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Evaluation of Chemical Amendments for pH and Redox Stabilization in Aqueous Suspensions of Three California Soils

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Many chemically and biologically important trace element, heavy metal, and organic contaminant reactions in soils are constrained by pH and redox conditions and changes in these conditions can significantly affect reaction rates. Although closed-system, batch methods have been used for many years to study contaminant reaction kinetics, redox conditions in such suspensions for extended durations have not been well evaluated. We tested a suite of readily available chemicals for their ability to buffer pH and redox potential (E_H) of anaerobic soil–water (1:4 or 1:5) suspensions at specific levels under closed conditions. After initial titration, 20 m*M* Good buffers (e.g., PIPES) were used to stabilize the pH of the soil suspensions within ±0.3 units of the target pH (5.8–8.9) for a period of at least 8 d. The ability of redox active chemicals, such as $NaNO_3$, Fe(III)nitrilotriacetic acid (NTA), Ti(III)NTA, Fe and Mn oxyhydroxides, cysteine-HCl, dithiothreitol, and ascorbic acid, to stabilize E_H at specific levels (400 to −300 mV) depended heavily on the initial concentration of the chemical as well as the organic C and N status of the soil. Redox stabilization in most soils was typically achieved within a period of 3 to 4 wk. Iron(II)/Fe(III) ratios in 1 *M* HCl soil extracts and S(−II)/SO_4^{2-} ratios in filtrates generally reflected the redox condition as measured by the Pt electrode. This investigation demonstrates that the pH and E_H of enclosed soil–water suspensions can be buffered for long periods across a wide range of conditions using soluble chemicals alone.

Abbreviations: AIW, artificial irrigation water; DDW, distilled deionized water; Eh, redox potential.

The speciation of many soil nutrients, trace elements, heavy metals, and organic contaminants is greatly affected by the pH and oxidation–reduction (redox) status of the soil–water system. As master variables, pH and redox describe thermodynamically which chemical species should dominate a system at equilibrium. Typically, the measured activities for particular chemical species in aqueous environments do not match those based on thermodynamic calculations of pH and redox measurements. Chemical reactions may be impeded by thermodynamic and kinetic barriers, and the soil–water system itself may shift from various states of redox disequilibria due to ever-changing environmental conditions, such as those resulting from seasonal inputs of O_2 and organic C from plant metabolism. Microbial activity is directly linked to the redox disequilibria of the soil, as bacteria, fungi, and other microorganisms oxidize organic C and other electron donors while reducing O_2 and other electron acceptors. Although it is usually considered to be a descriptive or operational parameter, the redox status of a soil may indicate possible chemical and microbial reaction pathways that a contaminant can undergo. The effect of redox on the kinetics for a particular contaminant reaction (e.g., degradation) is often highly uncertain due to complex interactions between physical, chemical, and biological processes in a soil–water system. Consequently, the study of reaction rates for individual contaminants under different pH and redox conditions is vital for assessing toxicity risks and remediation strategies in environments vulnerable to changing conditions.

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Redox Characterization

Unlike pH, which can be accurately assessed in natural waters using a pH electrode, characterization of the redox status of a soil–water system is less straightforward. Theoretically, redox potential can be expressed as the negative logarithm of the dissolved electron activity in a solution (pε). Since the actual concentration of dissolved electrons in solution is exceedingly low ($\sim 10^{-55}$ *M*), direct measurement of the electron activity, or redox potential, is impractical (Hostetler, 1984; Thorstenson, 1984). More appropriately, electrons in environmental systems are exchanged directly between oxidized and reduced molecules in gases, liquids, and solids in accordance with electrochemical gradients. Hence, the redox status of a natural system may be best characterized by comprehensively analyzing all of the soluble oxidized and reduced species present within the system. Summation of the different oxidized and reduced redox-active species may

then yield capacity-type parameters, such as oxidative (OXC) or reductive (RDC) capacities (Scott and Morgan, 1990), which can be used qualitatively for contaminant reaction investigations. The terminal electron accepting processes (TEAPS) method extends this concept further by also quantifying dissolved H_2 concentrations, which can be indicative of dominant redox couples in aqueous systems (Chapelle et al., 1995; Lovley and Goodwin, 1988). Depending on the concentrations of the different redox couple species and dissolved H_2, the redox condition of a groundwater or soil–water system can then be described as being dominated by O_2-, NO_3-, Fe-, or S-reduction or methanogenic processes.

For many investigations, the time and expense involved with a complete analysis of relevant redox species or reaction intermediates, such as with the OXC or TEAPS approaches, may be prohibitive. A simpler, yet less definitive, technique to estimate the redox condition of a system is to measure the redox potential (Eh, in millivolts) of the solution with an inert Pt, Au, or glassy C electrode. The electronic potential developed between the surface of the electrode and the solution, calibrated against a reference electrode and corrected to the standard H_2/H^+ electrode, theoretically reflects the summation of all redox couples in contact with the surface. In reality, only Fe(II)–Fe(III) and possibly S(–II)–S(0) redox couples have been found to show accurate responses, or electronic reversibility, with Pt electrode surfaces, and only at relatively high concentrations ($>10^{-5}$ *M*; Nordstrom, 2000). For other redox couples, the exchange current densities are insufficient to induce accurate electrode responses to changing concentrations (Tratnyek and Macalady, 2000). In such cases, and even for Fe(II)–Fe(III) and S(–II)–S(0) couples at concentrations $<10^{-5}$ *M*, the electrode may respond more toward mixed chemical potentials or toward contaminants, such as oxides or sulfides, on the electrode surface (Nordstrom, 2000).

Notwithstanding these limitations, redox electrodes can provide a valuable alternative to direct measurement of all possible redox couples or reaction intermediates for evaluating redox conditions (Westall, 2000). For example, NO_3^-–NH_4^+, Mn(IV)–Mn(II), and Fe(III)–Fe(II) redox couple transitions were found to occur at distinct and relatively well separated Pt electrode readings in flooded soils, both during reduction and oxidation processes (Patrick and Jugsujinda, 1992). Similarly, transformations between different oxidation states for some inorganic contaminants, such as Se, As, and Cr, have been found to correspond well with differences in Pt electrode readings (Masscheleyn et al., 1990, 1991, 1992). Used in conjunction with chemical analysis, electrode measurements of the solution redox potential may help further constrain the redox condition of the soil–water system.

Methods to Poise pH and Redox Levels in Soils

Chemical equilibrium and kinetic reactions in soil–water systems are typically conducted by placing a soil or sediment into a stirred batch reactor or a flow-through column (Dragun, 1993; Guenzi et al., 1989; Jayaweera and Biggar, 1996; Masscheleyn et al., 1990; Patrick et al., 1973; Petrie et al., 1998). The pH control is achieved by connecting a pH electrode to a potentiometer and dispenser, which delivers acid or base into the reactor or flow lines as needed if values fall outside a designated range. Similarly, the redox level (E_H in millivolts) of the suspension is controlled using a Pt electrode and reference cell connected to the potentiometer and solenoid to deliver gases (N_2, O_2, or H_2) into the system, the latter two gases being strong oxidants and reductants, respectively. Such infrequent pulses of strong oxidants (O_2) or reductants (H_2) create a soil–water redox environment that is inevitably disrupted from chemical equilibrium. Other redox couples, such as Fe(II)–Fe(III), or S(–II)–SO_4, may or may not respond accordingly in the short period of the experiment due complex reactions involving aqueous and solid phases. Similarly, the microbial ecology of the soil may be disrupted by these strong redox perturbations, thereby altering any enzymatically catalyzed reactions. Thus, reactions involving ambient redox couples and microorganisms may not truly reflect the redox condition imposed by gas introduction as measured by the Pt electrode. In addition, gas introduction into the soil–water reaction vessel could potentially disrupt concentrations of other chemically important gases, such as CO_2, which is directly linked to many biological reactions and solid-phase carbonate equilibria. Thus, alternative approaches are needed to control pH and redox in soil systems.

One alternative approach involves direct addition of pH- and redox-buffering chemicals to closed soil–water suspensions. Basic and dibasic phosphate solutions can be used as buffers across the pH range of 4 to 8, and carbonates, acetates, borates, etc., may be used for other pH ranges. Such buffers can be consumed as biological nutrients or may be inhibitory (or toxic) to microorganisms. They may also precipitate or react with nutrient media, thereby rendering them unsuitable as biological buffers (Good et al., 1966). Because of these limitations, Good et al. (1966) developed relatively unreactive, organic-sulfonate pH buffers for use in microbial investigations. To our knowledge, few applications of these buffers to environmental soil–water investigations have been reported.

Chemical amendments have often been used to manipulate redox conditions of natural and artificial environments to study contaminant release or uptake in soils, or to remediate contaminants in subsurface and wastewater treatment systems. For example, sodium ascorbate and borohydride were used to manipulate E_H and pH in soil slurries to predict As release from contaminated sites (Chatain et al., 2005). As a remediation method, reduced Fe(II), combined with sodium dithionite, was found to best reduce soluble Cr(VI) to relatively insoluble $Cr(OH)_3$ in chromite ore processing waste (Su and Ludwig, 2005). Similar metal reductants, such as zerovalent Fe (Bigg and Judd, 2000; Singh et al., 1999) or ferrous sulfide (Butler and Hayes, 1998; Patterson et al., 1997) have also been found to be effective redox modifiers in contaminant remediation investigations. In addition to these reductants, organic chemical modifiers (or complexing agents), such as dithiothreitol (DTT), cysteine, and Ti(III) citrate (Brock and O'Dea, 1977; Cleland, 1964; Jones and Pickard, 1980; Lay and Levina, 1996; Lewis et al., 1996; Singh et al., 1999; Zehnder and Wuhrmann, 1976) have also been used in microbial–enzymatic redox and contaminant degradation studies. Clearly, a wide range of inorganic and organic chemicals have the potential to alter the redox status of soil–water systems.

Accordingly, the primary objective of this study was to evaluate whether single additions of dissolved chemicals or suspended solids could buffer the pH and redox of closed, anaerobic soil–water suspensions over long durations. For pH control, we focused our investigations on the Good buffers, as they were designed to be stable, nontoxic, and unreactive toward most microbes (Good et al., 1966). For redox control, we evaluated a wide range of redox-active chemicals, falling in the general categories of dissolved gases, soluble inorganics, soluble organics, suspended Fe or Mn (hydr)oxides, and reduced S compounds, for their effects on the soil–water redox potential as measured by Pt combination electrodes and by soluble and 1 *M* HCl extractable Fe(II)/Fe(III) and soluble S(–II)/SO_4^{2-} ratios.

