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Anaerobic Metabolism in Tidal Freshwater Wetlands: III. Temperature Regulation of Iron Cycling

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Abstract Understanding the ecological processes that regulate the production and fate of methane (CH_4) in wetland soils is essential for forecasting wetland CH_4 emissions. Iron reduction is an important carbon mineralization pathway that is capable of suppressing CH_4 production in freshwater wetlands, but our understanding of temperature regulation of iron oxide respiration and the subsequent impacts on CH_4 production is limited. We tested the hypothesis that temperature regulates iron reduction rates indirectly through differential effects on Fe(II) oxidation versus Fe(III) reduction, which ultimately determines the size of the microbially labile, poorly crystalline Fe(III) pool. Our study indicates that rates of iron reduction are more sensitive to changes in temperature than rates of iron oxidation, which creates imbalance in the relative proportion of Fe(II) and Fe(III) in the poorly crystalline soil iron pool as temperatures change. Our results suggest that warmer temperatures can cause the Fe (III) oxide pool to decline, limiting the Fe(III) supply to iron reducers and relieving competition for organic carbon with methanogens.

Keywords Iron cycling · Iron oxidation · Iron reduction · Microbial respiration · Tidal freshwater wetland · Temperature response · Jug Bay Sanctuary

Introduction

The instantaneous emission of methane (CH_4) has 25 times the global warming potential of CO_2 over the ensuing 100 years (Forster et al. 2007). Wetland ecosystems are a globally significant source of this potent greenhouse gas, contributing between 40 and 62 % of total CH_4 emissions (Neef et al. 2010), and current increases in CH_4 emissions from wetlands are an important positive feedback on contemporary climate change (Anisimov 2007). Because of the many challenges faced when forecasting wetland CH_4 emissions, there is an ongoing need for mechanistic research on the ecological processes that regulate the production and fate of CH_4 in soils. Here we address temperature regulation of iron oxide respiration, a potentially important facet of forecasting wetland CH_4 emissions that has been largely overlooked.

Flooded or saturated wetland soils are characterized by anaerobic conditions in which molecular oxygen is largely unavailable as a terminal electron acceptor (TEA) for carbon mineralization. In the absence of oxygen, wetland soil microbes use alternative TEAs to support the final step of anaerobic carbon mineralization. Alternative TEAs are generally consumed in a sequential order based upon thermodynamic yield: NO_3^- (denitrification), Mn(IV) (manganese reduction), Fe(III) (iron reduction), and SO_4^{2-} (sulfate reduction). Methanogenesis is competitively suppressed by the microbes that consume these TEAs, and this suppression is a key control of CH_4 production in wetland ecosystems (Megonigal et al. 2004). Iron reduction is an important carbon mineralization pathway that competitively suppresses CH_4 production in freshwater wetland soils (Lovley and Phillips 1987a; Achtnich et al. 1995; Neubauer et al. 2005).

The ability of iron-reducing bacteria to outcompete methanogens is dependent on the size of the poorly crystalline iron oxide pool (hereafter "iron oxide pool") (Roden and Wetzel 1996; Thamdrup and Canfield 1996; Thomsen et al. 2004). In turn, the size of the iron oxide pool is regulated by the balance between reduction of Fe(III) to Fe(II), and oxidation of Fe(II) to Fe(III). In freshwater anaerobic wetland environments, nearly all Fe(III) reduction is mediated

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by iron-reducing bacteria (Lovley and Phillips 1986). In addition to limitation by iron oxides, iron reduction can be limited by the availability of organic carbon (Roden and Wetzel 2002). By comparison, Fe(II) oxidation occurs through both abiotic and biotic (i.e., microbial) pathways and is often limited by oxygen availability (Neubauer et al. 2002).

Neubauer et al. (2005) reported a seasonal shift in the dominant pathway of anaerobic carbon mineralization from iron reduction in early summer to methanogenesis in late summer. They hypothesized that variation in plant-mediated regeneration of iron oxides was responsible. Specifically, they suggested that root oxygen loss (ROL) from an actively growing plant community regenerated iron oxides, allowing iron reduction to dominate during the period of peak plant productivity in early summer. Following the start of plant senescence in late summer, ROL declined and iron oxides were depleted by iron reduction. Only under these Fe(III)-limited conditions was methanogenesis released from competitive suppression by microbial iron reduction. See Neubauer et al. (2008) for a review of the mechanisms that couple iron oxidation and reduction in the rhizosphere.

Recent work by Keller et al. (2012, this volume) demonstrates that seasonal shifts between iron reduction-dominated and methanogenesis-dominated anaerobic respiration can occur in the absence of an actively growing plant community, suggesting that factors beyond plant activity can also mediate the outcome of microbial competition for TEAs in wetland soils. Specifically, they found evidence that CH_4 production was suppressed by iron reduction both early and late in the growing season when soil temperatures were low, but not during the summer. Because this occurred in their no-plant treatment, the work suggests that temperature may regulate the competitive outcome between these microbial respiration processes.

