The role of iron and reactive oxygen species in the production of CO_2 in arctic soil waters

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Abstract

Hydroxyl radical (•OH) is a highly reactive oxidant of dissolved organic carbon (DOC) in the environment. •OH production in the dark was observed through iron and DOC mediated Fenton reactions in natural environments. Specifically, when dissolved oxygen (O_2) was added to low oxygen and anoxic soil waters in arctic Alaska, •OH was produced in proportion to the concentrations of reduced iron (Fe(II)) and DOC. Here we demonstrate that Fe(II) was the main electron donor to O_2 to produce •OH. In addition to quantifying •OH production, hydrogen peroxide (H_2O_2) was detected in soil waters as a likely intermediate in •OH production from oxidation of Fe(II). For the first time in natural systems we detected carbon dioxide (CO_2) production from •OH oxidation of DOC. More than half of the arctic soil waters tested showed production of CO_2 under conditions conducive for production of •OH. Findings from this study strongly suggest that DOC is the main sink for •OH, and that •OH can oxidize DOC to yield CO_2. Thus, this iron-mediated, dark chemical oxidation of DOC may be an important component of the arctic carbon cycle.

1. Introduction

Hydroxyl radical (•OH) is one of the strongest oxidants in the environment and thus plays important roles in the oxidation of organic carbon in the atmosphere and in surface waters (Faust and Hoigné, 1990; Mopper and Zhou, 1990; Vaughan and Blough, 1998; Page et al., 2014). Most research on •OH as an oxidant of organic carbon has been done in sunlit environments, where •OH is produced by photochemical processes (e.g., Goldstone et al., 2002; Southworth and Voelker, 2003; Vermilyea and Voelker, 2009; Page et al., 2014). Recent work has focused on the light-independent 'dark' pathway for •OH production during redox reactions likely involving reduced iron (Fe(II)) or dissolved organic carbon (DOC) as electron donors (Fig. 1; Burns et al., 2010; Page et al., 2012, 2013; Minella et al., 2015; Tong et al., 2016). Oxidation of Fe(II) or reduced DOC by oxygen (O_2) can produce hydrogen peroxide (H_2O_2; Fig. 1; Haber and Weiss, 1932; Stumm and Lee, 1961; Page et al. 2012). Once produced, H_2O_2 can react with remaining Fe(II) or reduced DOC to yield •OH (Fig. 1). Therefore, •OH is produced where Fe(II) and reduced DOC are present, suggesting that •OH is an important oxidant in these environments.

The predecessors to •OH production, Fe(II) and reduced DOC, are common products of anaerobic microbial respiration in waterlogged soils or lake sediments (Klapper et al., 2002; Lipson et al., 2010). When waterlogged soils are flushed with oxygenated water, or at the oxic-anoxic boundary in soils or sediments, •OH may be produced (Burns et al., 2010; Page et al., 2012, 2013; Minella et al., 2015; Tong et al., 2016). Prior research observed •OH production from soil waters draining the dominant vegetation types of the low Arctic in proportion to concentrations of reduced soil water constituents including Fe(II) and DOC (Page et al., 2013). In addition, •OH production from aeration of soil waters increased along a gradient of low to high reducing conditions from dry upland soils to wet lowland habitats in the Arctic (Page et al., 2013). Specifically, soil waters draining from wet sedge vegetation had the highest reducing conditions (i.e., highest electron donating capacities from high concentrations of reduced constituents; Page et al., 2013). Upon introduction of O_2, wet sedge soil waters produced the greatest •OH compared to soil waters from dry upland areas low in reduced constituents (Page et al., 2013). It was estimated that together Fe(II) and reduced DOC accounted for ~ 90% of the electron donating capacity of those soil waters, which contain low concentrations of other potential reductants like sulfide (Page et al., 2013). Thus, Fe(II) and reduced DOC were inferred to be the main reductants of O_2 yielding •OH in arctic soil waters (Fig. 1; Page et al., 2013). Studies in lake water and lake sediments have also concluded that Fe(II) and reduced DOC were the main electron donors upon introduction of O_2 in the production of •OH (Minella et al., 2015; Tong et al., 2016). However, the relative importance of Fe(II) versus DOC as electron donors to yield •OH in natural systems is unknown, in part because concentrations of Fe(II) and DOC often co-vary in soils or sediments (Page et al., 2013).

Determining the relative importance of Fe(II) versus reduced DOC as electron donors to produce •OH requires quantifying the fraction of the total electron donating capacity *in-situ* in soils or sediments from the oxidation of Fe(II) versus the oxidation of reduced DOC. Total electron donating capacity of Fe(II) (i.e., electrons released from Fe(II) oxidation) can be quantified, but the redox moieties within DOC are poorly characterized and thus difficult to isolate and quantify in natural soils (Aeschbacher et al., 2012; Wallace et al., 2017). However, comparison of electrons released from the oxidation of Fe(II) to Fe(III) alone to the total electron donating capacity (i.e., electrons released from all reduced constituents) may identify the relative importance of Fe(II) versus DOC as the electron donors to produce •OH; this method would be particularly effective in environments where other potential reductants are present at much lower concentrations.

