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Optimized high-throughput methods for quantifying iron biogeochemical dynamics in soil

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ABSTRACT

Iron (Fe) redox cycling and sorption/complexation reactions influence numerous soil biogeochemical processes, and the precise, rapid, and low-cost determination of reactive Fe pools is critical for understanding these dynamics. Colorimetric methods are often used to measure Fe, yet assay conditions vary widely among studies, and the robustness of these methods and their potential interferences remain poorly characterized. Here, we developed optimized ferrozine methods (modified from water and sediment protocols) to determine Fe concentrations in three common soil extractions: reduced (Fe(II)) and oxidized Fe (Fe(III)) in 0.5 M HCl (Fe_{HCl}), and Fe extracted by citrate-ascorbate (Fe_{ca}) and citrate-dithionite (Fe_{cd}). These methods were adapted for 96-well microplates by employing increased buffer concentrations and longer incubation times relative to published cuvette methods. Iron quantitation was sensitive to the final pH of the reaction mixture and duration of incubation period, factors that have varied widely in previous studies. We obtained consistent results with an assay pH near 7, half hour incubations for Fe_{HCl}, and 1 h incubations for Fe_{ca} and Fe_{cd}. These ferrozine methods compared favorably with inductively coupled plasma optical emission spectrometry (ICP-OES) across a broad range of soils, including Oxisols, Mollisols, and Inceptisols with as much as 18% organic C. Iron determination in HCl extractions from 158 tropical forest soil samples with widely varying C content was not influenced by dissolved organic carbon (DOC) or phenolics at lower Fe concentrations (< 2.5 mg g^{-1} soil), and showed only minor effects ($< 6\%$ overestimation) at higher Fe concentrations. This was likely due to co-variation between Fe and DOC, which measured as high as 691 mg C L^{-1} in a cloud forest soil extract. These microplate-based ferrozine methods can be applied to quantify several reactive soil Fe phases with high precision and throughput, minimal interference, and low cost relative to ICP-OES.

1. Introduction

Iron (Fe) is the most abundant redox-active element in Earth's lithosphere, and is commonly present in soils in ferric (Fe(III)) and ferrous (Fe(II)) oxidation states under aerobic and anaerobic conditions, respectively. Iron redox cycling is closely coupled to the biogeochemical dynamics of carbon (C), phosphorus (P), nitrogen (N), and cations. These phenomena have previously received greatest attention in wetlands and marine sediments. However, Fe redox cycling can also be highly significant in relatively well-drained surface soils of terrestrial ecosystems, and is duly receiving increased research attention (Chacon et al., 2006; Dubinsky et al., 2010; Fimmen et al., 2008; Hall et al., 2016; Schulz et al., 2016; Thompson et al., 2006; Yang and Liptzin, 2015). Reactive Fe phases are critical sorbents for soil organic matter and nutrients, yet they can also potentially catalyze organic matter decomposition via multiple mechanisms. As a consequence, Fe pools can explain significant variation in both C stabilization and decomposition rates across scales ranging from soil microsites to the globe (Hall and Silver, 2013, 2015; Kögel-Knabner et al., 2008; Kramer et al., 2012). Inexpensive, high-throughput analytical methods could enhance our capacity to quantify rapid changes in reactive soil Fe pools and associated biogeochemical dynamics. In addition, it is well established that Fe can interfere with colorimetric nitrate determination (Colman et al., 2007; Davidson et al., 2008; Yang et al., 2012). Thus, it is often necessary to measure Fe in soil extracts to assess potential interference even when Fe is not the main focus of a given study.

Chemical extractions are commonly used to quantify the abundance of operationally-defined soil Fe phases (Loeppert and Inskeep, 1996; Thompson et al., 2011; Wagai et al., 2013). For instance, a weak HCl extraction (0.5 or 1 M) solubilizes adsorbed and some solid phase Fe(II) species (including siderite and green rust) and a reactive fraction of Fe (III) minerals (Fredrickson et al., 1998), allowing for separate quantification of Fe(II) and Fe(III) (Lovley and Phillips, 1987). Citrate-ascorbate solution effectively dissolves short-range-ordered (i.e., poorly

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crystalline) Fe oxides via reduction in proportion to a pool that is potentially reducible by microbes (Hyacinthe et al., 2006; Reyes and Torrent, 1997). Citrate-dithionite solution dissolves total free Fe oxides via reduction, including both crystalline and poorly crystalline phases (Loeppert and Inskeep, 1996). These three chemical extractions are useful to quantify reactive soil Fe phases that are strong potential predictors of soil biogeochemical dynamics (Hall and Silver, 2015; Peretyazhko and Sposito, 2005).