Temperature may regulate Fe(III) reduction rates indirectly through differential effects on Fe(II) oxidation versus Fe(III) reduction that ultimately determine the size of the iron oxide pool. If Fe(III) reduction rates increase faster with rising temperature than Fe(II) oxidation rates, the size of the iron oxide pool should decline from winter to summer even in the absence of growing plants. We hypothesized that Fe(III) reduction is more sensitive to temperature than Fe(II) oxidation, resulting in temperature-driven changes in the size of the poorly crystalline iron oxide pool (Fig. 1). Such an effect would represent a potential mechanism for temperature-driven seasonal oscillations in rates of iron reduction and methanogenesis that operates independently of plant activity, and could represent an important control of CH_4 dynamics in freshwater wetlands.

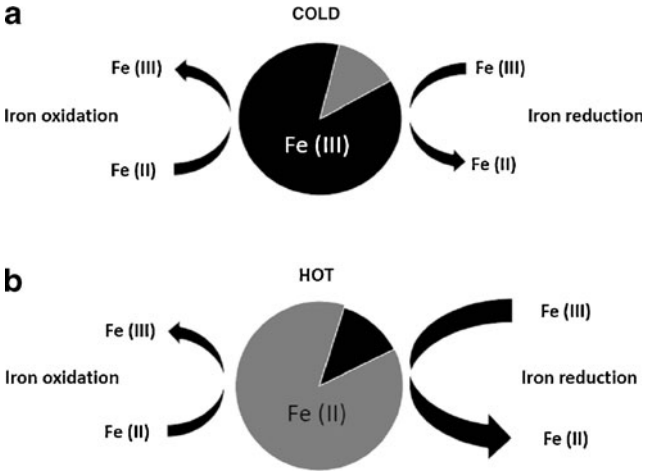

Fig. 1 This diagram illustrates our central hypothesis. The *pie chart* represents the speciation of the total poorly crystalline iron pool. The difference (or lack thereof) in the *size of the arrows* going from cold (**a**) to hot (**b**) represents the change in rate due to the effects of temperature. The schematic suggests that oxidation is relatively insensitive to temperature, such that the difference in rates between hot and cold incubations is relatively small compared to reduction. Likewise, reduction is relatively more temperature sensitive. The net effect is for cool temperatures to favor a larger Fe(III) pool and warm temperatures to favor a larger Fe(II) pool

Methods

Site Description and Soil Sampling

Jug Bay Wetland Sanctuary encompasses a large, interconnected network of tidal freshwater wetlands located on the Patuxent River, Anne Arundel County, Maryland, USA. These tidal wetlands typically receive two high tides daily and are dominated by a wide variety of wetland plant species, including *Peltandra virginica*, *Pontederia cordata*, *Polygonum arifolium*, *Typha* spp, *Nuphar lutea*, and *Zizania aquatica*.

The soils used in this study were collected from an unvegetated mudflat (N 38°46.877′, W 076°42.802′) in order to minimize the legacy effects of plant activity via ROL on the poorly crystalline iron oxide pool. Soils collected at this location in February 2012 had a pH of 6.59±0.042 (mean± SE, n=5), moisture content of 66±0.3 %, organic matter content of 18.0±0.65 % (measured by loss on ignition at 450 °C), and porewater sulfide content of 0.70±0.04 μM (n=6). Previous work in the same freshwater marsh has shown that anaerobic respiration is dominated by iron reduction and methanogenesis (Neubauer et al. 2005). Sulfate reduction contributes ≤2 % of the total anaerobic metabolism, which is consistent with low porewater sulfate concentration (<0.03 mM, Neubauer et al. 2005). Blocking sulfate reduction with sodium molybdate showed that iron reduction is entirely mediated by biotic reduction versus chemical reduction (Neubauer et al. 2005). For this reason, we did not explicitly explore temperature effects on iron–sulfur interactions.

We took an initial set of cores in January 2009 that were 10 cm diameter by 40 cm deep, capped on the top and bottom in the field to minimize oxygen exposure. This soil was used to determine the temperature responses of potential rates of iron oxidation and reduction. In September 2009, we collected additional cores from the same location and in the same manner to investigate the effects of iron oxide pool size on rates of iron reduction. A portion of this soil (referred to as "stock") was sieved (2.0 mm) and stored anaerobically at 4 °C for later use. In January 2010, additional soil (referred to as "fresh") was collected in bulk from the top 20 cm of the same location to use in a third study that focused on temperature and carbon effects on iron oxide pool size.