Once •OH is produced in soils or sediments, its fate and consequences for carbon cycling are poorly understood. From studies of •OH in simulated surface waters containing high concentrations of •OH and DOC, •OH is expected to rapidly oxidize DOC (Voelker and Sulzberger, 1996). However, in natural waters, soils, or sediments, DOC may compete with chloride, bromide, or carbonates as a sink for •OH (Buxton et al., 1988; Lipson et al., 2010; Page et al., 2013). Recent work showed that in high DOC surface waters low in salts or carbonates, DOC was the main sink for •OH (Page et al., 2014). While DOC is expected to be the sink for •OH in surface waters or soils of the Arctic, the products of the oxidation of DOC by •OH can yield several organic or inorganic compounds (Goldstone et al., 2002).

•OH reacts with DOC by addition (i.e., hydroxylation) or hydrogen atom abstraction producing organic and hydroperoxyl radicals (Sulzberger and Durisch-Kaiser, 2009). Those radicals may initiate additional degradation of DOC, ultimately forming partially-oxidized or degraded aromatic or aliphatic compounds (Westerhoff et al., 1999; Waggoner et al., 2015), low molecular weight organic acids, or CO_2 (Goldstone et al., 2002). Using artificially generated •OH and simulated natural waters, Goldstone et al. (2002) reported a yield of 0.3 mole of CO_2 from the oxidation of DOC by 1 mole of •OH. Page et al. (2013) used this laboratory yield to estimate that the amount of CO_2 produced from •OH in natural soil waters could be on the same order of magnitude as the amount of CO_2 produced by bacterial respiration of DOC in surface waters of the Alaskan Arctic. Page et al. (2013) concluded that oxidation of DOC by •OH could be an important source of CO_2 in boreal and arctic regions given the vast stores of organic carbon residing in waterlogged soils conducive to redox cycling. Similarly, Hall and Silver

(2013) suggested oxidation of DOC by •OH to CO_2 is important in tropical soils where they observed a strong, positive correlation between Fe(II) oxidation and CO_2 production. However, the effects of •OH on the fate of DOC in natural systems remain poorly understood because no study has directly measured the CO_2 produced from oxidation of DOC by •OH in soils or soil waters.

The objectives of this study were to (1) determine the relative importance of Fe(II) versus reduced DOC in the production of •OH, and (2) determine whether oxidation of DOC by •OH produces CO_2 in natural soil and surface waters. To address these knowledge gaps on the controls of dark •OH production and its fate, we measured concentrations of •OH produced upon introduction of air to low-O_2 and anoxic soil waters and to oxic surface waters in the Alaskan Arctic. We also quantified production of H_2O_2, an expected key reactant produced from oxidation of either Fe(II) or reduced DOC in low-O_2 and anoxic waters. To identify the relative importance of Fe(II) versus DOC as electron donors (or other reductants present in natural waters), we quantified the oxidation of Fe(II) to Fe(III) alongside changes in total electron donating capacity upon introduction of O_2. Finally, we quantified production of CO_2 from the oxidation of DOC by •OH in soil waters.

2. Study sites and sampling strategy

Soil and surface water samples were collected May – August 2015 and July – September 2016 near Toolik Lake Field Station on the North Slope of Alaska in the arctic tundra (Fig. 2). The objective in sampling surface waters was to verify the conceptual model for dark •OH production, i.e., that dark •OH is produced upon introduction of O_2 to low-O_2 waters (Fig.1; Page et al. 2013). Thus, we would not expect to detect high •OH production in the oxic surface waters near Toolik Lake, in contrast to the low O_2 soil waters in this region. Study sites for soil water sample collection represented the dominant land surface ages and vegetation types (Hobbie and Kling, 2014; Table S1) and were expected to differ in soil water chemistry primarily due to variability in calcium carbonate (Keller et al., 2007). Soil water samples were collected from younger glacial surfaces (Itkillik I, ~60,000 yr BP (years Before Present) and Itkillik II, ~14,000 yr BP), and from an older glacial surface (Sagavanirktok, ~250,000 yr BP). In addition, soil waters were collected on the Arctic Coastal Plain in northern Alaska and from areas adjacent to glacial-fed rivers. Soil waters on younger surfaces were expected to have higher pH and conductivity than waters on older surfaces due to weathering and depletion of calcium carbonate over geologic time (Keller et al., 2007). Soil waters collected on the Arctic Coastal Plain or next to glacial-fed rivers (the Sagavanirktok and Saviukviayak Rivers) were also expected to have higher pH and conductivity than soil waters sampled near Toolik in this and prior work (Fig. S4; Table S1; Page et al., 2013) due to calcareous loess deposits (Walker and Everett, 1991). Soil waters sampled from the younger and older landscape ages were collected from the two dominant ecosystem types: the upland tussock tundra and the lowland wet sedge tundra (Muller et al., 1999). The dominant vegetation above all soil waters collected on the coastal plain and near the Sagavanirktok River was wet sedge tundra. Soil waters collected near the Saviukviayak River (*also spelled as* Saviukviak) were collected beneath birch-willow vegetation.