MATERIALS AND METHODS

Collection and Preparation of Soils

Surface (0–20 cm) soils from an agricultural field near Broadview, CA (Lillis, a very-fine, smectitic, thermic Halic Haploxerert), were brought back to the laboratory, where they were air dried and ground to pass through 0.85-mm (20-mesh) sieve. Surface soils were also collected from the USDA-ARS field station at Parlier, CA (Hanford, a coarse-loamy, mixed, superactive, nonacid, thermic Typic Xerorthent), and the USDA-ARS field station near Brawley, CA (Imperial, a fine, smectitic, calcareous,

Table 1. The pH buffers used in 1:5 Lillis soil–water suspensions.

Good buffer, formula	Suspension concentration	Appropriate pH range
	m*M*	
MES, $C_6H_{13}NO_4S$	20	5.5–7.0
PIPES, $C_8H_{16.5}N_2O_6S_2$	20	6.1–7.5
HEPES, $C_8H_{18}N_2O_4S$	20	6.8–8.2
TAPS, $C_7H_{17}NO_6S$	20	7.7–9.1
CHES, $C_8H_{17}NO_3S$	20	8.6–10.0

hyperthermic Vertic Torrifluvent), placed on ice, then transported to the laboratory where they were mixed 1:5 (w/v) with artificial irrigation water (see below) and placed into 160-mL serum bottles under anaerobic (N_2) conditions within 24 h. Tests with redox buffers were conducted immediately after mixing (see below).

Dried, ground, and sieved soils from each location were analyzed for total C and inorganic C using a UIC coulometer (UIC, Inc., Joliet, IL) and HCl titration (Loeppert and Suarez, 1996) and for total N using a Leco CN-2000 carbon-nitrogen analyzer (Leco Corp., St. Joseph, MI). Soil surface areas were determined using the EGME method (Cihacek and Bremner, 1979), and particle size was determined by the hydrometer technique (Gee and Bauder, 1986). Free Fe and Al were determined by extracting dried soils with citrate and dithionite (Coffin, 1963) and analyzing the total soluble Fe and Al using a Thermo Jarrell Ash inductively couple plasma-optical emission spectrometer (Thermo Electron, Madison, WI). Dried, ground, and sieved soils were mixed 1:5 (w/v) with distilled, deionized water (DDW) and shaken on a reciprocal shaker for 12 h. Each soil–water extract was centrifuged at 6700 × *g* for 20 min, and the decanted aqueous phase was passed through a 0.2-µm filter. Electrical conductivity of the filtrates was measured by conductivity cell (Amber Scientific, Belmont, WA) and alkalinity was measured by acid titration. Soluble anions were analyzed with a Dionex ICS-2000 system using an IonPac AS18 column (Dionex Corp., Sunnyvale, CA).

Preparation of pH and Redox Chemical Solutions

Stock solutions of pH and redox buffers were made by dissolving reagent-grade chemicals in DDW. For pH control, 500 m*M* stock solutions of Good buffers were prepared and titrated to target pH values using HCl or NaOH (Table 1). The solutions were bubbled with N_2 for 1 h then sterilized by passing through a sterile 0.2-µm filter. A 10% solution of chloramphenicol was made by dissolving 2.5 g reagent grade chloramphenicol in 25 mL ethanol, which was then transferred to a serum bottle, stoppered, and flushed with N_2.

Redox-active chemical stock solutions were made similarly by dissolving reagent-grade chemicals in DDW (Table 2). Iron (III) nitrilotriacetic acid [Fe(III)NTA] was prepared by first adding 1.78 g of NTA to 10 mL of DDW. While stirring, the solution was brought to pH 7 with NaOH to dissolve the NTA. To this solution, 4.33 g oF $FeCl_3{\cdot}6H_2O$ was added and the pH was adjusted slowly to 7.0 with 1 *M* $NaHCO_3$. The solution was brought up to 100-mL volume with DDW then bubbled under N_2 for 1 h and filter sterilized. Similarly,

Table 2. Redox reagents used in 1:5 soil–water suspensions.

Type of redox agent	Amendments	Approximate primary redox half reaction	E_w^0 of redox half reaction†	Reference‡	Tested concentrations
			V		m*M*
Gas	NaCl–N_2				5.0 (NaCl)
	NaCl–O_2	$O_2(g) + 4H^+ + 4e^- \leftrightarrow 2H_2O$	0.81	4	1.0, 5.0
	NaCl–H_2	$2H^+ + 2e^- \leftrightarrow H_2$	−0.42	4	0.5, 2.5
Soluble anion (Fe^{3+}or Ti^{3+})	sodium fumarate, $Na_2C_4H_2O_4$	Fumarate ↔ succinate	0.10	4	5.0
	$NaNO_3$	$NO_3^- + 10H^+ + 8e^- \leftrightarrow NH_4^+ + 3H_2O$	0.36	3	0.5, 2.5, 5.0
	$FeCl_3{\cdot}6H_2O$	$Fe^{3+} + e^- \leftrightarrow Fe^{2+}$	0.77§	7	5.0
	$FeCl_2{\cdot}4H_2O$	$Fe^{3+} + e^- \leftrightarrow Fe^{2+}$	0.77§	7	5.0
	$FeSO_4{\cdot}4H_2O$	$Fe^{3+} + e^- \leftrightarrow Fe^{2+}$	0.77§	7	5.0
	Fe(III)nitrilotriacetic acid (NTA)	Fe^{3+}–NTA + $e^- \leftrightarrow Fe^{2+}$–NTA	0.38	1,2	0.5, 2.5, 5.0
	Ti(III)NTA	Ti(IV)citrate + $e^- \leftrightarrow$ Ti(III)citrate	−0.48	6	0.5, 2.5, 5.0
Organic	ascorbic acid, $C_6H_8O_6$	dehydro-L-ascorbic acid + $2e^- \leftrightarrow$ ascorbic acid	0.08	4	0.5, 2.5, 5.0
Solid-phase Fe or Mn(hydr)oxide	ferrihydrite, $Fe_5HO_8{\cdot}4H_2O$	$Fe(OH)_3$ (ferrihydrite) + $e^- \leftrightarrow Fe^{2+}$	−0.1–0.1	1	5.0
	goethite, α-FeOOH	α-FeOOH (goethite) + $e^- \leftrightarrow Fe^{2+}$	−0.27	1	5.0
	hematite, Fe_2O_3	α-Fe_2O_3 (hematite) + $e^- \leftrightarrow Fe^{2+}$	−0.28	1,2	5.0
	magnetite, Fe_3O_4	α-Fe_3O_4 (magnetite) + $e^- \leftrightarrow Fe^{2+}$	−0.32	1,2	5.0
	birnessite, MnO_2	½β-MnO_2 + $2H^+ + e^- \leftrightarrow$ ½Mn^{2+} + H_2O	0.56	1	0.5, 5.0
Sulfur species	cysteine·HCl, $C_3H_7NO_2S{\cdot}HCl{\cdot}H_2O$	cystine + $e^- \leftrightarrow$ 2cysteine-HCl	<−0.20	4	0.5, 2.5, 5.0
	dithiothreitol (DTT), $C_4H_{10}O_2S_2$	oxidized dithiothreitol ↔ dithiothreitol	−0.33§	5	0.5, 5.0
	$Na_2S{\cdot}9H_2O$	$SO_4^{2-} + 9H^+ + 8e^- \leftrightarrow HS^- + 4H_2O$	−0.19	1	0.5, 5.0
	sodium hydrosulfite (dithionite), $Na_2S_2O_4$	$H_2SO_3 + 5H^+ + 6e^- \leftrightarrow HS^- + 3H_2O$	−0.02§	7	2.5
	Na_2SO_4	$SO_4^{2-} + 9H^+ + 8e^- \leftrightarrow HS^- + 4H_2O$	−0.19	1	2.5

†Approximate reduction potentials for redox half reactions relevant to soil and microbial systems at pH 7, 25°C, concentration <10 m*M*.

‡ 1, Thamdrup (2000); 2, Straub et al. (2001); 3, Patrick et al. (1996); 4, Hewitt (1950; 5, Cleland (1964); 6, Zehnder and Wuhrmann (1976); 7, Lide (1990–1991).

§ Standard-state conditions.

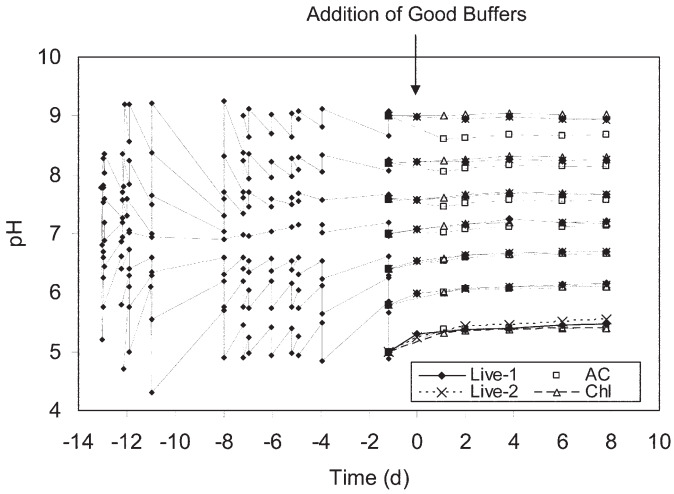

Fig. 1. Stability of pH in Lillis soil suspensions after titration with HCl or NaOH (Days −13 to −1.2) and separation and enclosure into serum bottles followed by addition of 20 m*M* Good Buffers (Day 0). Symbols represent live (solid and hatched), heat-killed (AC, open square), or antibiotic (Chl, open triangle) samples.

Ti(III)NTA was prepared by first adding 6.19 g of NTA to 10 mL of DDW. The pH was slowly raised to 6.5 with NaOH to dissolve the NTA, bringing the volume to 35 mL. To this solution, 10 mL of 20% $TiCl_3$ (Fisher Catalog no. ST43–500) was added, and the pH was slowly adjusted to 6.5 with 1 *M* $NaHCO_3$. The solution was diluted to 100 mL, then bubbled with N_2 for 1 h. The pH of the solution was then adjusted to 6.8, after which the solution was filter sterilized. We chose to use NTA as the complexing, stabilizing ligand for Ti(III) to mimic the Fe(III)NTA reagent, although other ligands, such as oxalate, could be used. Although NTA may be less bioavailable (or metabolized) in comparison to citrate, which is often used in microbial redox studies with Ti(III) (Jones and Pickard, 1980; Zehnder and Wuhrmann, 1976), it has been observed to be biodegraded in soils, albeit at low levels under O_2–deficient conditions (Tiedje and Mason, 1974).