The colorimetric ferrozine method has long been used to quantify Fe in water and sediment samples (Lovley and Phillips, 1987; Stookey, 1970; Viollier et al., 2000), and also increasingly in terrestrial soils (e.g. Peretyazhko and Sposito, 2005; Thompson et al., 2006). Ferrozine (3-(2-pyridyl)-5,6-bis(4-phenylsulfonic acid)) enables separate quantification of Fe(II) and Fe(III), and provides a simple, low-cost alternative to inductively coupled plasma optical emission spectrometry (ICP-OES) for total Fe analysis following reduction of Fe(III) to Fe(II). Ferrozine reacts with Fe^{2+} to form a relatively stable magenta-colored complex with maximum absorbance at 562 nm (Stookey, 1970). However, several studies demonstrated that organic matter may interfere with spectrophotometric Fe determination—problems that may be exacerbated in C-rich soils relative to water or sediments. For example, complexation of Fe by humic substances may cause underestimation by ferrozine (Veverica et al., 2016; Yamamoto et al., 2010). On the other hand, Fe(II) in humus-rich samples might be overestimated by the ferrozine method due to autoreduction of Fe(III) (Verschoor and Molot, 2013). These artifacts may partly result from the use of different matrices and reagents in the ferrozine-based assays. For example, the various solutions (water and soil extractions) used in previous studies differed in pH, and buffer concentrations have varied from 10 mM to at least 0.5 M (Pullin and Cabaniss, 2003; Viollier et al., 2000). Also, different buffers (e.g. sodium acetate and HEPES) have been used. Effects of pH on the rate and stability of Fe(II)-ferrozine complexation in different sample types have received little attention, but may be important. The original ferrozine method specified a one-minute incubation time before recording absorbance values (Stookey, 1970). Some studies have applied the ferrozine assay without explicit mention of incubation times, while others have used incubations of 10 min or longer (Viollier et al., 2000; Pepper et al., 2010; Keller and Takagi, 2013). The duration of incubation period has been shown to influence the development of Fe(II)-ferrozine complexes (Im et al., 2013), and could affect unintentional Fe(III) reduction by the ferrozine reagent (Pullin and Cabaniss, 2003). Based on the above, identifying potential interferences, matrix effects, and the importance of assay conditions may be critical for accurate quantification of soil Fe using ferrozine.

Here, we developed optimized ferrozine methods, modified from water and sediment protocols (Lovley and Phillips, 1987; Viollier et al., 2000), to determine Fe concentrations in three common soil extractions. We measured reduced (Fe(II)) and oxidized Fe (Fe(III)) in 0.5 M HCl (Fe_{HCl}), total Fe extracted by citrate-ascorbate (Fe_{ca}), and total Fe extracted by citrate-dithionite (Fe_{cd}). We also compared them with total Fe analysis by ICP-OES, which is widely used to measure samples with differing matrix compositions. Given concerns about the interference of soil organic matter on Fe determination, we examined samples greatly differing in organic matter content. As Fe(II) was shown to bind to oxygen-containing (e.g. phenolic) functional groups (Catrouillet et al., 2014), we also explored potential interferences from phenolics. Additionally, we downscaled our ferrozine-based assay from cuvettes to a 96-well microplate to increase throughput, and demonstrated the importance of pH and time effects on Fe quantification.