3. Methods

3.1. Soil and surface water collection and characterization

Soil water samples were collected below the ground surface using a stainless steel needle attached to a plastic syringe with a 3-way valve. The needle and syringe were triple rinsed with soil water before collection of bubble-free water to avoid introduction of oxygen (O_2) from air into the syringe. Water was pulled from the ground through the needle slowly to minimize collection of soil particles. Once the syringe was filled, soil water was transferred from the syringe to black BOD bottles, overfilling the bottle to minimize introduction of O_2 from air, and then stoppering. Surface waters were collected by dipping the BOD bottle into the water after triple rinsing the bottle with sample. Temperature, pH, and conductivity on unfiltered soil or surface water samples were measured in the field immediately after collection. For each soil or surface water sample collected, a subset of the water was filtered in the field using pre-combusted Whatman GF/F filters for analysis of dissolved organic carbon (DOC) concentrations. Water for analysis of cation concentrations was also collected in the field by filtering a subset of the sample through sample-rinsed Whatman 0.45 μm polypropylene filters. Subsamples for DOC and cation analysis were preserved with 6 N HCl. Subsamples for DOC and cations were stored in the dark at 4 ºC until analysis. Filtered and preserved samples for DOC and cation analysis were analyzed on a Shimadzu TOC-V analyzer (CV ~ 5% on duplicate samples or standards; Kling et al., 2000) and a Perkin Elmer ICP (CV ~ 3% on duplicate samples or standards), respectively.

After collection in the field, BOD bottles of surface and soil waters were transferred to an anoxic glove box (97% ultrapure nitrogen, 3% ultrapure hydrogen atmosphere) at Toolik Field Station (within 30 minutes to six hours after collection for sites farthest away from the station). Dissolved oxygen (DO) concentrations were measured in the glove box using an optical DO probe (YSI; 1% standard error). Soil and surface waters were analyzed for electron donating capacity (EDC), total iron and Fe(II), •OH, H_2O_2, and CO_2 production at room temperature (Fig. 3, details *below*). For each analysis (EDC, iron, •OH, H_2O_2, CO_2) sample waters were split into triplicates for initial, control, and treated subsamples. All values reported for EDC, iron, •OH, H_2O_2, and CO_2 are mean ± standard error from the triplicate samples. The chemical composition of unamended surface waters might have changed slightly if the waters were under-saturated with O_2 before sampling. Unamended soil waters were likely close representations of the chemical composition of water collected in the field, given the short duration between collection and analysis and the limited exposure to the atmosphere or to light.

3.2. Electron donating capacity and iron oxidation

For quantification of initial electron donating capacity (EDC), total iron, and Fe(II) from soil and surface waters, subsamples were analyzed immediately after filtration (0.2 µm Sterivex filter) in the anoxic glove box (initial; Fig. 3). EDC, total iron, and Fe(II) were quantified again after filtered subsamples were oxidized by O_2 for 24 hours (+air; Fig. 3). EDC was measured colorimetrically using 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+•; Page et al., 2013). For the EDC measurements, soil waters were often diluted by 2 - 200 fold with aerated MilliQ water (deionized water further purified to achieve a resistivity of 18.2 MΩ and treated by UV to reduce residual organics). Total iron and Fe(II) concentrations were quantified colorimetrically by the ferrozine method (Stookey, 1970). Soil waters often had to be diluted 2 - 80 fold in aerated MilliQ water due to high Fe(II) concentrations. Absorbance for both EDC and iron were measured on spectrofluorometer (Aqualog, Horiba Scientific), at 734 nm and 562 nm, respectively, using 1 cm pathlength cuvettes. Change in EDC and Fe(II) between initial and oxidized waters represent electrons released from oxidation of all reduced constituents and electrons released from oxidation of Fe(II), respectively, in surface and soil waters.

To test whether ABTS+• could detect electron donating capacity from the high concentrations of Fe(II) in these soil waters, the EDC was measured over the range of Fe(II) concentrations using ferrous iron solutions, in the form of ferrous ammonium sulfate. Ferrous ammonium sulfate solutions were prepared in 0.01 N HCl at concentrations of Fe(II) observed in these soil waters (Page et al., 2013; Fig. S1). At Fe(II) concentrations above 50 µM, ABTS+• did not detect all electrons that could be donated from Fe(II), leading to lower EDC than expected based on the Fe(II) concentration (Fig. S1). This underestimate in EDC was likely due to complexation of Fe(II) with the high concentration of phosphate (~2.7 mM) in the ABTS+• buffer, resulting in less Fe(II) available to donate electrons to ABTS+•. This test of ABTS+• with Fe(II) standard ferrous iron solutions suggests that ABTS+• may underestimate electrons donated from Fe(II) in surface or soil waters with Fe(II) concentrations > 50 µM.

