $\text{WO}_4^{2-}$  on Fe(III) reduction as much less oxalate-extractable Fe(II) was initially present in high-N-W control slurries than high-N controls (compare Fig. 5b and Fig. 2b). Nonetheless, the pronounced decrease in the  $\text{NO}_3^-$  reduction rate from  $0.68 \text{ mmol kg}^{-1} \text{ h}^{-1}$  in the high-N slurries (Fig. 2d) to  $0.14 \text{ mmol kg}^{-1} \text{ h}^{-1}$  in the high-N-W treatment (Fig. 5d) indicates active nitrate reductase in our system. Molybdoenzymes of *E. coli* were inactivated by similar levels of  $\text{WO}_4^{2-}$ , resulting in a drastic decline in nitrate reductase activity (Scott et al., 1979; Smith, 1983). In addition, negligible  $\text{NO}_3^-$  reduction in the high-N-sterile treatment (Fig. 2d) further substantiates the important role of nitrate reductase, which is inactivated by autoclaving (Sorensen, 1982).



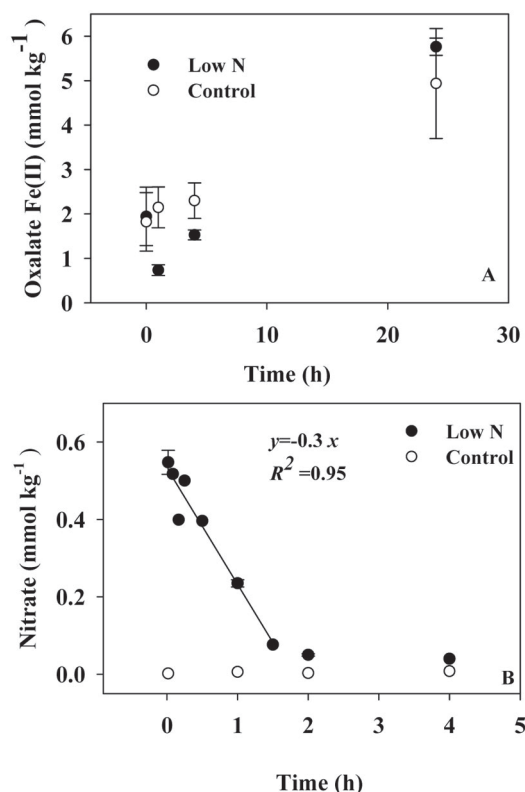
**Fig. 3. Michaelis-Menten kinetic plot in anoxic soil slurries of the Sadler silt loam. The line is a nonlinear fit of the Michaelis-Menten equation to the data. Error bars represent the standard deviation from the mean for duplicate or triplicate runs.**

The saturation behavior displayed in  $\text{NO}_3^-$  reduction rates (Fig. 3) further supports a biological reduction of  $\text{NO}_3^-$  by the nitrate reductase enzyme. This has been ascribed to limiting soil organic C concentrations for heterotrophic denitrifiers (Bowman and Focht, 1974; Kohl et al., 1976). This could have been the case in our experimental systems because no C additions were made and the native soil organic C level was used ( $\sim 1.3\%$ ). The apparent  $K_m$  value ( $70 \mu\text{mol L}^{-1}$   $\text{NO}_3^-$ ) was lower than that reported elsewhere (Bowman and Focht, 1974; Kohl et al., 1976), suggesting greater affinity of  $\text{NO}_3^-$  in our soil. This could reflect differences in soil organic C availability,

soil type, and experimental conditions.

It is conceivable that  $\text{NO}_3^-$  consumption was also due to Fe(III)-reducing bacteria that are able to simultaneously reduce  $\text{NO}_3^-$  and Fe(III), with a preference toward  $\text{NO}_3^-$  (DiChristina, 1992; Krause and Nealson, 1997). Several *Geobacter* species have been shown to reduce  $\text{NO}_3^-$  (Coates et al., 1996; Senko and Stolz, 2001) without the involvement of Mo-containing enzyme systems based on sustained nitrate reductase activity in the presence of up to  $2 \times 10^4 \mu\text{mol L}^{-1}$   $\text{WO}_4^{2-}$  (Murillo et al., 1999).