Temperature Effects on Potential Rates of Iron Oxidation

Soil cores collected in January 2009 were transported directly from the field to the laboratory and placed in an anaerobic chamber (>95 % N_2, <5 % H_2; COY Laboratories, Michigan, USA). Soil from the 5–15 cm depth of all cores were pooled, homogenized, and sieved (2.0 mm) to remove undecomposed plant materials (e.g., roots). The soil was then divided for use in separate experiments aimed at quantifying the temperature response curves of potential iron reduction and potential iron oxidation. Soil slurries in all the experiments were prepared at a soil-to-water ratio of ~0.6, which we found produced repeatable rate determinations (data not shown). Approximately 300 g of field-moist soil was added to 500 mL N_2-purged deionized water and stirred for 5 min. This slurry was then divided into five flasks, one for each temperature treatment. To maintain soil particles in suspension while being subdivided, the slurries were continually mixed with a stir bar while samples were withdrawn using a pipettor. Each flask was subdivided into 45 centrifuge tubes (15 mL), each containing ~3 mL of soil slurry. All of the tubes were then brought out of the chamber and exposed to ambient atmosphere (~23 °C), returning headspace [O_2] to ambient. The centrifuge tubes were loosely capped to allow additional headspace exchange with atmosphere and placed on shaker tables (180±10 rpm) in incubators set to 3, 9, 14, 19, and 30 °C. Samples were allowed to incubate in the dark at these temperatures. Four replicate tubes were sacrificed for the measurement of extractable Fe(II) at regular intervals until the pool of Fe(II) was depleted, which was less than 2 h for the warmer treatments and 6 h for the coldest treatment.

Rates of Fe(III) reduction and Fe(II) oxidation were both quantified as changes in the Fe(II) pool over time. Although we expected most of the Fe(II) to be in the dissolved phase of this freshwater soil, we accounted for sorbed Fe^{2+} and iron sulfide by extracting soils with a weakly acidic solution of 0.2 M ammonium oxalate and 0.2 M oxalic acid (AOOA, modified from the methods of Phillips and Lovley (1987)). Soil slurries were transferred into the anaerobic chamber and 10 mL of AOOA extract was added to each centrifuge tube. Tubes were tightly capped, removed from the anaerobic chamber and shaken on an orbital shaker at 250 rpm for 4 h in the dark. The samples were then centrifuged for 10 min at 3,500 rpm and a 25-μL subsample of the supernatant was analyzed for Fe(II) using the ferrozine method (Lovley and Phillips 1987b). Although AOOA also extracts poorly crystalline Fe(III) (i.e., ferrihydrite), the ferrozine reaction is specific to Fe(II). The remaining supernatant was carefully decanted and the soil pellet was dried to a constant mass at 60 °C. The mass of AOOA sorbed to the pellet was negligible and not accounted for in the dry soil mass. Rates of oxidation were measured as the loss of Fe(II) through time. Multiple volumes of 5 and 10 mM anaerobic stock solutions of $FeCl_2$ were extracted using the same protocol described above to generate standard curves for these extracts.

Temperature Effects on Potential Rates of Iron Reduction

Soil was prepared as described above and stored anaerobically at 4 °C for several days. It was then spread onto a tray and exposed to the ambient atmosphere for 24 h to ensure a pool of poorly crystalline iron oxide that was large enough to measure Fe(III) reduction. Based on our visual assessment of soil color, this length of time was long enough to oxidize the Fe(II) on soil surfaces, but not long enough to oxidize deeper layers of soil. The bulk soil moisture content at the end of the exposure was 76 % (similar to the water content when it was collected) and the soil supported Fe(III) reduction rates comparable to previously published rates from the same site (Neubauer et al. 2005). This soil was then returned to the anaerobic chamber where it was homogenized. In order to avoid potential carbon limitation, 500 mL of N_2-purged 2 mM dextran (a dextrose polysaccharide that is a fermentable carbon source) was added to 300 g of soil (51 mg labile C/g wet soil) and stirred for 5 min. This slurry was then divided into five flasks, one for each temperature treatment (3, 9, 14, 19, and 30 °C); each flask was subdivided into four 60-mL serum bottles, each containing ~20 mL of soil slurry. Bottles were capped using gray butyl septa, removed from the anaerobic chamber, and flushed with N_2 for 30 min to remove the headspace H_2 introduced by the anaerobic chamber. Preliminary testing (data not shown) showed no difference in rates of reduction due to the effects of shaking, so samples were incubated in the dark without being shaken. Subsamples from each bottle were removed at 0.08, 0.2, 0.9, 1, 2, 4, and 6 days for measurements of AOOA-extractable Fe(II) as described above. Rates of Fe(III) reduction were quantified as the increase in extractable Fe(II) over time.

Temperature and Carbon Effects on Iron Oxide Pools

We designed this experiment to explore the interactive effects of temperature and carbon limitation on the poorly crystalline Fe(III) pool, by incubating soils with either ample ("Amended") or reduced ("Unamended") levels of labile carbon. We refer to this as the "coupled experiment" because the conditions of the experiment were conducive to simultaneous Fe(II) oxidation and Fe(III) reduction, as evidenced by both net gains and net losses of Fe(III) over the course of the incubations. This is in contrast to the temperature response experiments described above in which conditions allowed either Fe(II) oxidation (i.e., fully aerobic) or Fe(III) reduction (i.e., fully anaerobic), but not both.