3.3. •OH production

Terephthalate (TPA) was used as a probe to quantify •OH production in this study, the same probe as used previously in these soil waters (Page et al., 2013). During summer 2015, unfiltered subsamples of soil water were analyzed for •OH production following protocols described in Page et al. (2013). Although Page et al. (2013) reported no difference in •OH production between unfiltered versus filtered soil waters, during the summer of 2016 soil waters were filtered before •OH analysis (0.2 µm Sterivex filters) to minimize potential biological •OH production. The initial •OH production was quantified upon addition of soil or surface water samples to O_2-free TPA, with TPA present in excess (initial; Fig. 3). O_2-free TPA was prepared by bubbling with nitrogen. The solution was then stored in the dark in the glove box. Initial •OH production was quantified after 24 hours to allow for any •OH initially present in the sample to react with TPA. Production of •OH from oxidation of reduced constituents by O_2 was quantified by adding soil or surface waters to O_2- free TPA that was then exposed to O_2 by adding air (+air; Fig. 3). These aerated samples were allowed to react for 24 hours (+air; Fig. 3) and stirred every hour for 12 hours. After 24 hours the •OH concentrations in the initial and oxidized (+air) waters were determined using standard additions to the samples of 0, 25, and 50 nM 2-hydroxyterephthalic acid (hTPA, the product of TPA reaction with •OH; Page et al., 2010) to account for matrix effects. hTPA was quantified on an Acquity Ultra High Performance H-Class Liquid Chromatography (uPLC; Waters, Inc.) with fluorescence detection (excitation 250 nm, emission 410 nm) on an Acquity uPLC BEH C_{18} column (2.1 x 50 mm; 1.7 µm). The yield for hTPA formation from •OH reaction with TPA was assumed to be 35% (Page et al., 2010).

3.4. Hydrogen peroxide production

During summer 2016, a subset of soil waters was analyzed for H_2O_2 using the Amplex Red method (Burns et al., 2012, Cory et al., 2016) on the uPLC (excitation 565 nm, emission 587 nm). Undiluted, 0.2 µm-filtered soil waters were added to aerated Amplex Red reagents (+air; Fig. 3) to allow for oxidation of soil water. There was no control for the +air treatment for H_2O_2 production due to the inability to limit introduction of O_2 to the samples with addition of Amplex Red reagents. However, verification of the presence of H_2O_2 was conducted by addition of catalase to soil water, which rapidly decomposes H_2O_2 to water and O_2. Thus, soil water containing 3 mg L^{-1} catalase (+catalase, +air; Fig. 3) should yield no H_2O_2 upon introduction of air. H_2O_2 produced during oxidation in the presence and absence of catalase was quantified one hour after addition of soil water to the Amplex Red reagent. H_2O_2 was quantified using standard additions (500 - 2500 nM of H_2O_2 added) with three replicates per concentration of added H_2O_2, after subtraction of the background signal from Amplex Red alone.

3.5. CO_2 production

To quantify CO_2 production from the introduction of an oxidant to soil water, nitrogen-sparged aliquots of H_2O_2 were added to soil waters to achieve a final concentration of 50 or 100 µM H_2O_2 (+ H_2O_2; Fig. 3). Controls were amended with the same volume of O_2-free MilliQ water to account for any change in dissolved CO_2 due to introduction of MilliQ water (control;

Fig. 3). Both controls and amended vials had no headspace. In addition, four different soil waters were oxidized with a range of H_2O_2 concentrations (5 - 300 μM). After letting the control or +H_2O_2 soil waters react for 24 hours at room temperature in the dark, soil waters were analyzed for dissolved inorganic carbon (DIC) concentration using a DIC analyzer (Apollo, Inc.). Change in DIC between oxidized and control soil waters represents CO_2 produced by H_2O_2 during the 24 hour oxidation.

•OH production was also quantified from each soil water oxidized by H_2O_2 (Fig. 3) to test the hypothesis that the production of CO_2 was due to oxidation of DOC by •OH. However, absolute •OH production may have differed between the same +H_2O_2 soil water used to quantify CO_2 versus to quantify •OH due to differences in methodological constraints for detection of DIC versus •OH. The volume of soil water and thus concentration of Fe(II) exposed to the same concentration of H_2O_2 differed between the undiluted subsample of soil water quantified for CO_2 production, versus the diluted subsample of the same soil water quantified for •OH production. This is because quantification of •OH requires soil water to be diluted (~17-fold) with added TPA, to ensure that TPA is present in excess of other constituents that may scavenge •OH (see *methods* above; Page et al., 2010). These methodological constraints resulted in the same concentration of H_2O_2 added to higher concentrations of soil water constituents in the soil waters used to test CO_2 production compared to the soil waters used to quantify •OH production (Fig. 3, S3). The ratio of H_2O_2 added per mol Fe(II) in sample waters was higher in the subsamples used to quantify •OH production compared to the subsamples used to quantify changes in CO_2. Thus, it is possible that •OH production is higher in the subsample used to quantify CO_2 production compared to the subsample used to measure •OH. However, we assume that dilution does not affect trends in •OH production between soil waters; that is, a soil water exhibiting relatively high •OH production compared to another soil water will do so independent of dilution. When relating trends in CO_2 production versus •OH production from soil waters amended with H_2O_2, •OH production is expressed as the "relative" •OH (Fig. 7, 9).