### Inhibitory Effects due to Concomitant Iron(III) and Nitrate Reduction Coupled to Iron(II)



**Fig. 4. Time course of (A) oxalate-extractable Fe(II), and (B) solution  $\text{NO}_3^-$  for the low-N treatment and low-N control soil slurries. Error bars represent the standard deviation from the mean for duplicate or triplicate runs.**

## Reoxidation by Nitrite

If  $\text{NO}_3^-$  functioned strictly as an inhibitor of Fe(III) reduction by nitrate reductase, then one would expect Fe(II) levels to remain constant during  $\text{NO}_3^-$  reduction as pointed out by Obuekwe et al. (1981) and observed in sediment slurries by Sorensen (1982). In our studies, however, Fe(II) levels in both dissolved and oxalate-extractable fractions were not constant during the  $\text{NO}_3^-$  reduction process, but dropped below the corresponding controls. Inhibition of Fe(II) production in high N and low N was most pronounced whenever  $\text{NO}_3^-$  was still present in solution (Fig. 2a, 2b, 2d, 4a, and 4b, Table 4). These results indicate that Fe(II) was oxidized during  $\text{NO}_3^-$  reduction and contributed to inhibiting Fe(II) production. The contributions of Fe(II) oxidation to  $\text{NO}_3^-$  reduction were 6 to 10% in the high-N and 50 to 90% in the low-N slurries (Table 4). Additional support for Fe(II) oxidation was based on the greater levels of dissolved Fe(III) produced in high-N treatments when compared with the high-N control (Fig. 2c). This evidence indicates that the nitrate reductase competitive model was not the only mechanism operating in the inhibition of Fe(II) formation by  $\text{NO}_3^-$ .

Iron(II) production in control bottles did not appear to level off during our experiments, suggesting that microbial Fe(III) reduction was proceeding at the time when  $\text{NO}_3^-$  was added (Fig. 2a, 2b, 4a, and 4b). Secondary reactions involving chemical Fe(II) reoxidation by  $\text{NO}_2^-$  formed during concomitant Fe(III) and  $\text{NO}_3^-$  reduction may explain the inhibitory effects in Fe(II) production (Komatsu et al., 1978; Obuekwe et al., 1981). The greatest contribution of Fe(II) oxidation to  $\text{NO}_3^-$  reduction was found in the low-N slurries (Table 4). This may be ascribed to the initial stoichiometric excess of oxalate-extractable Fe(II) over  $\text{NO}_3^-$  (3.5:1) in this treatment (Fig. 4a and 4b), whereas in high-N experiments, the ratio was 0.2:1 (Fig. 2b and 2d). This would provide more available Fe(II) to chemically react with  $\text{NO}_2^-$  produced as an intermediate during heterotrophic reduction of  $\text{NO}_3^-$ . Nitrite is a versatile ligand in that it can coordinate to a metal via the N or O atoms, with bonding on the N forming a stronger complex (Shriver et al., 1994).

One possible form of reactive Fe(II) that may chemically reduce  $\text{NO}_2^-$  in our soil systems is  $\text{FeCO}_3(\text{s})$  (Table 3). Using data compiled by Bard et al. (1985), one can compute a favorable thermodynamic driving force for oxidation of siderite by  $\text{NO}_2^-$  to poorly crystalline Fe(III) hydroxide ( $\Delta G^\circ_{\text{r}} = -128 \text{ kJ mol}^{-1}$ ):