Other soluble redox-active reagents were prepared by dissolving reagent-grade chemicals in DDW followed by bubbling with N_2 and sterilization through autoclaving or filtration. In some cases, the pH of the stock solutions required adjustment to circumneutral pH with $NaOH_{(aq)}$, $Na_2CO_{3(aq)}$, or HCl. Solid-phase stock suspensions of α-FeOOH (goethite) (CAS no. 51274-00-1), α-Fe_2O_3 (hematite) (CAS no.1317-60-8) and Fe_3O_4 (magnetite) (CAS no. 1317-61-9) were obtained from Strem Chemicals Inc. (Newburyport, MA), and MnO_2 from J.T. Baker Chemical Co. (Phillipsburg, NJ). The suspensions were prepared by adding the reagent-grade solids to DDW and bubbling under N_2. A suspension of ferrihydrite was prepared by quickly titrating a solution of $FeCl_3$ to pH 7.5 with NaOH, which precipitated ferrihydrite (Hansel et al., 2003). After equilibrating for 4 h, the solid-phase ferrihydrite was repeatedly rinsed and centrifuged to remove excess salts, then brought back into suspension with DDW and bubbled with N_2.

Preparation of Soil–Water Suspensions

To test the ability of the Good buffers to stabilize pH after titration, 50 g of dried Lillis soil was added to each of seven bottles inside a Coy Anaerobic Chamber (Grass Lake, MI) filled with N_2/H_2 (97.5:2.5). The soils in each bottle were mixed with 180 mL of artificial irrigation water (AIW) containing: NaCl (0.292 g L^{-1}), $MgCl_2{\cdot}6H_2O$ (1.016 g L^{-1}), $CaCl_2{\cdot}2H_2O$ (0.735 g L^{-1}), and Na_2SO_4 (1.775 g L^{-1}). The soil–water suspensions in the seven separate bottles were titrated to either pH 5.0, 5.8, 6.4, 7.0, 7.6, 8.2, or 9.0 by manual additions of HCl or NaOH during a period of 12 d (an arbitrary period of titration time to overcome the majority of acid–base buffering of the soils), after which time the final solution volume was brought to 200 mL with DDW. While vigorously stirring, 50 mL from each soil–water suspension was then transferred into each of four 160-mL serum bottles, which were sealed with butyl rubber stoppers. The bottles were flushed with N_2 for 15 min, and Good buffers at the target pH were added to each to achieve a final concentration of 20 m*M* (Day 0, Fig. 1). The Good buffers used in this study were MES [2-(*N*-morpholino)ethanesulfonic acid], PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)], HEPES [*N*-(2-hydroxyethyl) piperazine-*N*′-2-ethanesulfonic acid)], TAPS (*N*-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid) and CHES [2-(cyclohexylamino)ethanesulfonic acid]. From each set of four bottles, one was autoclaved twice (heat killed), one had 0.04% chloramphenicol added (antibiotic), and two were left alone (live controls). The purpose of the autoclaved sample was to strongly prohibit microbial activity and to minimize microbial effects on pH buffering; similarly, the antibiotic chloramphenicol was used to minimize microbial growth effects on pH. The pH of the soil suspensions was monitored inside the anaerobic chamber by transferring 2.0-mL aliquots of the soil–water suspension using a 16G needle-syringe into polyethylene test tubes and inserting a pH combination electrode (Thermo Electron Corp., Orion no. 9103BN).

The ability of various chemical amendments to buffer soil suspension E_H was initially tested by weighing out 6.0 g of dried Lillis soil into 50-mL polypropylene centrifuge tubes. The soils were shaken overnight with 30 mL of AIW without SO_4^{2-} (AIW–S), which contained: NaCl (1.461 g L^{-1}), $MgCl_2{\cdot}6H_2O$ (2.033 g L^{-1}), and $CaCl_2{\cdot}2H_2O$ (0.735 g L^{-1}). This procedure significantly decreased soluble SO_4^{2-}, which could have been used as an electron acceptor by bacteria. After shaking, the soil suspensions were centrifuged and the solutions decanted before introduction of the soil pellets into the anaerobic chamber. In this and all subsequent experiments, H_2, a strong reductant, was flushed out of the chamber by repeatedly purging with ultrapure N_2. Although trace amounts of O_2 remain inside the chamber in the absence of H_2 (which react with O_2 on the palladium catalyst to form H_2O), we determined that this small amount had little effect on redox measurements. The moist soils were mixed with 30 mL of AIW–S and placed on a rotary shaker inside the glove bag. To each 50-mL tube containing the soil–water suspension, soluble or solid-phase chemicals were added to achieve 5.0 m*M* concentrations. The amendments included DDW (control), dissolved $NaNO_3$, Na_2SO_4, $Na_2C_4H_2O_4$ (fumarate), lactate (1.67 m*M*), $Fe(III)Cl_3{\cdot}6H_2O$, $Fe(II)Cl_2{\cdot}4H_2O$, $Fe(II)SO_4{\cdot}4H_2O$, or Fe(III)NTA, and solid–solution suspensions of $Fe_5HO_8{\cdot}4H_2O$ (ferrihydrite), α-FeOOH (goethite), α-Fe_2O_3 (hematite), Fe_3O_4 (magnetite), or MnO_2(s).

During the next 25 d, the pH and E_H were measured using combination electrodes directly immersed into the soil–water suspension. Redox potentials were measured using a Corning combination Pt electrode (no. 476080) and were corrected for temperature and deviations from a modified Zobell solution (Nordstrom, 1977), and then adjusted to the standard H_2/H^+ electrode. Polarization of the Pt electrode often occurred soon after taking a few measurements in soil suspensions at markedly different E_H values. To overcome this effect, the Pt electrode was soaked for 5 to 10 s between measurements first with a Zobell-type $K_3Fe_3(CN)_6$–$K_4Fe_2(CN)_6{\cdot}2H_2O$ solution followed by 0.5 *M* HCl and DDW. We hypothesize that this technique depolarized the electrode surface and brought about a quicker response to oxidizing or reducing samples than if rinsed with HCl or DDW alone. All E_H measurements were taken either exactly at 5 min or when the reading stabilized (±1 mV min^{-1}), whichever came first.

Additional tests with the above redox amendments, as well as Ti(III)NTA, S, and ascorbic acid reagents, were conducted with fresh Hanford and Imperial surface soils. Besides using freshly collected soil,

Table 3. Characteristics of soils used in pH and redox investigations.

Parameter	Lillis	Hanford	Imperial
Surface area, $m^2\ g^{-1}$	138.4	11.4	147.4
Moisture, %	—	9.2	21.9
Sand, %	37.8	58.2	6.1
Silt, %	22.1	30.5	41.6
Clay, %	40.1	11.4	52.4
Free Fe, %	0.99	0.40	0.71
Free Al, %	0.80	0.25	0.67
Organic C, %	0.47	0.82	0.43
Inorganic C, %	0.07	0.04	1.36
Total N, %	0.11	0.08	0.07
1:5 (w/v) H_2O extracts†			
pH	7.42	7.27	7.87
Electrical conductivity, dS m^{-1}	0.41	0.13	0.61
Alkalinity, $mmol_c\ L^{-1}$	1.83	1.01	3.25
NO_3^-, $mmol_c\ L^{-1}$	0.27	0.29	0.30
SO_4^{2-}, $mmol_c\ L^{-1}$	0.33	0.08	2.17
PO_4^{3-}, $mmol_c\ L^{-1}$	0.03	0.04	<0.01

† Reported values are averages of three extracts.

the major difference between these tests and the tests with the Lillis soils was that the artificial irrigation water was modified to include SO_4^{2-} and K^+ (AIW+S2): NaCl (1.402 g L^{-1}), $MgCl_2{\cdot}6H_2O$ (1.016 g L^{-1}), $CaCl_2{\cdot}2H_2O$ (0.735 g L^{-1}), KCl (0.074 g L^{-1}), and $NaSO_4$ (0.355 g L^{-1}), while keeping salinity nearly constant (~4 dS m^{-1}). The soils were mixed 1:5 (w/v) with AIW+S2 in 160-mL glass serum bottles, accounting for the ambient moisture percentage, and sealed with butyl rubber stoppers. After adding chemical amendments, all bottles were placed onto an orbital shaker (80 rpm) residing outside the anaerobic chamber. For E_H and pH measurements, the bottles were brought into the N_2–filled anaerobic chamber and 2.0 mL of soil–water suspension was removed by 16G needle-syringe and transferred to 15-mL polyethylene test tubes. The pH and E_H were measured by combination electrodes as described above.

Iron and Sulfur Analyses

Immediately after mixing the Hanford or Imperial soils with the AIW+S2 media, 2.0-mL soil–water suspensions were removed from select bottles by needle-syringe and transferred into centrifuge vials. The tubes were centrifuged at 2245 × *g* for 10 min, and solutions were decanted. The sediment vials were then transferred to centrifuge tubes filled with 30 mL of 1 *M* HCl that was previously made anaerobic by bubbling with N_2 for 1 h. The soil pellets were shaken in the 1 *M* HCl for 16 h to extract labile Fe(III) oxides, Fe(II) monosulfides, greigite, and small amounts of Fe silicates (Heron et al., 1994; Lovley and Phillips, 1986). After shaking, the soil extracts were centrifuged for 20 min at 4300 × *g* and filtered through 0.2-µm nylon filters. Separate soil–water suspensions were centrifuged, decanted, and dried at 105°C overnight to adjust the 1 *M* HCl extract data to a dry-weight basis. Following the 34-d monitoring, additional soil–water samples were removed for similar 1 *M* HCl extraction. The Fe(II) and Fe(II) + Fe(III) concentrations of the HCl extracts were measured by the ferrozine method (Hansel et al., 2003), and were used to provide a general estimate of the labile Fe solid phases that resulted from the anaerobic incubations.