Because no study has directly measured CO_2 production from oxidation of DOC by •OH in any natural water, we first tested this reaction by exposing a reference isolate of terrestrially-derived DOC to •OH produced by the Fenton reaction (i.e., •OH was produced by reaction of Fe(II) with H_2O_2). Suwannee River Fulvic Acid (SRFA), a reference DOC isolate obtained from the International Humic Substances Society (IHSS), served as a terrestrial end-member of DOC representing carbon derived from decomposed plant matter and soils (http://humic-substances.org). SRFA solutions were prepared by dissolving freeze-dried solid SRFA in air-equilibrated MilliQ water. The SRFA solution had a final pH of 5.2, a DOC concentration of 2310 μM, and 60 μM Fe(II) added in the form of ferrous ammonium sulfate. The pH of the SRFA solution and the ratio of DOC to Fe(II) were similar to soil waters sampled in the field (Table 1). A range of H_2O_2 concentrations was added to the SRFA + Fe(II) solution (5 to 300 μM H_2O_2), and compared to a control with no H_2O_2 added. After 24-hour oxidation, CO_2 and relative •OH production were measured as described above.

3.6. Soil core collection

Soil cores were collected with a SIPRE coring auger from six different sites during summer 2015. Seven cores were collected at each site (Fig. 2) to yield a minimum of 4 kg wet soil per depth analyzed. Soils collected from cores were split by depth into the annually thawed, shallow organic mat (5-50 cm; the "active layer"), and the deeper permafrost layer that included both organic and mineral soil horizons (95-105 cm). Immediately after collection soils were placed into Ziploc bags and frozen at -20 ºC until thawed for further experiments.

3.7. Soil leachates

Frozen soil (250 grams) was added to 1 L of MilliQ water and allowed to incubate in the dark at room temperature in an anoxic glove box for two weeks. The amount of soil added and incubation time were chosen to generate soil leachates that contained similar chemistry (pH and conductivity) and concentrations of Fe(II), EDC, and DOC comparable to soil waters collected in the field. Following incubation, soil water leachates were 0.2 µm filtered (Sterivex), split into initial, control, and treatment triplicate subsamples, and analyzed for EDC, total iron, Fe(II), •OH, and CO_2 as described above. Soil water leachates were tested alongside soil waters sampled in the field to increase the dataset of CO_2 and relative •OH production in this study.

4. Results

4.1. Surface and soil water chemistry

On average, soil waters were mildly acidic ($pH = 5.6 \pm 0.7$) and contained low O_2 (29 ± 5 µM DO) (Table 1). The average specific conductivity in soil waters was 408 ± 104 µS cm^{-1} (Table 1), and the average concentration of DOC was 1769 ± 262 µM (Table 1). Average iron concentrations were 245 ± 57 µM for total iron and 225 ± 56 µM for Fe(II) (Table 1). The average EDC upon introduction of air to soil waters was 192 ± 30 µM electrons released (Table 1). Waters leached from soils incubated in the lab (i.e., soil leachates) had lower conductivity, but similar pH and similar concentrations of DOC, EDC, and iron as compared with soil waters collected in the field (Table 1). On average, surface waters had higher pH (6.7 ± 1.7; Table 1), higher DO concentrations, lower specific conductivity, and lower concentrations of DOC, EDC, and iron compared to soil waters sampled in the field and soil water leached in the laboratory (Table 1).

The change in EDC upon addition of O_2 to soil waters relative to the initial value was a measure of electrons released from oxidation of reduced constituents (Fig. 4). Likewise, the change in Fe(II) concentration upon introduction of air to soil waters relative to the initial value was a measure of electrons released from Fe(II) oxidation (Fig. 4). There was a significant, positive correlation between electrons released from Fe(II) oxidation and total number of electrons released from oxidation of all reduced constituents (slope = 0.72 ± 0.02, $p < 0.0001$; Fig. 4), suggesting that Fe(II) was the main source of electrons released upon oxidation.