In order to ensure that iron reduction was not limited by Fe (III) availability in the Amended incubations, we regenerated Fe(III) by exposing stock soil that had been stored wet at 4 °C to the ambient atmosphere for several hours. As explained above, the length of this exposure to atmospheric O_2 was based on changes in soil color, with the goal of regenerating Fe(III) on exposed soil surfaces while maintaining wet, anaerobic interior soil areas and their native microbial communities. As a precaution, this soil was amended with fresh soil in a 20:1 ratio (stock/fresh) to refresh the microbial community. A final mass of 36 g of mixed stock and fresh soil (moisture content 67 %) was homogenized and slurried with 58 mL of either N_2-purged deionized water ("Unamended") or N_2-purged 2 mM dextran ("Amended") as described above (soil-to-water ratio ~0.6). The initial poorly crystalline iron concentration was 343.1± 7.84 μmole gdw^{-1} (mean±SE, n=3) in the Unamended treatment and 346.4±39.7 μmole gdw^{-1} (n=5) in the Amended treatment. Each slurry was then divided into 45 15-mL centrifuge tubes (~1.5 mL of slurry in each tube). The slurries were removed from the anaerobic chamber and exposed to ambient atmosphere (~23 °C) to return the headspace [O_2] to ambient; they were loosely capped to allow additional headspace exchange with atmosphere. Fifteen tubes for each of the three temperature treatments (4, 14, and 26 °C) were placed into their respective incubators in the dark; five were sacrificed at 0, 13, and 26 days (Amended) and 0, 20, and 34 days (Unamended) for the measurement of extractable iron.

In this particular experiment, it was important to track the size of the total poorly crystalline iron pool and the fraction that was Fe(II) or Fe(III). To conveniently quantify these fractions, we used a slightly modified version of a method by Lovley and Phillips (1987b) that extracts poorly crystalline Fe(II) and Fe(III) with 0.5 M HCl, then reduces Fe(III) to Fe (II) with hydroxylamine in the same extract. The concentration of Fe(III) is calculated as the difference in [Fe(II)] before and after the hydroxylamine reduction step. Replicate tubes were sacrificed for each measurement, amended with 10 mL of 0.5 M HCl, shaken on an orbital shaker at 250 rpm for 1 h, and then centrifuged. First, a sample of the supernatant was taken for [Fe(II)] determination using the ferrozine method. Next, 1 mL of 0.25 M hydroxylamine hydrochloride in 0.25 M HCl was added to the ferrozine–supernatant solution and incubated for 24 h to reduce the acid-soluble Fe(III) to Fe (II) prior to being measured colorimetrically at an absorbance of 562 nm. HCl and AOOA both extract poorly crystalline iron pools (Kostka and Luther 1994), but "poorly crystalline" is operationally defined because the procedures extract somewhat different specific pools. For this reason, comparisons should not be made between the potential rate experiments and the coupled experiment with respect to the size of poorly crystalline Fe(III) pools; however, conclusions can be drawn about their respective response to temperature because the same method was used within a given temperature experiment. The relative proportions of Fe(II) and Fe(III) were calculated as a percentage of the total poorly crystalline acid-extractable iron pool.

Effects of Iron Oxide Pool Size on Rates of Iron Reduction

This experiment was designed to study the ability of our methods to detect iron reduction as a function of the size of the poorly crystalline iron oxide pool. Our goal was to determine the minimum initial concentration of poorly crystalline iron oxide that will yield a measurable rate of reduction. Iron oxides for this experiment were generated from anaerobic stock soil (403.8±4.27 μmol poorly crystalline Fe gdw^{-1}, mean ± SE, n=20) by exposure to ambient air for several days to regenerate soil-surface iron oxides. As in the other experiments, the soils were not allowed to dry long enough to cause significant O_2 exposure in the soil interior. The soil was then homogenized by sieving (2.0 mm) and slurried with deoxygenated, deionized water. In order to create treatments that spanned a range of initial [Fe(III)], we added small amounts of the oxidized soil slurry to carbon-amended anaerobic stock soil in proportions yielding 1, 2.5, 5, and 10 % oxidized soil based on wet weight (61–67 % moisture content). We created slurries from these mixtures using deoxygenated, deionized water (soil-to-water ratio ~0.6), sealed the vials with gray butyl septa, and flushed the headspace for 30 min with N_2. Five replicate slurries were established for each treatment level and all samples were incubated in the dark at 20 °C. Subsamples were collected on days 0, 1, 2, and 3 for the measurement of acid-extractable poorly crystalline Fe(II) and total poorly crystalline (Fe(II) + Fe(III)) using 0.5 M HCl and 0.25 M hydroxylamine hydrochloride in 0.25 M HCl as described above. Rates of iron reduction were calculated as the increase in extractable Fe(II) over time.