After the 34-d incubation, filtrates were collected for S analyses by centrifuging 4.0-mL soil–water suspensions in two microcentrifuge vials at 2245 × *g* for 5 min in the anaerobic chamber. For dissolved S^{2-} preservation, 2.0 mL of the centrifuged suspension was immediately filtered (0.2 µm) and added into glass vacuum tubes containing 2.0 mL of sulfide antioxidant buffer, which consisted of: NaOH (80.0 g L^{-1}), ascorbic acid (35.0 g L^{-1}), and Na_2EDTA dihydrate (67.0 g L^{-1}). The remaining filtrate solution was refrigerated for anion analyses by ion chromatography. Sulfide

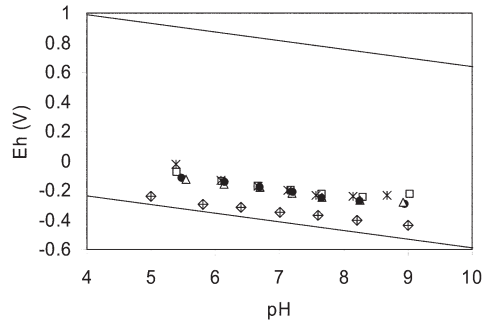

Fig. 2. The pH and Eh values of Lillis soil suspensions on Day 0 (plus symbols) and Day 8 (small symbols: controls [closed circles and open triangles]; autoclaved [cross-hatched]; chloramphenicol [open squares]). Theoretical E_H–pH values for water in equilibrium with H_2 gas partial pressure = 2.53 kPa at the initial pH are also indicated (diamonds).

concentrations were measured using a silver–sulfide ion-specific electrode (Fisher no. 300762). Standards were diluted from a $Na_2S{\cdot}9H_2O$ stock solution that was calibrated against a 0.10 *M* $Pb(NO_3)_2$ solution. Dissolved SO_4^{2-} was analyzed using a Dionex ion chromatography system, which consisted of an AS11 column with 25 m*M* NaOH eluent and an ED40 conductivity detector.

RESULTS AND DISCUSSION

Soil Characteristics

Lillis and Imperial soils are high in surface area while Hanford soils are low, generally reflecting particle size distribution (Table 3). Hanford soils have nearly double the organic C content as Lillis or Imperial soils, the latter having higher inorganic C and slightly lower total N. Chemistries of the water extracts indicate that Imperial soils are slightly more alkaline and saline than Lillis soils, and that Hanford soils generally contain low total dissolve solids but high organic C. Although NO_3 levels in the extracts are similar for the three soils, phosphate levels for the Imperial soils are below the detection level (0.01 $mmol_c\ L^{-1}$).

pH Titration and Stabilization of Lillis Soils

The pH of acid- or base-titrated Lillis soil suspensions was considerably stabilized on addition of 20 m*M* Good buffers, although a slight drift from the target values was observed under acidic conditions (pH 5.0, 5.8, and 6.4) and in the autoclaved (heat-killed) replicates, particularly at higher pH conditions (pH > 7.0) (Fig. 1). The natural buffering ability of the soil constituents continually shifted the pH toward the equilibrium condition, which was approximately pH 7.8 in unbuffered soil–water samples. From the titration data (before Day 0), the greatest pH stability occurred in the pH 7.0 treatment. After titration and addition of Good buffers, the slight 0.1 to 0.3 unit pH shift downward for autoclaved samples in the pH 7.0 to 9.0 range probably resulted from a release of acid-generating constituents during heating; nevertheless, the drop in pH was momentary, with pH remaining stable thereafter.

Besides measuring pH variations in these samples, we also monitored E_H after the 12-d acid–base titration and after the 8-d equilibration with Good buffers. The measured E_H after titration corresponded well with pH and an equilibrium H_2 gas partial pressure of 2.53 kPa, with values ranging from –240 mV at pH 5.0 to –434 mV at pH 9 (Fig. 2). After flushing the bottles with N_2 and mixing the soil–water suspensions with 20 m*M* Good buffers, the

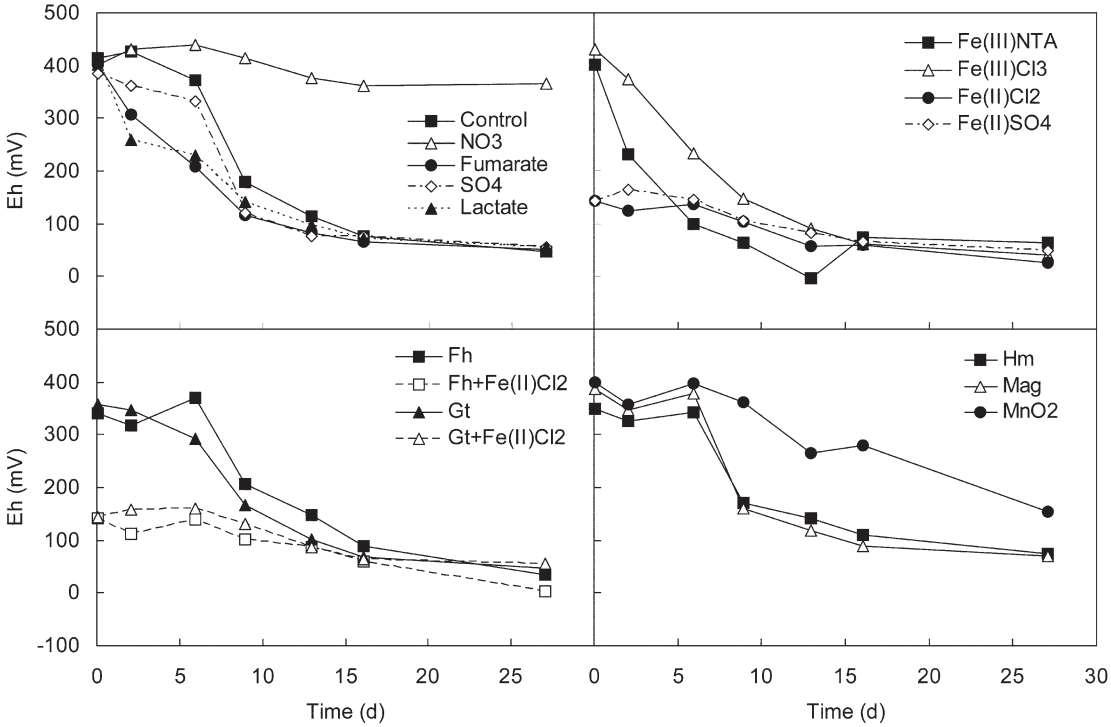

Fig. 3. Changes in E_H with time for Lillis soil suspensions with 5 m*M* soluble or solid-phase suspension concentrations of various chemical amendments. Sample labeled "Control" had no amendment, and the concentration for the lactate sample was 1.67 m*M*. Abbreviations: ferrihydrite (Fh), goethite (Gt), hematite (Hm), magnetite (Mag). Samples labeled +Fe(II)Cl2 were amended with 1 m*M* $FeCl_2$ in addition to 5 m*M* solid-phase suspension.

pH stabilized (Fig. 1), while E_H values increased by approximately 100 mV at pH 5.8 to 150 mV at pH 8.9, reflecting the loss of H_2. By using this technique, we can deduce that pH and E_H variations for strongly reducing soils will be best constrained in the pH range of 6 to 8.2, which encompass most agricultural soils. For extreme pH perturbations outside this range, variations will be greater due to natural pH and redox buffering of the soil constituents, such as bioavailable organic matter, colloidal Fe and surface-bound Fe^{2+}, Fe–Al (hydr)oxides, aluminosilicates, etc.

Chemical Amendment Effects on Redox

Lillis Soils

Our first set of experiments to observe redox stability following the addition of redox-active chemical amendments was conducted with soil–water suspensions using dried Lillis soils. Redox levels (E_H) in the soil–water suspensions with AIW–S media dropped to <100 mV by Day 15 for all but NO_3^-– or MnO_2–amended samples (Fig. 3). The most rapid decreases in E_H were observed for samples amended with fumarate or lactate, yet differences between these and the SO_4^{2-}–amended samples or the control became negligible after 15 d. Redox levels for $FeCl_3$–amended samples were approximately 100 mV higher than Fe(III)NTA-amended samples during the first 15 d. The NTA^{3-} ligand probably enhanced solubility and bioavailability of Fe^{3+} over that of the Cl^- anion, thereby increasing Fe(III) microbial reduction and Fe(II) formation and lowering redox values. For samples amended with $FeCl_2{\cdot}2H_2O$ or $FeSO_4{\cdot}6H_2O$, E_H began at approximately 150 mV and dropped to 0 to 50 mV by 27 d. Although Fe^{2+} can quickly adsorb onto clays and other soil mineral surfaces, sufficient Fe^{2+} remained in solution to drive Pt electrode readings lower. Essentially no differences in E_H were observed between the control and samples amended with Fe (hydr)oxides, and additions of $Fe(II)Cl_2$ to ferrihydrite- and goethite-amended samples did not deviate significantly from the Fe(II)-amended samples without the ferric (hydr)oxides. The sample amended with MnO_2 showed intermediate behavior, with a slow gradual decrease in E_H from 400 mV on Day 0 to 150 mV on Day 27.

Hanford and Imperial Soils

Our next set of experiments was designed to test a wider range of redox-active chemical amendments on the soil–water redox condition, as measured by the Pt electrode and by Fe and S analyses, with freshly collected Hanford or Imperial soils. These amendments included many of those used for the Lillis soils as well as reduced S complexes, ascorbic acid, and Ti(III)NTA. For comparison purposes, we simultaneously tested Hanford and Imperial soils, which vary significantly in surface area, C content, alkalinity, and soluble phosphate content (Table 1). We also evaluated Fe^{2+}–Fe^{3+} and HS^-–SO_4^{2-} redox couples in these soils to compare measured E_H to chemical status.

Platinum Electrode Response. Large differences were observed in redox potentials during 34 d for the Hanford and Imperial soil–water suspensions amended with redox-active gases, soluble NO_3, Fe(III)- or Ti(III)-NTA complexes, or ascorbate (Fig. 4), and solid-phase Fe and Mn (hydr)oxides or soluble S compounds (Fig. 5). Not surprisingly, measured redox potentials deviated substantially from the values in Table 2 of the oxidation–reduction potential for the listed half-reactions at pH 7, 25°C, at concentrations <10 m*M*.