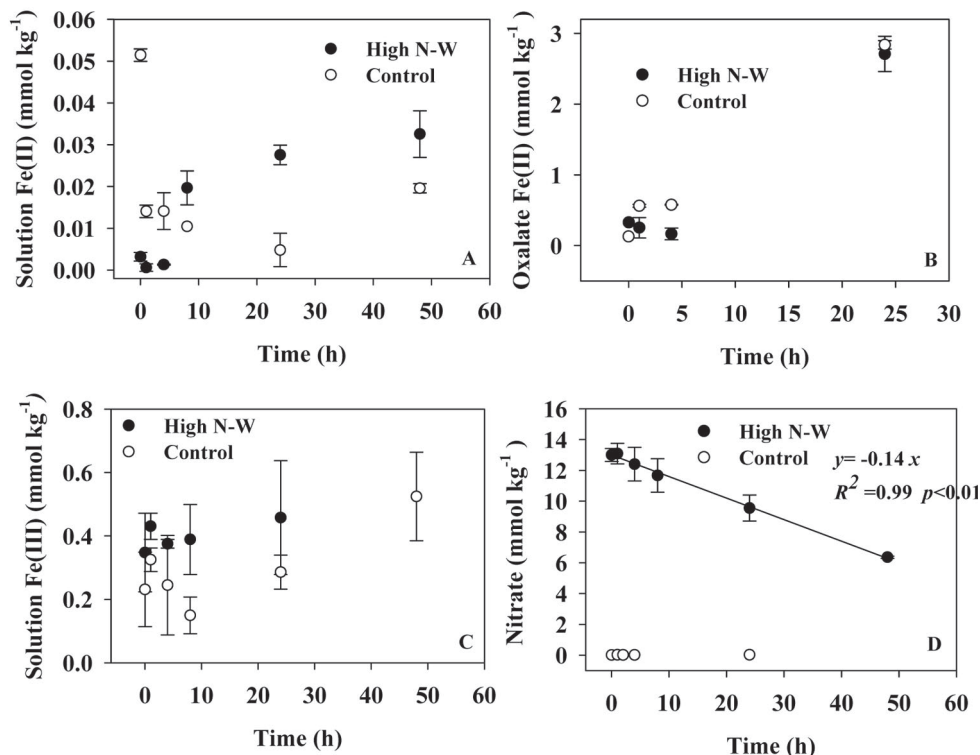
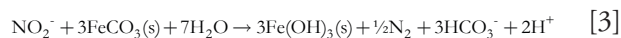


Fig. 5. Time course of (A) solution Fe(II), (B) oxalate-extractable Fe(II), (C) solution Fe(III), and (D) solution  $\text{NO}_3^-$  for high-N-W treatment and high-N-W control soil slurries. Error bars represent the standard deviation from the mean for duplicate or triplicate runs.



It is not clear whether the overall reaction in Eq. [3] occurs at a rate relevant to our time series data. The importance of siderite oxidation by  $\text{NO}_2^-$  as a possible pathway in inhibiting Fe(II) production merits further study.

Another form of Fe(II) that may be present in our systems is adsorbed Fe(II). A wide variety of sorption sites in the Sadler soil [kaolinite, HIV, and possibly Fe(III) (hydr)oxide minerals] would be available for Fe(II) at pH values encountered in this study (Table 3). For example, Kukkadapu et al. (2001) reported that Fe(II) produced during biological reduction of a sediment-derived clay fraction resorbed to kaolinite. Adsorbed Fe(II) as an inner sphere complex to oxo functional groups is a strong chemical reductant, reflected by a lowered reduction potential when compared with dissolved Fe(II) (Wehrli, 1990; Luther et al., 1992). Nitrite is known to be heterogeneously reduced by adsorbed Fe(II) forms, at estimated rates of  $\sim 1$  to  $3.5 \mu\text{mol L}^{-1} \text{NO}_2^- \text{min}^{-1}$  at pH 8 (Van Cleemput and Baert, 1983; Sorensen and Thorling, 1991). These rates are generally faster than the rate of  $\text{NO}_3^-$  reduction in our studies ( $0.2$ – $1 \mu\text{mol L}^{-1} \text{NO}_3^- \text{min}^{-1}$ , Fig. 2d and 4d), and may explain why  $\text{NO}_2^-$  was not detected as an intermediate. These reactive forms of Fe(II) may have been rapidly oxidized to Fe(III) by  $\text{NO}_2^-$  and may explain the inhibition in Fe(II) production.

## Inhibitory Effects due to Autotrophic, Nitrate-Dependent Iron(II) Oxidation

Autotrophic bacteria may also be responsible for the inhibiting effect of  $\text{NO}_3^-$  on Fe(II) production by coupling

$\text{NO}_3^-$  reduction to Fe(II) oxidation (Straub et al., 1996). In our experiments, the immediate oxidation of Fe(II) following  $\text{NO}_3^-$  addition resulted in accelerated production of dissolved Fe(III) in high-N slurries (Fig. 2a–2d). The dissolved Fe(III) is probably chelated by dissolved organic matter. Although Fe(II) oxidation could also be due to the chemical pathway mentioned above, no loss of  $\text{NO}_3^-$  was noted in the high-N–sterile slurries (Fig. 2d), nor was the production of Fe(III) (data not shown). Klüber and Conrad (1998) reported immediate production of Fe(III) coupled to  $\text{NO}_3^-$  reduction under anoxic conditions and attributed this finding to bacterial Fe(II) oxidation. Enrichment cultures of  $\text{NO}_3^-$ -dependent Fe(II) oxidizers produce poorly crystalline ferrihydrite [ $\text{Fe}(\text{OH})_3(\text{s})$ ] as the primary product of Fe(II) oxidation (Straub et al., 1996).