Statistical Analysis

Iron oxidation proceeded too quickly to allow subsamples to be taken from single tubes over time, so we sacrificed whole

tubes (n=4) at each time point. The [Fe(II)] of the four replicate tubes were averaged for each time point; these averages were fit to a linear regression of [Fe(II)] versus time to calculate the potential rate of iron oxidation for each temperature treatment. Iron reduction proceeded slowly enough to allow us to repeatedly subsample single vials over time. For these reduction data, a separate linear regression was fit to the relationship of [Fe(II)] versus time for each bottle, yielding four rate estimates for each temperature treatment that were averaged. In all cases, the linear section of the [Fe(II)] versus time relationship was used in the regression analysis, yielding regressions fit to 3–7 points (oxidation) or 6–9 points (reduction) for each curve. Iron reduction became non-linear after 3 days above 19 °C and after 4 days below 14 °C due to Fe(III) limitation, while iron oxidation became non-linear at 10–20 μmol Fe(II) gdw^{-1} due to Fe(II) limitation (data not shown). The rates derived from these regression analyses were fit to the Arrhenius equation in order to quantify temperature responses. Regressions were conducted using Systat SigmaPlot software, version 11.0 (San Jose, CA, USA).

Results

Temperature Effects on Potential Oxidation and Reduction

Rates of iron reduction were linear (r^2>0.90) for all but the 3 °C incubations, where rates were very low (2–5 μmol Fe gdw^{-1} day^{-1}) and r^2 values ranged from 0.4 to 0.8 (Fig. 2a). Rates of iron oxidation were linear (r^2>0.94) at all temperatures (Fig. 2b). Across the 3 to 30 °C temperature range used in these experiments, potential rates of Fe(III) reduction increased from 4 to 36 μmol Fe gdw^{-1} day^{-1}, while potential rates of Fe(II) oxidation increased from 351 to 1,710 μmol Fe gdw^{-1} day^{-1}. Although the temperature responses for iron reduction and oxidation were approximately linear over the temperature range, both increased most rapidly between 9 and 14 °C (Fig. 2c). Based on Arrhenius plots, iron reduction (E_a= 60 kJ mol^{-1}) was 50 % more sensitive to temperature than iron oxidation (E_0=41 kJ mol^{-1}) ($y_{reduction}$=−7187.4×+27.47, r^2= 0.95, P=0.005; $y_{oxidation}$=−4948.1×+24.03, r^2=0.85, P= 0.015; Fig. 2d).

Temperature and Carbon Effects on Soil Iron Pools

In the coupled experiments, Unamended soils (no additional carbon) showed an increase in the Fe(III) oxide pool size from an initial proportion of 26 to ~90 % in all temperature treatments (Fig. 3d). In contrast, Amended soils (additional labile carbon) showed a differential response to temperature, with relatively small net changes in the Fe(III) oxide pool at 4 °C (decrease of 3 %) and 14 °C (increase of 7 %), and a large net decrease of 36 % at 26 °C (Fig. 3b). Changes in the absolute sizes of the poorly crystalline Fe(II) and Fe(III) pools showed the same result (Fig. 3a, c).

Effects of Iron Oxide Pool Size on Rates of Iron Reduction

Iron reduction rates were sensitive to the initial pool of poorly crystalline iron oxide (Table 1). We did not detect Fe(III) reduction as an accumulation of acid-extractable Fe (II) in the treatment with the smallest poorly crystalline Fe (III) pool (211 μmol gdw^{-1} or 51 % of the total poorly crystalline pool). Iron reduction was detected with slightly larger pools of Fe(III) (≥217 μmol gdw^{-1} or 55 % of the poorly crystalline pool).

Discussion

Our study indicates that iron oxidation and reduction respond differently to changes in temperature, creating imbalances that result in seasonal variations in the size of the poorly crystalline iron oxide pool. Rates of iron reduction were more sensitive to changes in temperature than rates of iron oxidation (Fig. 2d), suggesting that reduction will increase at a faster pace than oxidation as soils warm and decrease more quickly as soils cool. These differential temperature sensitivities have the potential to impact the relative proportion of Fe(II) and Fe(III) in the total Fe pool (Fig. 3). In the coupled experiment, where labile organic carbon (electron donor) was not limiting (Amended treatment), the size of the Fe(III) pool was largest in soils incubated at the coolest temperatures (≤14 °C). Presumably, cool temperatures caused iron reduction to decrease more than iron oxidation, allowing Fe(III) to accumulate. Conversely, soils incubated at the warmest temperature had the smallest iron oxide pool because rates of iron reduction increased more quickly than oxidation, drawing down the Fe(III) oxide pool.

Although temperature sensitivity played a major role in regulating the size of the poorly crystalline iron pool (coupled experiment), the availability of carbon was also an important control. The process of microbial respiration via iron reduction can be limited by either electron donors (organic carbon) or electron acceptors (Fe(III)). Regardless of temperature, the pool of oxidized iron in the carbon-limited incubations increased over time because iron reducers were limited by electron availability (Fig. 3c, d). In contrast, when carbon was not limiting, warmer temperatures resulted in a rapid draw down of the poorly crystalline Fe(III) oxide pool by a metabolically active, iron-reducing microbial population (Fig. 3a, b).