2. Materials and methods

2.1. Soil collection and extractions

In the first experiment, we used surface soils (0–10 and 10–20 cm depths, approximately representing A and B horizons) from sites with differing topography, rainfall and parent materials in the Luquillo Experimental Forest, Puerto Rico, to capture a range of soil Fe(II) and Fe(III) and organic matter concentrations. Soils from these sites are characterized by high rates of Fe redox cycling (Hall et al., 2013) and also represent high variation in C content (from 2% to as much as 18% C for cloud forest samples). These soils (total n = 158) included Ultisols, Oxisols and Inceptisols in the USDA soil taxonomy; see Hall and Silver (2015) for further information about samples and sites. Soil subsamples (3 g dry mass equivalent) were immersed in a 1:10 ratio with 0.5 M HCl in the field and then shaken for 1 h. Extractions were centrifuged and the supernatant solution filtered to 0.22 μm for analysis of Fe(II), Fe(III), dissolved organic C, and phenolics as described below. Solutions were stored in the dark at 4 °C for analysis within two weeks of collection.

For the second and third experiments, samples were collected from Mollisols under corn/soybean cultivation that differed in topographic position and drainage characteristics in the Walnut Creek watershed (41°75′N, 93°41′W) in the Des Moines Lobe geological region of north-central Iowa, USA (Cambardella et al., 1994). Topographic depressions in this region known as “prairie potholes” flood periodically, providing an ideal environment for Fe redox cycling. For the first experiment, we used 0–20 cm (plow layer) soils from ridge, slope, and depression positions. Samples were incubated under three different moisture levels (field capacity to saturation) to produce different levels of HCl-extractable Fe(II). Soils were then extracted in 0.5 M HCl as described above. Extractions were centrifuged at 10,000 rcf to remove colloids and the supernatant solution carefully decanted to a clean container (opaque HDPE bottle) for dark storage at 4 °C to prevent photo-reduction of Fe(III) and photo-oxidation of Fe(II). Subsequent analyses (data not shown) demonstrated that the 0.22 μm filtration step described above for the Puerto Rican samples could be omitted if extractions were centrifuged at 10,000 rcf. Total Fe in these samples did not vary over 6 months, suggesting their long-term stability.

For the third experiment, field-moist soils were extracted with sodium citrate-ascorbate solution to measure easily reducible Fe oxides (Fe_{ca}) (Reyes and Torrent, 1997), and air-dried and ground subsamples were extracted with sodium citrate-dithionite solution to estimate total free Fe oxides (Fe_{cd}) (Loeppert and Inskeep, 1996). In these two extractions, ascorbate and the dithionite are the respective reductants, and citrate can accelerate reductive dissolution by forming surface complexes with reduced Fe (Loeppert and Inskeep, 1996; Reyes and Torrent, 1997). Field-moist soils were used for the citrate-ascorbate extractions given that soil drying may enhance Fe crystallization (Hall and Silver, 2015). For the Fe_{ca} measurements, soil subsamples (1.5 g dry mass equivalent) were added to freshly prepared citrate-ascorbate solution in a 1:30 ratio, vortexed, and then shaken in the dark for 16 h. For the Fe_{cd} measurements, air-dried and ground subsamples (0.5 g) were mixed with 0.5 g of sodium dithionite ($Na_2S_2O_4$), 6 g of sodium citrate ($Na_3C_6H_5O_7{\cdot}2H_2O$), and 30 mL of deionized water. The slurry was vortexed and shaken in the dark for 16 h. Samples for Fe_{ca} and Fe_{cd} analysis were centrifuged for 10 min at 10,000 rcf and the supernatant solution decanted to a clean HDPE bottle for dark storage at 4 °C.

2.2. Experiment design

The first experiment evaluated the robustness of our ferrozine method to potential interferences from dissolved organic matter and phenolics in 0.5 M HCl extractions of the Puerto Rican soil samples described above. We measured Fe(II) and Fe(III) using a ferrozine method modified from Lovley and Phillips (1987) and Viollier et al. (2000). To measure Fe(II), we combined 100 μL of the soil extract and 100 μL of nanopure water with 2 mL color reagent (1 g L^{-1} Ferrozine in 50 mM HEPES buffer adjusted to pH 8 with NaOH) in a 1 cm cuvette. This achieved a final solution pH of 7.0 ± 0.1 to minimize ferrozine-catalyzed reduction of Fe(III) (Pullin and Cabaniss, 2003). Hydroxylamine hydrochloride is a strong reductant thought to quantitatively