The redox-inactive 5.0 m*M* NaCl amendment, with N_2 gas in the bottle headspace, served as a reference, or control, for each soil. Changes in E_H for the Hanford soil control were similar to

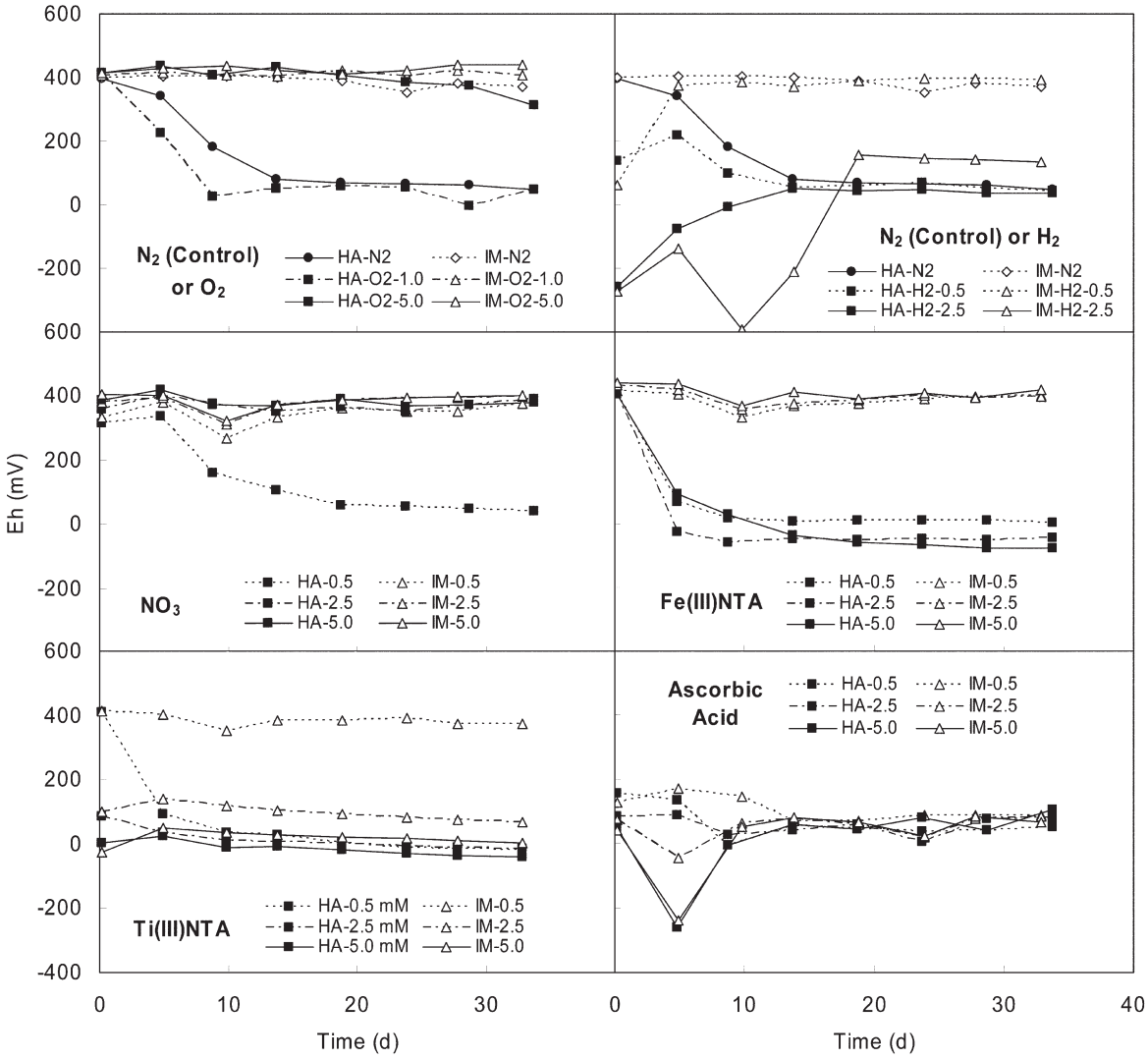

Fig. 4. Changes in E_H with time for Hanford (HA) and Imperial (IM) soil–water suspensions for samples with varying amounts of gases (O_2 or H_2) and soluble Fe, Ti, or ascorbate (as ascorbic acid). Concentrations (in m*M*) are listed following sample label.

changes observed in the Lillis control (Fig. 3), with levels stabilizing at approximately 50 mV after 24 d. Only a slight deviation from moderately oxidizing conditions (400 mV) was observed for the Imperial control sample or O_2–amended samples during the study. In contrast, a large drop in E_H was observed for the Hanford soil sample amended with 1 m*M* $O_{2(g)}$. We attribute this drop to stimulation of facultative microorganisms (possibly due to higher organic content in Hanford soils), which rapidly consumed the O_2 electron acceptor and which initially enhanced the onset of reducing conditions. At higher O_2 concentrations, E_H levels remained at 400 mV until Day 34, when a drop to 300 mV was observed. Redox potentials for the H_2–amended Hanford soils equilibrated at approximately 40 mV after starting out moderately reducing (150 mV for 0.5 m*M*) and very reducing (–270 mV for 2.5 m*M*). For Imperial soils, both concentrations of H_2 initially induced low redox potentials (80 and –280 mV, respectively), yet by Day 33, E_H increased by at least 300 mV for each. For NO_3–amended Hanford soils, the decreases in E_H were similar to that of the control for only the 0.5 m*M* concentration, with 2.5 and 5.0 m*M* levels remaining oxidizing (~400 mV). As expected based on the O_2 data, redox potentials for the NO_3–amended Imperial soils remained above 350 mV for all concentrations.

The addition of Fe(III)NTA and Ti(III)NTA amendments to Hanford soils induced slightly lower redox potentials by Day 34 compared with the control, with final potentials ranging from 3 to –80 mV (Fig. 4). In contrast, almost no evidence for Fe(III)NTA reduction and highly concentration-dependent Ti(III)NTA responses were observed for the Imperial soil samples. Decreases in redox potentials in the Hanford soils probably correlate to formation of soluble Fe(II), since the Pt electrode is highly responsive to the Fe(III)–Fe(II) couple. Under ambient conditions, equimolar concentrations of Fe(III)NTA and Fe(II)NTA, which may occur at some point on reduction, should result in E_H values of 350 mV (Straub et al., 2001). This was not observed, as E_H values rapidly decreased to <100 mV across the 0.5 to 5.0 m*M* Fe(III)NTA concentration range. Titanium in the +III oxidation state, stabilized by strong ligands such as citrate, is occasionally used as a strong redox buffer for microbial media (Jones and Pickard, 1980; Zehnder and Wuhrmann, 1976). Thus, it is not surprising that Ti(III) at these concentration levels lowered the soil–water redox potentials to –15 to –40 mV in the Hanford soils. The suggested

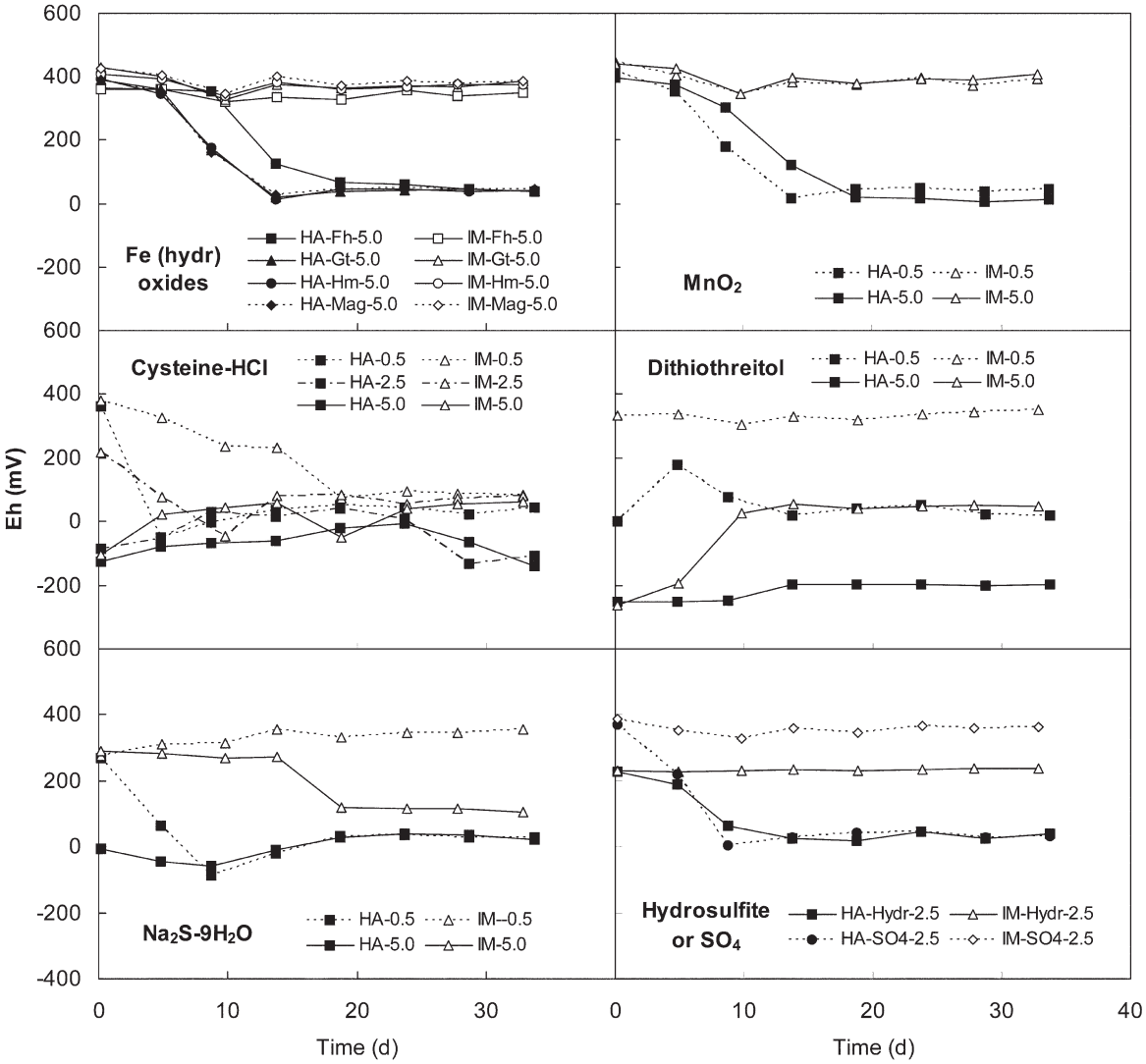

Fig. 5. Changes in E_H with time for Hanford (HA) and Imperial (IM) soil–water suspensions for samples with varying amounts of Fe or Mn (hydr)oxides (as solid–liquid suspensions) and S compounds. Abbreviations for Fe (hydr)oxides are ferrihydrite (Fh), goethite (Gt), hematite (Hm), and magnetite (Mag). Concentrations (in m*M*) are listed following sample label.

concentration of 1 to 4 m*M* Ti(III) citrate for microbial media should result in E_H values below −300 mV (Jones and Pickard, 1980); hence, complexation reactions of Ti(III)NTA with soil particles probably affected the redox condition of the soil. In contrast to the Hanford soils, redox potentials for Imperial soils showed a strong dependence on Ti(III)NTA concentration, with lower values occurring at higher concentrations. Surprisingly, redox levels remained relatively stable (i.e., <100 mV variation) over the duration of the tests.