4.2. Trends in •OH production

Production of •OH ranged from undetectable to 20 ± 7.9 μM (N = 77) for soil waters oxidized by O_2 (air; Fig. 3), and from undetectable to 50 ± 0.3 μM (N = 93) for soil waters oxidized with H_2O_2. •OH production was significantly, positively correlated with the initial EDC ($R^2 = 0.70$, $p < 0.0001$; Fig. 5A) and initial Fe(II) in soil waters tested ($R^2 = 0.66$, $p < 0.0001$; Fig. 5B). However, there was no significant correlation between •OH production and Fe(II) oxidation over the 24-hour aeration period (Fig. S2). There was also no significant correlation between •OH production and other water chemistry parameter (e.g., conductivity, carbonate; data not shown). •OH production was higher on average from soil waters sampled on older or fluvial land surfaces compared to younger surfaces or the coastal plain (Table S1). Surface water samples had low production of •OH upon introduction of O_2, consistent with the relatively high dissolved O_2, low EDC, and low Fe(II) concentrations in these waters compared to soil waters (Table 1; Fig. 5A, B).

4.3. H_2O_2 concentrations in soil waters

The average H_2O_2 concentration after one hour oxidation by O_2 in soil waters was 21 ± 11 μM. H_2O_2 concentration in soil waters after oxidation by O_2 was generally higher for soil waters with low initial O_2 concentration (Fig. 6). As a qualitative confirmation of H_2O_2 production from aeration of soil waters, H_2O_2 concentrations were compared between a filtered soil water leachate oxidized by O_2 in the presence and absence of catalase, an enzyme that rapidly decomposes H_2O_2 to water and O_2. In the absence of catalase, the soil water produced 2.7 ± 0.40 µM H_2O_2 after one hour exposure to air (data not shown). In the presence of catalase, there was no detectable H_2O_2 produced from the soil water.

4.4. •OH and CO_2 production from soil waters amended with H_2O_2

All soil waters amended with H_2O_2 showed significant production of relative •OH compared to the control (no H_2O_2 addition; Figs. 3, 7A, 7B). For soil waters amended with a range of H_2O_2 concentrations, relative •OH production was positively, linearly correlated with the concentration of H_2O_2 added (Fig. 7A). The slope of the relationship between H_2O_2 added and relative •OH produced varied between the soil waters and leachates tested, ranging from 0.08 ± 0.01 ($p < 0.01$) to 0.01 ± 0.002 ($p < 0.01$) (Fig. 7A). •OH production was significantly higher in the soil water amended with H_2O_2 in the absence of catalase, compared to unamended soil waters and to the soil water containing H_2O_2 and catalase (Fig. 8).

Most soil waters amended with H_2O_2 showed a significant increase in DIC (i.e., CO_2 produced) compared to control soil waters (65% of 92 soil waters tested, Fig. S3). The remaining soil waters showed no detectable production of CO_2 (35% of soil waters tested). The production of CO_2 from all soil waters was not significantly correlated with EDC, total iron and Fe(II), or •OH production (Fig. S3). However, CO_2 produced was significantly, positively correlated with increasing concentrations of H_2O_2 added to four soil waters (Fig. 7B). The slope of the linear relationship between H_2O_2 added and CO_2 produced differed by soil water, ranging from 0.39 ± 0.09 ($p < 0.1$) to 0.04 ± 0.002 ($p < 0.0001$) (Fig. 7B). There was no systematic pattern between the slopes of the linear relationship of CO_2 produced and H_2O_2 added for soil waters by date, site, or between soil water versus soil leachate. For the solution of Suwannee River Fulvic Acid (SRFA) containing Fe(II), there was also a significant, positive correlation between CO_2 produced and H_2O_2 added (Fig. 7B). The slope of the relationship for CO_2 produced and H_2O_2 added for SRFA + Fe(II) was within the range of slopes observed for the natural soil waters containing similar concentrations of DOC and Fe(II) and amended with the same range of H_2O_2 concentrations (Fig. 7B).

For any soil water amended with H_2O_2, both relative •OH production and CO_2 production increased with increasing H_2O_2 concentration added (Fig. 7A, 7B). Therefore, for all sample types, sites, and dates, CO_2 production was significantly positively correlated with the •OH produced by adding H_2O_2 (Fig. 9). The slopes of the relationship between CO_2 production and relative •OH production varied between the soil waters and leachates, and SRFA + Fe(II) solution, as did the slopes representing relative •OH production and H_2O_2 added to different soil waters (Fig. 7A, 7B).

5. Discussion

5.1. Conditions favorable for dark •OH production in arctic soil waters

Overall, our main results (1) extend the findings of •OH production by aeration of soil or surface water to a wider range of water chemistry, (2) demonstrate that Fe(II) is the dominant electron donor supporting •OH production, and (3) provide direct, multiple lines of evidence for the production of CO_2 from the oxidation of DOC by •OH in natural waters. The first main result is consistent with prior work demonstrating that •OH is produced from aeration of soil or lake waters containing reduced constituents (Page et al., 2013; Minella et al., 2015). These findings support the conceptual model proposing •OH production from the oxidation of Fe(II) or reduced DOC by dissolved O_2 (Fig. 1; Page et al., 2012). Consistent with this conceptual model and prior work (Page et al., 2013), in this study there was little •OH production in oxic surface waters containing lower EDC, lower Fe(II), and lower DOC, while in low-O_2 waters with higher EDC, higher Fe(II), and higher DOC the •OH production was significantly, positively correlated with initial EDC and Fe(II) (Fig. 5A, B).