Potential rates of Fe(II) oxidation were orders of magnitude greater than potential rates of iron reduction (Fig. 2c), reflecting the fact that iron reduction is largely a microbial process,

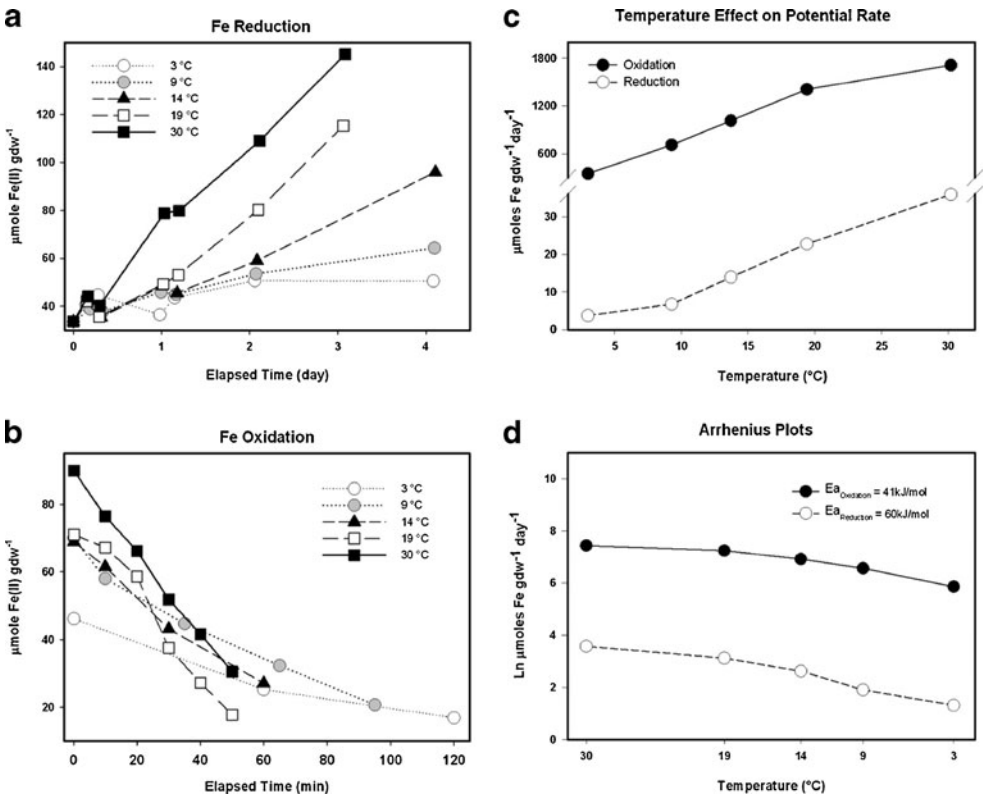

Fig. 2 Temperature responses of potential rates of iron reduction and iron oxidation. Changes in AOOA-extractable [Fe(II)] over time are plotted separately for each temperature manipulation (**a**, **b**). Rates of iron oxidation and iron reduction are plotted as simple functions of temperature (**c**) and as Arrhenius plots (**d**). The *x*-axis for the Arrhenius plots was calculated as $1/T$ (K), but is illustrated as T (°C) for comparisons to the treatments described in the text

while iron oxidation is both microbial and autocatalytic. Iron oxidation kinetics vary as a function of $[O_2]$, with high $[O_2]$ yielding very rapid rates at circumneutral pH through the autocatalytic pathway (Neubauer et al. 2002). Because the incubations for potential rates of iron oxidation were shaken in a fully oxic environment, we strongly suspect the dominant oxidation pathway was autocatalytic. This is not likely to be the case in circumneutral wetland soils where Fe(II)-oxidizing bacteria are abundant (Emerson et al. 1999; Weiss et al. 2003, 2005). We do not have a reason to suspect that the temperature