Measured redox potentials for ascorbic acid amendments were similar for both Hanford and Imperial soils (Fig. 4), indicating that the reactivity and buffering ability of low concentrations of ascorbic acid remain high even in a dense soil–water suspension. Redox potentials were highly concentration dependent, with initial readings of 154, 84, and 57 mV for Hanford soils and 127, 72, and 43 mV readings for Imperial soils for 0.5, 2.5, and 5.0 m*M* concentrations, respectively. At 5 d, E_H levels for the 5.0 m*M* concentration dropped significantly, to −260 and −240 mV for Hanford and Imperial soils, respectively, but rose quickly thereafter. After 15 d, E_H stabilized to 50 to 100 mV for each soil type, regardless of the initial concentration. It is possible that the ascorbic acid–dehydroascorbate acted catalytically with other oxidants and reductants in the soil–water suspension, as low concentrations yielded similar Pt electrode readings to higher concentrations after 14 d.

As was found in experiments with Lillis soils, solid-phase suspensions of Fe (hydr)oxides did not induce significantly different redox potentials compared with the NaCl–N_2 controls for either Hanford or Imperial soils (Fig. 5). For Hanford soils with these amendments, reducing conditions were evident after 4.8 d, with readings stabilizing around 45 mV thereafter. For the Imperial soils, reducing conditions were not observed as readings remained between 340 and 440 mV. In any of the three soils, redox potentials do not approach equilibrium conditions between Fe^{2+} and the solid-phase Fe (hydr)oxides (Table 2) under anoxic conditions (Straub et al., 2001; Thamdrup, 2000). We hypothesize that equilibration between the (hydr)oxide surfaces and Fe^{2+} is impeded by surface complexation with soil organics or that equilibration times between the Fe^{2+} and the Fe (hydr)oxide minerals are longer than the experimental time period.

Manganese(IV) oxides are typically considered to be strong oxidants in soils (Table 2), and are known to oxidize a number of trace elements on adsorption, e.g., Se(IV) → Se(VI) (Scott and

Morgan, 1996) and As(III) → As(V) (Manning et al., 2002). We were surprised to observe that MnO_2 did not inhibit reduction or elevate redox potentials significantly above that of the Fe (hydr)oxides or the NaCl control for Hanford soils. Only a slight delay in reduction was observed for the 5.0 m*M* MnO_2–amended sample between Days 7 and 15. The lack of redox buffering for this strong, solid-phase oxidant is surprising given that, during a period of 27 d, Pt electrode readings for the first set of experiments using Lillis soils were intermediate, only drifting down to ~150 mV at experiment termination (Fig. 3). Inhibition of redox buffering in these samples may partially be explained by poisoning of MnO_2–reactive surfaces by reduction, release, and readsorption of Mn^{2+} onto active sites, such as that observed in pentachlorophenol oxidation by MnO_2 suspensions (Petrie et al., 2002).

As expected, chemical amendments containing reduced S moieties drove redox potentials down for both Hanford and Imperial soil–water suspensions, particularly at higher concentrations (Fig. 5). Redox potentials for cysteine·HCl amendments showed strong initial concentration dependence for both soils. For the Hanford soils, the concentration dependence on E_H remained up until Day 34, while Imperial soil E_H levels converged at 60 to 80 mV regardless of concentration. Another commonly used reductant for microbial media, dithiothreitol, also showed strong concentration dependence for both soils. Redox potentials stabilized at 19 and 350 mV (0.5 m*M* initial concentrations), and −198 and 49 mV (5.0 m*M* initial concentrations) for Hanford and Imperial soils, respectively. The $Na_2S{\cdot}9H_2O$ showed slight concentration dependence initially for Hanford soils, yet final readings stabilized at ~20 mV for both 0.5 and 5.0 m*M* concentrations. Initially there was little concentration effect for $Na_2S{\cdot}9H_2O$ with Imperial soils, but by experiment termination a 250-mV difference was observed in final E_H levels between 0.5 and 5.0 m*M* concentrations. Sodium hydrosulfite ($Na_2S_2O_4$) decreased redox potentials in Hanford soils by approximately 200 mV lower than Imperial soils, possibly because of the overall greater microbial activity and organic C content in Hanford soils. Redox potentials for Na_2SO_4–amended samples were very similar to NaCl controls for both soils, indicating that any additional SO_4 reduction was not accompanied by decreases in electrode readings.

Iron and Sulfur Analyses. To further evaluate changes in the redox status of the two soils with the different chemical amendments, we analyzed the redox speciation of two major redox couples, Fe(II)–Fe(III) in 1 *M* HCl extracts and S(−II)–SO_4^{2-} in aqueous filtrates. Although the 1 *M* HCl extracts and filtrates for S species were taken 14 and 22 d, respectively, after the end of the last Pt electrode measurement, we expect that the majority of biochemical changes induced by the chemical amendments would have already occurred by this time and only minor variations after Day 34 would be expected.

As an extractant, low- to moderate-strength HCl (e.g., 0.5 or 1.0 *M*) may be useful as a general indicator of the redox status of soils and sediments, since it can remove the more easily soluble Fe(III) oxides, Fe(II) monosulfides, greigite, and minor amounts of Fe silicates (Heron et al., 1994; Lovley and Phillips, 1986). Consequently, we used the Fe(II)/Fe(III) ratios in 1.0 *M* HCl as a rapid, overall (aqueous plus labile solid phase) redox indicator for the soil–water suspensions.

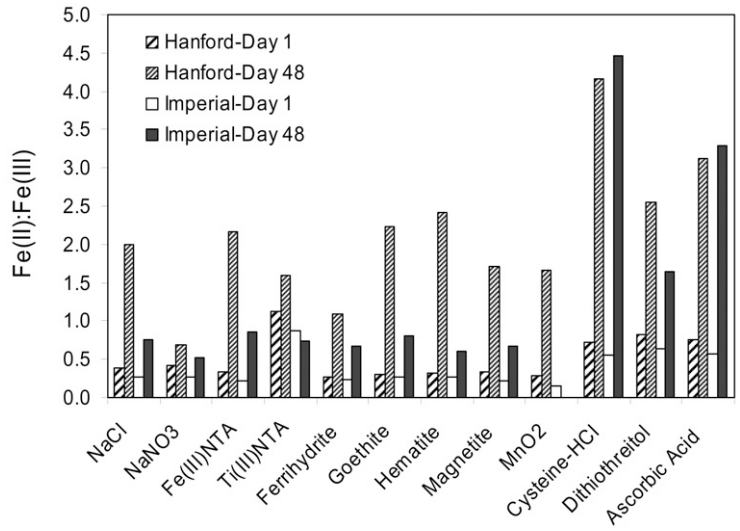

Fig. 6. Ratios of 1 *M* HCl extractable Fe(II)/Fe(III) on Days 1 and 48 for the 5.0 m*M* soluble or suspended solid-phase chemical amendments. Note missing value for Imperial soil amended with MnO_2, Day 48.

Figure 6 shows the relationships of the reduced couples Fe(II)–Fe(III) for 1 *M* HCl filtrates taken on Days 1 and 48 for samples amended with select soluble or solid-phase suspensions at 5 m*M* concentrations. The Fe(II)/Fe(III) ratios on Day 1 were all below 0.5, except for samples amended with Ti(III)NTA, cysteine·HCl, dithiothreitol, and ascorbic acid. These reductants clearly induced a rapid, direct chemical reduction of Fe(III) in the soil particles compared with the other amendments, which were probably dominated by microbial Fe(III) reduction. In general, Fe(II)/Fe(III) ratios on Day 1 were slightly higher for Hanford soils than they were for Imperial soils. Since Imperial soils were higher in citrate–dithionite-extractable Fe content (Table 3), it appears that these soils contain much higher levels of Fe(III) [and lower Fe(II)/Fe(III) ratios] than the Hanford soils. This observation suggests that Imperial soils exist in a more highly oxidized state than Hanford soils, even though both are well-drained surface soils, or that the Imperial soils contain more crystalline, less reactive Fe(III) (hydr)oxides, such as goethite, than the Hanford soils.

Over the 48-d test period, most samples showed moderate to large increases in Fe(II)/Fe(III) ratios (Fig. 6). The single exception to this observation was for the Ti(III)NTA-amended Imperial soils, which had a slight decrease in the Fe(II)/Fe(III) ratio. Only minor increases were observed in 5.0 m*M* $NaNO_3$–amended samples for both soils, reflecting substantial Fe(III) reduction inhibition by this level of NO_3^-. Relative increases in Fe(II)/Fe(III) ratios were of similar scale for Fe and Mn (hydr)oxides as they were for the NaCl controls for either soil. Ferrihydrite-amended samples showed suppression in the relative increases in Fe(II)/Fe(III) ratios. The reasons for this are unclear, but may be due to a greater solubility of ferrihydrite in 1 *M* HCl (releasing higher levels of Fe^{3+}) over that of the other Fe (hydr)oxides within the 18-h extraction period. Additionally, even though redox levels for many of the tested Imperial soil slurries did not change according to the Pt electrode, slight increases in Fe(II)/Fe(III) in 1 *M* HCl extracts for Imperial soils during 48 d suggests possible Fe(II) production that was not detected in solution or by Pt electrode measurements.