Our first result demonstrates that reducing conditions (i.e., high EDC and thus high concentrations of electron donors) that support •OH production can be found across a wider range of pH and conductivity in anoxic and low-O_2 arctic soil waters than previously observed (Page et al., 2013). In this study, the subset of soil waters sampled on the Arctic Coastal Plain and adjacent to glacially-fed rivers (Fig. 1) had significantly higher pH and specific conductivity than soil waters sampled near Toolik in this and prior work (Table S2; Fig. S4; Page et al. 2013). Soil waters of the Coastal Plain and adjacent to glacial-fed rivers had higher pH and specific conductivity due to calcareous loess deposits (Walker and Everett, 1991). Reducing conditions are observed across a range of pH and conductivity in arctic soil waters due to the presence of permafrost at a shallow depth and flat topography that prevents drainage of water and leads to saturated, low-O_2 soils (Lipson et al., 2012; Lipson et al., 2013). Anoxic or low-O_2 soils lead to strongly reducing conditions that accelerate the buildup of high concentrations of electron donors such as Fe(II) in arctic soils. For example, previous work showed that arctic soil waters spanning a wide range of pH and conductivity contain concentrations of reduced Fe(II) ranging from 100 to 10,000 μM (Keller et al., 2007; Lipson et al., 2010; Pokrovsky et al., 2013; Page et. 2013; Herndon et al., 2015; Reyes and Lougheed, 2015).

The yield of •OH from the oxidation of reduced constituents such as Fe(II) or reduced DOC may depend strongly on pH. At circumneutral pH, the oxidation of Fe(II) may yield oxidants other than •OH (Remucal and Sedlack, 2011). For example, at pH 7, oxidation of Fe(II) may result in production of ferryl iron (Fe(IV)) as well as •OH, which would lower the ratio of •OH produced per mol Fe(II) oxidized (Vermilyea and Voelker, 2009; Remucal and Sedlack, 2011). Studies of the •OH production from Fe(II) oxidation suggest that at low pH (~5), production of •OH from Fe(II) oxidation is more likely than production of Fe(IV) (Remucal and Sedlack, 2011). In contrast to Fe(II) oxidation, the effect of pH on the yield of •OH from oxidation of reduced DOC has not been studied. It has been shown that at high pH the oxidation of reduced moieties within DOC is more favorable than at low pH (Aeschbacher et al., 2012), suggesting that oxidation of DOC to produce •OH might be more likely at higher pH. Overall, the yield of •OH from oxidation of Fe(II) or reduced DOC is expected to be higher in natural waters with low pH than in waters with higher pH where there could be production of ferryl ion in addition to •OH. Consistently, in our study •OH production was generally higher in mildly acidic soil waters (pH ~ 6; Fig. S4), while •OH production was generally lower in soil waters with pH > 7.5 (Fig. S4).

5.2. Oxidation of Fe(II) controls •OH production in arctic soil waters

5.2.1. Fe(II) was the main electron donor to O_2

The second main result of this study provides strong evidence that Fe(II) was the main electron donor to O_2 upon aeration of soil waters, and thus the main control on •OH production in soil waters of the Alaskan Arctic. The significant, linear correlation between the total electrons released and the electrons released from Fe(II) oxidation upon addition of air to soil waters (Fig. 4) suggests the oxidation of Fe(II) accounts for the total electrons released during the oxidation. Assuming one mole of Fe(II) oxidized contributes one mole of electrons, the change in Fe(II) concentration should correspond 1:1 with the electrons released upon aeration.

The relationship between moles of electrons released from Fe(II) oxidation per moles of total electrons released supported this expectation, and had a slope of 0.72 ± 0.02 ($p < 0.0001$) (Fig. 4), suggesting that 7 out of 10 moles of electrons released came from Fe(II).