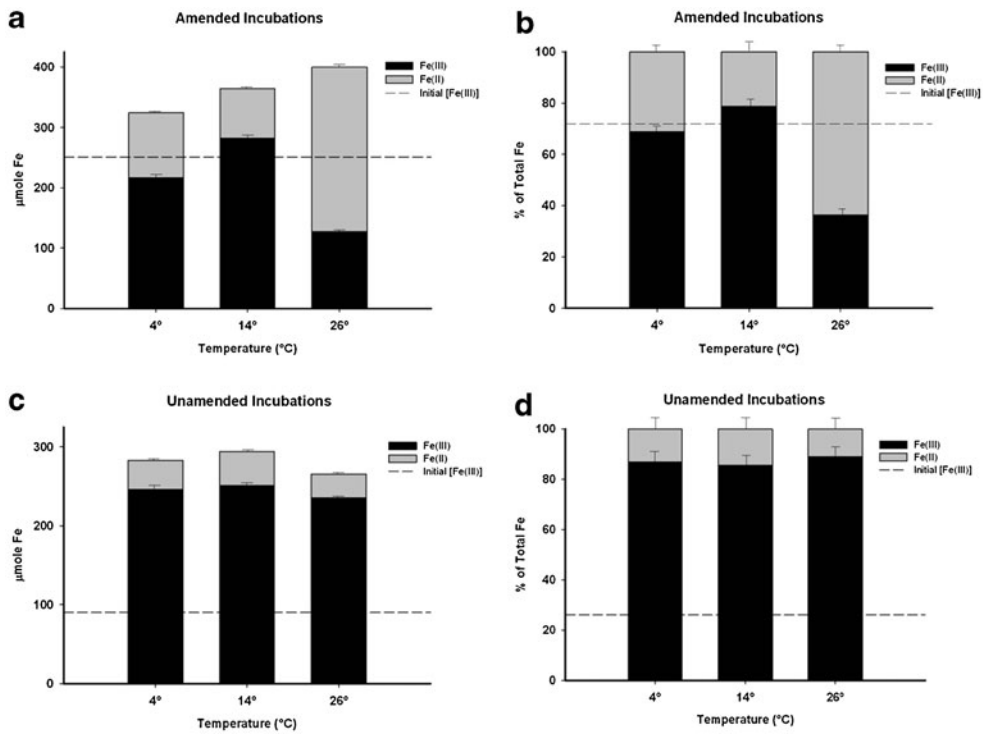

Fig. 3 Acid-extractable poorly crystalline iron on labile carbon-amended soils (**a**, **b**) and unamended soils (**c**, **d**). *Horizontal reference lines* indicate the initial poorly crystalline [Fe(III)]. Percent of total poorly crystalline iron (Fe(II) + Fe(III)) is presented as mean±SE (n=5) of the total available poorly crystalline iron pool at the time of sampling. The mean±SE of initial Unamended (**d**) and Amended (**b**) [Fe(III)] was 26± 0.8 % and 72±1.7 %, respectively

Table 1 Relationship of initial poorly crystalline Fe(III) pool size to total poorly crystalline iron pool and measurable rates of iron reduction

Oxidized soil (%)	Initial [Fe(II)] ($\mu mol/gdw^{-1}$)	Initial [Fe(III)] ($\mu mol/gdw^{-1}$)	Initial percent Fe(III) of total poorly crystalline Fe pool (%)	Iron reduction ($\mu mol\ Fe(II)\ gdw^{-1}\ day^{-1}$)
1	204	211	51	Not measurable
2.5	176	217	55	15±6
5	174	234	57	16±2
10	156	244	61	38±12

Rates of Fe(III) reduction are presented as mean±SE

response of the autocatalytic oxidation process measured here is different from the microbial oxidation process, but the issue should be considered in the future.

The coupled experiments were not shaken because our goal was to limit Fe(II) oxidation rates through O_2 limitation, simulating a field environment in which Fe(II) accumulates due to comparable rates of iron oxidation and reduction. We recognize that temperature effects on rates of O_2 diffusion may have contributed to the effects of temperature on iron reduction kinetics. Specifically, cooler temperatures may have allowed headspace O_2 to penetrate deeper into the soil slurry because of relatively low rates of heterotrophic respiration, relieving O_2 limitation and increasing the size of the Fe(III) pool. This mechanism for temperature regulation of iron oxide pool size is likely to also operate in saturated horizons of wetland soils where O_2 is supplied primarily through diffusion.

The difference in temperature sensitivity between iron reduction and oxidation provides a potential explanation for observations made at the same study site (i.e., their *mineral* site) by Keller et al. (2012, in this volume) and Emerson et al. (2012, in this volume). In both the presence and absence of plants, Keller et al. (2012) observed a mid-summer peak in the soil Fe(II) pool and seasonally high potential rates of iron reduction in early spring. Our data suggest that cold winter temperatures may have favored iron oxidation over reduction, allowing the accumulation of poorly crystalline iron oxide in the soils. As temperatures warmed in the spring, increased rates of iron reduction would have drawn down the iron oxide pool until iron reducers eventually became limited by iron availability (e.g., Rabenhorst and Castenson 2005), allowing methanogens to gain a competitive advantage over iron reducers for labile carbon. Indeed, Keller et al. (2012) observed near complete methanogenic conditions during peak summer months and Emerson et al. (2012) found the microbial communities were dominated by methanogens, suggesting there was no significant iron reduction activity. Temperature-driven changes in the iron oxide pools are also consistent with observations from two permanently saturated non-tidal freshwater wetlands, one of which showed a sharp decrease in the iron oxide pool size beginning in May that persisted through November (Roden and Wetzel 1996), and another that showed increasing porewater [Fe(II)] during the same time frame (Weiss et al. 2005).