Final 1 *M* HCl extractable Fe(II)/Fe(III) ratios for all chemically amended samples are shown in Fig. 7. For the Hanford soils,

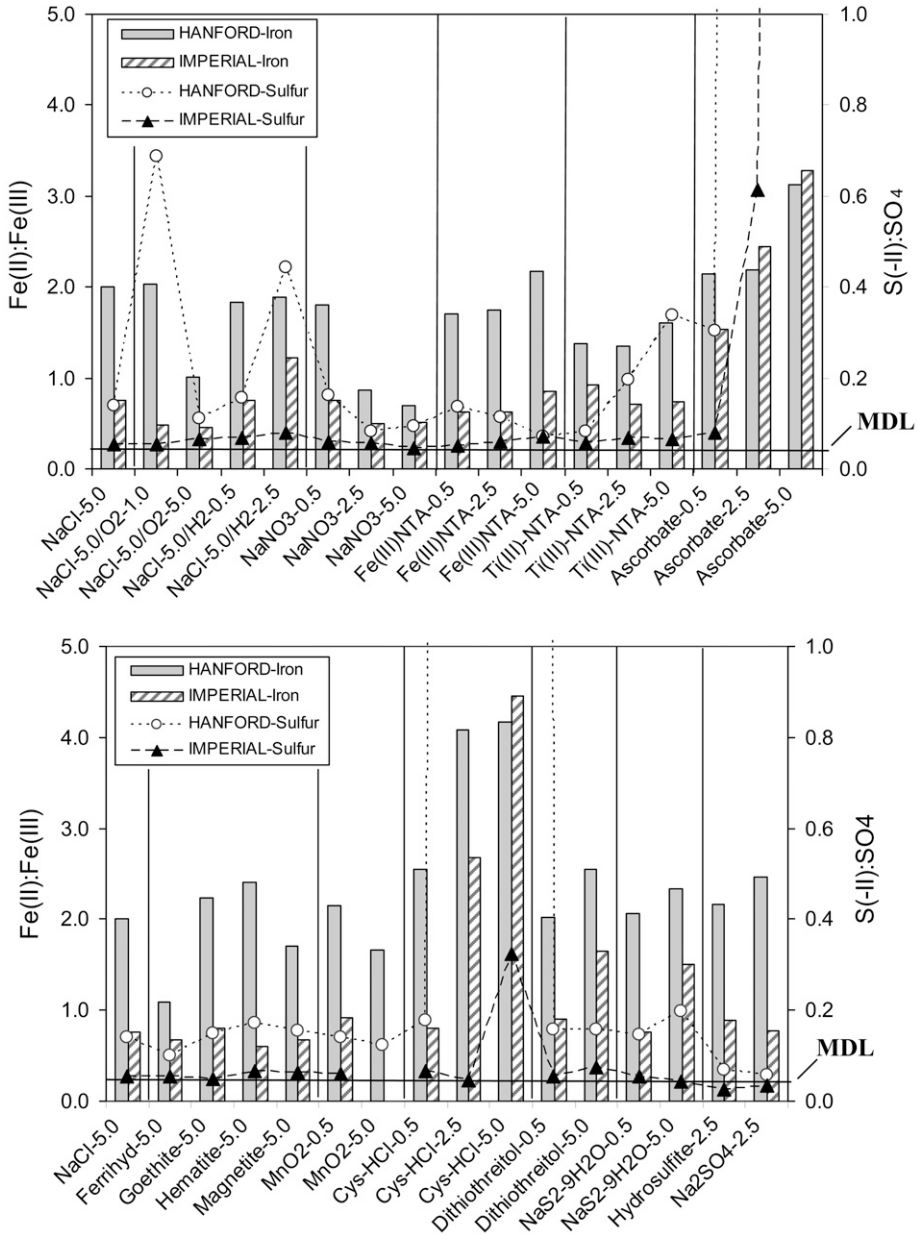

Fig. 7. Effects of chemical amendments on 1 *M* HCl extractable Fe(II)/Fe(III) (Day 48) and soluble S(−II)/SO_4 ratios (Day 56) for all Hanford and Imperial soil suspensions. Method detection limit (MDL) for S(−II)/SO_4^{2-} ratio is 0.05. Note missing values for Imperial Valley, MnO_2 amendment, Day 48.

the final Fe(II)/Fe(III) ratio of the control (2.0) is substantially greater than that found for the oxidizing amendments, 5.0 m*M* O_2 and 2.5 and 5.0 m*M* $NaNO_3$, and for the ferrihydrite amendment. Ratios of Fe(II)/Fe(III) >2.0 were observed in 5.0 m*M* ascorbic acid and 2.5 and 5.0 m*M* cysteine·HCl amendments, again reflecting chemical reduction of soluble and solid-phase Fe(III) beyond that which would be produced microbially. Surprisingly, the other strong reductants, dithiothreitol, $Na_2S{\cdot}9H_2O$, and $Na_2S_2O_4$ (sodium hydrosulfite), did not substantially increase this ratio compared with the control. For the Imperial soils, slight decreases in the Fe(II)/Fe(III) ratios were observed again for the oxidizing 1.0 and 5.0 m*M* O_2 and 2.5 and 5.0 m*M* $NaNO_3$ amendments compared with the control (0.75). All concentrations of ascorbic acid and 2.5 and 5.0 m*M* concentrations of cysteine·HCl dramatically increased the Fe(II)/Fe(III) ratio in the Imperial soils, while less substantial increases were observed for 2.5 m*M* H_2 or $Na_2S_2O_4$, 5.0 m*M* dithiothreitol, and 5.0 m*M* $Na_2S{\cdot}9H_2O$ amendments.

Evaluation of redox changes based on S(−II)/SO_4^{2-} ratios in aqueous filtrates is less straightforward, since the S^{2-} detection limit with the sulfide electrode was 1.5×10^{-4} *M*. Based on this background value, and an average dissolved SO_4^{2-} concentration of 3.34 m*M* for the Imperial soil–water suspensions, we estimate the detection limit for the S(−II)/SO_4^{2-} ratio to be 0.05. This value serves as a suitable basis for comparison of soluble S redox chemistries in the two soils. We must also note that the reduced S compounds, cysteine·HCl and dithiothreitol, did not induce any S electrode response beyond that of the blanks.

Dissolved S(−II) concentrations in nearly all Hanford soil–water filtrates were greater than 0.15 m*M*, indicating that bacterial SO_4^{2-} reduction was active in most, if not all, samples. Indeed, nearly all Hanford soil 1 *M* HCl extracts used for Fe(II)/Fe(III) ratio determination smelled of sulfide (after acidification). Only a few samples showed enhanced S(−II)/SO_4^{2-} ratios over that of the NaCl control (0.14), namely 1.0 m*M* O_2 (0.69), 2.5 m*M* H_2 (0.44), ascorbic acid (0.30, 12.87, and 51.15 for 0.5, 2.5, and 5.0 m*M*, respectively) and cysteine·HCl (0.18, 12.10, and 43.97 for 0.5, 2.5, and 5.0 m*M*, respectively) (Fig. 7). These samples also showed significant depletion of soluble SO_4^{2-} concentrations compared with the initial media concentration of 2.5 m*M*. In addition, mass balance of soluble S (2.5 m*M* based on initial SO_4^{2-} concentrations) was only observed in the most oxidizing samples: 5.0 m*M* O_2 and 2.5 or 5.0 m*M* $NaNO_3$. Excluding these and the most reduced samples, total soluble S averaged 1.76 ± 0.32 m*M* (n = 19), indicating that much of the S was sequestered within the solid phase, most likely as reduced sulfide precipitates. Total S for the 2.5 m*M* Na_2SO_4 and $Na_2S_2O_4$ amendments was approximately 3.7 m*M*, with about 3.5 m*M* being SO_4^{2-}, reflecting that SO_4^{2-} was added directly as an amendment (Na_2SO_4) or that some of the $Na_2S_2O_4$, which in solution forms HSO_3^- and $S_2O_3^{2-}$ (Cotton and Wilkinson, 1988), may have disassociated chemically or through bacterial dismutation to form SO_4^{2-}, e.g., $4SO_3^{2-} + H^+ \rightarrow 3SO_4^{2-} + HS^-$, or $S_2O_3^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+$ (Cotton and Wilkinson, 1988; Fuseler et al., 1996; Istok et al., 1999).

For the Imperial soils, enhanced S(−II)/SO_4^{2-} ratios over that of the control (0.05) were found only for 2.5 and 5.0 m*M* ascorbic acid (0.61 and 6.65, respectively) and 5.0 m*M* cysteine·HCl (0.32) (Fig. 7). For other samples, excluding $Na_2S_2O_4$ and Na_2SO_4 amendments, total dissolved S averaged 3.53 ± 0.30 m*M* (n = 26).

Of this dissolved S, 3.34 ± 0.29 m*M* was comprised of SO_4^{2-}, indicating that substantial dissolution of SO_4 salts occurred, since the concentration of SO_4^{2-} in the initial media was 2.5 m*M*.

In general, the presence of soluble sulfides in the soil–water suspensions signifies that SO_4–reducing conditions are present in most of the Hanford soil–water suspensions, while Imperial soils either sequestered much of the S^{2-} in the sediments or lacked any significant SO_4^{2-} reduction capability. Moreover, soluble S(–II) indicates that an excess of the reduced S(–II) occurred over that of soluble metal ions, which can form sulfide precipitates (e.g., FeS–FeS_2, MnS, etc.), or that S(–II) is in excess of other possible adsorbing or complexing substances (e.g., organics, clays, etc.).

Combined with Fe(II)/Fe(III) ratios from 1 *M* HCl extracts, which remove labile Fe, we can use the S(–II)/SO_4^{2-} ratios to indicate relative redox intensity effects from the different chemical amendments. Both redox couple ratios were significantly enhanced for ascorbic acid and cysteine·HCl amendments in the Hanford and Imperial soils, suggesting that even though these amendments were not always the most reducing according to Pt electrode readings, they stimulated the greatest chemical and microbial reduction in the soil–water system. Surprisingly, two of the strongest reducing agents, Ti(III)NTA and dithiothreitol, showed only minor increases in the final S(–II)/SO_4^{2-} or Fe(II)/Fe(III) ratios over that of the controls, but not both. Titanium(III)NTA induced a slight increase in S(–II)/SO_4^{2-} ratios for Hanford soils but not Imperial soils; similarly, dithiothreitol only stimulated slight increases in Fe(II)/Fe(III) ratios for both soils. Thus, the Pt electrode responses for these reductants may not always indicate the overall redox intensity of the soil–water system.