The Fe(II) used by  $\text{NO}_3^-$ -dependent Fe(II) oxidizers may originate from dissolved Fe(II) (Straub et al., 1996) or possibly siderite (Weber et al., 2001). In addition, structural Fe(III) in HIV (Fig. 1) that is reduced may provide the available Fe(II). Interestingly, a recent study showed that structural Fe(II) in smectite was available as an electron donor to support autotrophic Fe(II) oxidation by  $\text{NO}_3^-$  (Shelobolina et al., 2003). The structural Fe(II) to Fe(III) conversions mediated by bacteria occurred without the Fe being detached from the smectite.

It was expected that blocking nitrate reductase with  $\text{WO}_4^{2-}$  (high-N–W experiments) would isolate the contribution of the Fe(II)-oxidizing,  $\text{NO}_3^-$ -dependent autotrophic pathway. This would allow Fe(III) reduction to proceed and produce Fe(II), which then could participate in autotrophic  $\text{NO}_3^-$  reduction (Straub et al., 1996). As mentioned above, added  $\text{WO}_4^{2-}$  affected Fe(III) reductase and may explain the discrepancy between  $\text{NO}_3^-$  reduced and Fe(II) oxidized in high-N–W slurries (Fig. 5b and 5d, Table 4). Past studies have shown that inhibitors such as  $\text{WO}_4^{2-}$  have the potential to impact nontarget microorganisms when added to sediments (Oremland et al., 1989).

## SUMMARY AND CONCLUSIONS

It has long been known that fertilizer  $\text{NO}_3^-$  applications to flooded soil can result in N loss through denitrification. Our results show that soil Fe(III) reduction can be hindered as well and may result in temporary changes in soil physicochemical properties if  $\text{NO}_3^-$  fertilizer is added to Fe(III)-reducing soil. Addition of  $\text{NO}_3^-$  to soil under Fe(III)-reducing (anoxic) conditions inhibited Fe(II) production by preferential use of  $\text{NO}_3^-$  by nitrate reductase and anoxic Fe(II) oxidation. Processes that account for anoxic Fe(II) oxidation include concomitant Fe(III) and  $\text{NO}_3^-$  reduction coupled to reoxidation of Fe(II) by  $\text{NO}_2^-$  and  $\text{NO}_3^-$ -dependent, autotrophic Fe(II) oxidation. The role of these two processes in the inhibition in Fe(II) production became more important at high initial oxalate-extractable Fe(II) and  $\text{NO}_3^-$  concentrations. Thus, it may be important to consider the initial ratios of extractable Fe(II) to  $\text{NO}_3^-$  when trying to explain the mechanism of inhibition in Fe(II) production in anoxic soil environments. The inhibition was short-term in the sense that once  $\text{NO}_3^-$  was depleted, Fe(II) production resumed. An important consequence of  $\text{NO}_3^-$ -dependent Fe(II) oxidation is that the dissolved Fe(III) produced, even at micromolar levels, could behave as a kinetically labile oxidant.

It is difficult to separate the contributions of anoxic Fe(II) oxidation between  $\text{NO}_3^-$ -dependent, autotrophic Fe(II) oxidation and chemical reoxidation of Fe(II) by  $\text{NO}_2^-$  during concomitant Fe(III) and  $\text{NO}_3^-$  reduction on the basis of our data. One approach could include a prolonged incubation to exhaust microbially reducible Fe(III). Once Fe(II) production has stabilized,  $\text{NO}_3^-$  could be added to isolate Fe(II) oxidation. Perhaps addition of ferrozine to soil slurries to rapidly complex Fe(II) before  $\text{NO}_2^-$  oxidizes it would allow one to distinguish between chemical and biological processes. Furthermore, it would be interesting to follow Fe(II) and Fe(III) changes in the clay fraction to identify reactive species.

## ACKNOWLEDGMENTS

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