Importantly, this temperature-driven seasonal pattern can operate independently of plant activity, providing additional mechanistic insight into seasonal shifts in the dominant carbon mineralization pathway reported by other studies (e.g., Weiss et al. 2005). For example, in a study of seasonal iron cycling in saltmarsh sediments, Kostka and Luther (1995) found that [Fe(III)] was greatest during the coldest months. They suggested that iron oxides are produced year round from passive O_2 transport through plants to sediments, but that low winter microbial respiration prevented iron oxides from being consumed until summer. This explanation implies that iron reduction and oxidation respond differently to temperature, which our results confirm.

A previous study at our field site used voltammetric microelectrodes to study in situ redox species during the summer in vegetated and unvegetated soils (Ma et al. 2008). They reported that the iron pool in plant-free soils was 91 % Fe(II) and 9 % Fe(III), and concluded that iron reduction was the dominant anaerobic respiration pathway. Our data suggest that Ma et al. (2008) witnessed the Fe(III) pool at a seasonal low due to temperature effects on the iron oxide pool size. If iron reducers were active, our data suggest rates of iron reduction would have been too low to detect (Table 1). Although the relatively large Fe(II) pool indicates that iron reduction had occurred, our data and those of Keller et al. (2012) suggest this Fe(II) pool was most likely a remnant of microbial respiration activity that occurred during cooler months, earlier in the year.

Differences in iron oxide pool sizes can also explain why iron reduction rates are not measurable at times. A strong correlation between poorly crystalline Fe(III) oxide concentration and iron reduction rates has been documented (Thamdrup and Canfield 1996; Jensen et al. 2003), and studies have suggested that a minimum of 27–30 $\mu mol\ Fe(III)\ cm^{-3}$ of wet sediment is required for iron reduction to dominate carbon mineralization (Jensen et al. 2003; Thomsen et al. 2004). To better understand this relationship, we measured rates of iron reduction as a function of the relative proportion of Fe(III) to total poorly crystalline iron and found the two are related (Table 1). Although we were unable to detect iron reduction

in soils containing <217 μmol Fe(III) gdw^{-1}, we were able to measure an increase in the proportion of Fe(II) relative to the total pool (data not shown), suggesting that the process may have been occurring at a rate that was undetected by the widely used 0.5 M HCl extraction method. Based on our very limited sample, we suggest that iron reduction is unlikely to be an important pathway of microbial respiration in soils where [Fe (III)] is <200 μmol Fe(III) gdw^{-1} or <50 % of the total poorly crystalline iron pool, and that robust iron reduction rate estimates may require [Fe(III)] >200 μmol Fe(III) gdw^{-1}.

It is important to note that we cannot rule out the possibility of interplay among mechanisms, contributing to the effects of temperature demonstrated here. We expect that in nature, seasonal changes in the iron oxide pool size are due to changes in temperature, acting in concurrence with other mechanisms. Because iron oxides are regenerated abiotically in the presence of O_2, iron cycling in wetland soils can be stimulated in many ways. When labile organic matter is not limiting, bioturbation by benthic (Thomsen et al. 2004) and macrobenthic fauna (Gribsholt et al. 2003) yields sustained levels of high iron oxide concentration, which in turn allows iron reducers to outcompete sulfate reducers and methanogens. Actively growing plants regenerate iron oxides by introducing O_2 to rhizosphere soils directly through root oxygen loss (Weiss et al. 2003; Hyun et al. 2007), and indirectly through transpiration-driven withdraw of water from pore space (Dacey and Howes 1984). A dominant mechanism regulating iron cycling is wet–dry cycles associated with temporal variation in water table depth, which alternately favor net iron reduction (wet) or net iron oxidation (dry) by regulating soil [O_2] (Roden and Wetzel 1996; Whitmire and Hamilton 2008). Seasonal changes in each of these mechanisms are likely to act in conjunction with changes in temperature to regulate iron oxide pools. We emphasize that the temperature-driven mechanism we propose here is not applicable to purely anaerobic environments, or those in which key substrates are unavailable.

Conclusion

We report different temperature sensitivities for iron oxidation and reduction that provide insight into field studies that have observed seasonal oscillations in soil iron and methane dynamics. Our laboratory experiments suggested that falling temperatures favor Fe(II) oxidation more than Fe(III) reduction, promoting an accumulation of poorly crystalline iron oxides that are drawn down when soil temperatures rise. This pattern was caused by a combination of direct temperature effects on iron reduction and oxidation kinetics, and possibly an indirect effect of temperature on O_2 diffusion mediated by heterotrophic respiration. The pattern has important implications for methane production because it suggests that an increase in temperature can cause the microbially labile, poorly crystalline Fe(III) oxide pool to decline, limiting the Fe(III) supply to iron reducers and relieving competition for organic carbon with methanogens.

Climate change predictions suggest that average global temperatures will continue to increase, causing spring to arrive earlier and winter to arrive later. Considering only the predicted changes in temperature, our data suggest that the importance of iron reducers in suppressing methane production may decrease in a warmer world, affecting greenhouse gas emissions from freshwater wetlands.

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