Iron and Sulfur Controls on Platinum Electrode Readings

To further evaluate how well the Pt electrode estimates E_H of the soil–water system, we compared theoretical E_H values based on Fe^{2+} and Fe^{3+} or SO_4^{2-} and HS^- concentrations to the measured E_H for the Hanford soil–water suspensions. Soluble Fe concentrations in the soil–water filtrates taken on Day 35 were determined by the ferrozine method. Since spuriously high concentrations of soluble Fe(III) were also found using this method, due to colloidal $Fe(OH)_3$ passing through 0.2-µm pore-sized filters, we estimated Fe^{3+} concentrations based on solubilities of amorphous $Fe(OH)_3$ (analogous to ferrihydrite) or α-FeOOH (goethite). Dissolved Fe^{3+} concentrations, which are highly pH dependent, were estimated from the solubility relationships: amorphous $Fe(OH)_3(s) = Fe^{3+} + 3OH^-$, which has a logarithm of the equilibrium constant (log *K*) of –38.7, or α-FeOOH + $3H^+ = Fe^{3+} + 2H_2O$, which has a log *K* of 1.6 (Stumm and Morgan, 1981). The Nernst equation was used to estimate the redox potential based on dissolved Fe^{2+} and Fe^{3+} or SO_4^{2-} and HS^- concentrations using the following relationships, with reference E_H (E_H^0) values taken from Stumm and Morgan (1981):

$$Fe(OH)_3 + 3H^+ + e^- \leftrightarrow Fe^{2+} + 3H_2O\,;E_H^0 = +1.01\,V \quad [1]$$

$$Fe^{3+} + e^- \leftrightarrow Fe^{2+}\,;\,E_H^0 = +0.77\,V \quad [2]$$

$$SO_4^{2-} + 9H^+ + 8e^- \leftrightarrow HS^- + 4H_2O\,;E_H^0 = +0.24\,V \quad [3]$$

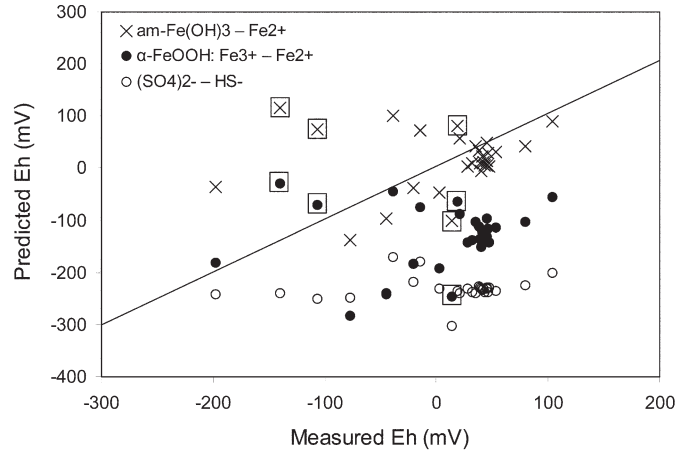

Fig. 8. Predicted vs. measured (Pt combination redox electrode) Eh based on soluble concentrations of Fe^{2+} (measured) and Fe^{3+} (estimated, in equilibrium with amorphous (am-)$Fe(OH)_3$ or α-FeOOH solid phase) for Hanford soil–water suspensions. The E_H comparisons based on the SO_4^{2-}–HS^- (measured) redox couple are also shown. Measured Fe^{2+} concentrations for highlighted (square-enclosed) samples were less than the detection limit (9.0×10^{-6} *M*) and were estimated at 1.0×10^{-6} *M*. Straight line indicates 1:1 correlation between measured and predicted E_H values.

For Eq. [1], the Nernst equation is

$$E_H = E_H^0 + \frac{2.303RT}{nF}\log\left[\frac{(H^+)^3}{(Fe^{2+})}\right] \quad [4]$$

where *R* is the universal gas constant (8.314510 J K^{-1} mol^{-1}), *T* is temperature (K), *n* is the number of electrons transferred in the half-reaction, and *F* is the Faraday constant (the charge per a mole of electrons = 9.6485309×10^4 C mol^{-1}). Equation [4] can be reduced to $E_H = 1.01 - 0.177pH - 0.059\log(Fe^{2+})$. Similarly, the Nernst equation for Eq. [2] reduces to $E_H = 0.77 + 0.059\log(Fe^{3+}) - 0.059\log(Fe^{2+})$, and Eq. [3] reduces to $E_H = 0.24 + 0.007375\log(SO_4^{2-}) - 0.066375pH - 0.007375\log(HS^-)$.

Comparisons between measured and predicted E_H values for Hanford soil–water suspensions are shown in Fig. 8. Correlations between predicted and measured E_H were best approximated by using the amorphous $Fe(OH)_3$–Fe^{2+} redox couple, particularly for samples falling in the range of 0 to 100 mV. Concentrations of Fe^{3+} based on α-FeOOH solubility were an order of magnitude lower than amorphous $Fe(OH)_3$, which subsequently underpredicted E_H in comparison to amorphous $Fe(OH)_3$ by approximately 100 mV. We attribute differences between measured and predicted E_H to the 5-min Pt electrode measurement interval. Typically, readings were taken at exactly 5 min after insertion of the electrode into the 2-mL soil–water suspension, and remarkable consistency for individual samples measured on different days was observed. In some cases, Pt electrode readings reached stability (i.e., < ±1 mV min^{-1}) within a few minutes. Yet in most cases, Pt electrode readings continued a slow downward drift beyond the 5-min interval, particularly for the more reducing samples. Ideally, the Pt electrode should be allowed to stabilize completely (which may take up to or longer than 30 min); however, time constraints prohibited us from allowing each sample to reach stability. Nevertheless, a 5- or 10-min interval has been stated as being sufficient time to obtain a suitable and reproducible

Pt electrode reading for natural waters (Hamilton et al., 2004), and our current results support this claim.

A few notable samples deviated substantially from the 1:1 measured/predicted E_H correlation line (square-enclosed samples in Fig. 8). For these samples, E_H values were calculated using 10^{-6} *M* Fe^{2+}, since soluble Fe^{2+} was undetectable (limit of detection 9.0×10^{-6} *M*). For cysteine·HCl^- (measured $E_H = -107$ and -140 mV) and dithiothreitol- (19 mV) amended samples, predicted E_H values were substantially greater than measured values. Sulfide production in these highly reduced samples probably precipitated much of the soluble Fe^{2+}; consequently, calculations overestimated E_H. We hypothesize that the Pt electrode is increasingly responsive to the SO_4^{2-}–HS^- redox couple at the lower E_H conditions, particularly in the absence of soluble Fe^{2+}. Nevertheless, E_H values based on measured SO_4^{2-} and HS^- concentrations were substantially lower than the predicted E_H values for all but 5.0 m*M* dithiothreitol (−200 mV). These observations confirm that the Pt electrode is most responsive toward the Fe^{3+}–Fe^{2+} redox couple, even at circumneutral pH, than it is toward the SO_4^{2-}–HS^- redox couple.

It is also noteworthy to compare the predicted vs. measured E_H values for the Fe(III)NTA- and Ti(III)NTA-amended samples, which fell into the measured E_H range of 3 to −80 mV. Simple E_H predictions based on soluble Fe^{2+} and Fe^{3+} concentrations, as determined by the ferrozine method and incorporated into Eq. [2], yielded E_H values within ±200 mV of the measured values. Predictions were not significantly improved in more comprehensive equilibrium models that allow for Fe–NTA or Fe–S interactions, such as with Visual MINTEQ (developed by J.P. Gustafsson and available at www.lwr.kth.se/English/OurSoftware/vminteq/index.htm), a Windows version of MINTEQA2 (Allison et al., 1991) (data not shown). Predictive equilibrium redox modeling for these samples is difficult due to complex interactions between Fe(II)/Fe(III)–NTA, Ti(III)–NTA and its oxidation products, Fe- or Ti-reduced sulfides, and NTA^{3-} with other soil constituents.

CONCLUSIONS

The study of pH and redox effects on contaminant reactions in soils typically requires frequent addition of strong acids, bases, oxidants, and reductants, all of which can perturb the pseudoequilibrium condition of the soil. We explored the ability of organic buffers and redox-active chemicals to stabilize the pH and E_H of aqueous suspensions of three soils, during a period of approximately 1 mo, using only single additions to minimize disturbances to the samples. We observed that after titrating soil–water suspensions to target pH ranges between 5.0 and 9, Good buffers were able to stabilize the pH during a period of 8 d at the equilibrium condition (pH 5.8–8.9), and perturbations such as autoclaving or the addition of antibiotics only shifted the pH from control values by approximately 0.3 units. We also found that commonly available reductants could stabilize the soil–water suspension E_H levels for extended periods of time, and that the final redox state was highly dependent on initial concentrations as well as on native soil conditions. A potential advantage of using single additions of chemical amendments to closed-system soil–water suspensions is that it allows simultaneous evaluation of chemical and microbial reactions across many stable pH and E_H conditions for individual soil samples. This lessens uncertainties in the pH or redox condition that can arise when comparing samples collected at different times or locations (heterogeneities), or when samples are altered by frequent acid–base or oxidant–reductant additions.

In terms of poising the soil–water system at target E_H values using chemical amendments, initial C (nutrient) and microbial activities will greatly dictate which chemicals, and what concentrations, will be necessary to achieve the desired E_H according to the Pt electrode. This was fully evident for many of the tested chemicals, which brought about different responses for the nutrient-rich and microbiologically active Lillis and Hanford soils compared with the highly oxidized, lower nutrient, and biologically suppressed Imperial soils. Also, E_H, as determined by the Pt electrode, does not always correspond with other redox indicators, such as described above for Ti(III)NTA and dithiothreitol. Nevertheless, it appears to be easiest to decrease E_H below that which will occur in anoxic controls by using strong, sulfidic reductants. Nitrate amendments serve well in stabilizing E_H around 300 to 400 mV, which is not much lower than E_H for oxidized (O_2–equlibrated) soils. Below control conditions (40–70 mV), Fe(III)NTA surprisingly stabilizes E_H levels in the range of 0 to −80 mV, and similar E_H ranges could also be found for Ti(III)NTA additions. Cysteine·HCl readily decreased redox potentials down to the −100 to −200 mV range, and Fe(II)/Fe(III) and S(−II)/SO_4^{2-} ratios also increased dramatically. Dithiothreitol induces similar E_H responses as cysteine·HCl, but not so for Fe(II)/Fe(III) and S(−II)/SO_4^{2-} ratios. Ascorbic acid appears to be a stronger reductant than Pt electrode readings would indicate. Although the E_H readings in both soils stabilized at approximately 50 mV, 5.0 m*M* concentrations induced a momentary drop in E_H on Day 5 to −250 mV, and highly elevated Fe(II)/Fe(III) and S(−II)/SO_4^{2-} ratios apparently are associated with the strong reducing capacity of this amendment.

Finally, we recognize that the addition of high concentrations of the oxidized and reduced forms of the chemicals may help to poise a system closer to the intended thermodynamic E_H^0 of the redox couple. In these instances, such as in the addition of 20 m*M* NO_3^- and 30 m*M* NO_2^-, unnaturally high concentrations of oxidants or reductants may force the microbial populations to consume all naturally available electron donors (and similarly electron acceptors if reductants are added) as they attempt to balance energy gradients. Depending on the type of contaminant investigations, these changes in nutrient status may be unwanted. Since each soil will be unique in chemical and nutrient condition, preliminary redox and pH buffering tests on the soils undergoing investigation are recommended.

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