5.2.2. Limitations of the EDC method to detect electrons released from Fe(II) oxidation

Some soil waters had a lower EDC than expected based on the amount of Fe(II) oxidized, and plotted substantially below the 1:1 line in Figure 4. This is likely due to interference in the EDC method that uses a phosphate buffer to minimize changes in pH (Aeschebacher et al., 2012). In the presence of high concentrations of Fe(II) in soil waters, a greater proportion of Fe(II) may complex with phosphate in the buffer solution, which may slow the oxidation of Fe(II) (Van der Grift et al., 2016). Thus, complexation of Fe(II) with phosphate may result in a lower EDC than expected based on the concentration of Fe(II) present. For example, when Fe(II) as the electron donor was added to MilliQ water in the form of ferrous ammonium sulfate, the EDC was lower than the concentration of Fe(II) when Fe(II) concentrations exceeded 50 µM (less than 1 mol of total electrons released per 1 mol of electrons released from Fe(II); see *methods* and Fig. S1). This result suggests that at high Fe(II) concentrations some of the iron complexes with phosphate, resulting in a lower EDC than expected based on the initial Fe(II) concentration. Because phosphate buffer was used only for the subset of soil waters analyzed for EDC, and was not used to quantify Fe(II) oxidation upon addition of air, there was no interference for the quantification of the oxidation of Fe(II). Thus, together these results strongly indicate that for the 78% of soil waters plotting on or below the 1:1 line for the electrons released from Fe(II) oxidation versus total electrons released (Fig. 4), Fe(II) was the most important electron donor upon oxidation of soil waters.

5.2.3. Contribution from other electron donors to •OH production

For the ~ 20% of soil waters plotting above the 1:1 line, electron donors other than Fe(II) may have contributed to the EDC upon aeration of soil water (Fig. 4). While oxidized iron could be reduced and re-oxidized multiple times (*section 5.4.1.*), most of the soil waters that plotted significantly above the 1:1 line had low Fe(II) concentrations suggesting the presence of electron donors other than iron (Fig. 4). Based on estimates of the fraction of DOC that may be reduced and on the concentrations of total manganese (Mn) in these soil waters, previous work proposed that alternate electron donors may include reduced DOC or Mn (Table 1; Page et al., 2013). Sulfide could be an additional electron donor (Wallace et al. 2017); however, concentrations of sulfide in the soil waters of this study are too low for sulfide to be a substantial source of electrons to produce •OH (Page et al., 2013). The expected reductants within the DOC pool are reduced quinone moieties, which may produced •OH upon oxidation by air via an organic Fenton reaction, with H_2O_2 expected to be an intermediate, similar to Fe(II) oxidation (Fig. 1; Page et al., 2012). Assuming the same fraction of reduced DOC in our samples as that determined for similar samples by Page et al. (2013), DOC could have accounted for 25% of the EDC on average from the soil waters in this study. Concentrations of total Mn were on average five-fold lower than total iron in these soil waters (Table 1; Page et al., 2013), suggesting that Mn was likely less important than Fe(II) or DOC as an electron donor to O_2 yielding •OH in most soil waters. However, at one site, concentrations of Fe(II) were significantly lower than the EDC (Fig. 4) and lower than total dissolved Mn (49 μM), suggesting that the oxidation of reduced Mn could have contributed to the EDC.

5.3. H_2O_2 production is consistent with a Fenton source of •OH

Here we show for the first time that H_2O_2 is produced upon introduction of O_2 to anoxic or low-O_2 soil waters (Fig. 6). H_2O_2 may be present in soils as a result of fungal activities that may produce H_2O_2 to degrade lignin by the Fenton reaction (Baldrian and Valaskova, 2008). While H_2O_2 has not been measured directly in soils before, others have reported dark H_2O_2 production or H_2O_2 concentrations in ponds, lakes, and coastal waters (Vermilyea et al., 2010; Cory et al., 2016; Zhang et al., 2016). Dark H_2O_2 production in low Fe(II), oxic surface waters was attributed to biological activity (Vermilyea et al., 2010; Cory et al. 2016; Zhang et al., 2016). H_2O_2 production in this study was not likely due to biological processes because soil waters were filtered to minimize microbial activity prior to oxidation (see *methods*). In addition, H_2O_2 likely reacts rapidly with the high Fe(II) concentrations in these soil waters (Table 1, Fig. 1), and thus any H_2O_2 biologically produced prior to filtration should not be stable. High concentrations of H_2O_2 similar to those in this study (µM range; Table 1; Fig. 6) have been reported for aerated sediments amended to contain high concentrations of Fe(II), where production of up to ~ 4 µM H_2O_2 in the amended sediments was attributed to the oxidation of Fe(II) by O_2 (Murphy et al., 2014). Thus, this direct evidence for the production of H_2O_2 upon aeration of soil waters rich in reduced constituents (Figs. 1, 6) is also evidence for an abiotic source of H_2O_2.

H_2O_2 production from aeration of soil waters was higher at low initial O_2 concentrations, suggesting that H_2O_2 production resulted from oxidation of reduced constituents such as Fe(II) or DOC (Fig. 6). This result is consistent with the well-studied production of H_2O_2 during the oxidation of Fe(II) or reduced DOC by O_2 (Haber and Weiss, 1932; Stumm and Lee, 1961; Voelker and Sulzberger, 1996; Page et al., 2012; Kluepfel et al., 2014). H_2O_2 produced by the oxidation of Fe(II) or reduced DOC likely subsequently oxidized Fe(II) in these high-iron soil waters, leading to production of •OH (Petigara et al., 2002).

5.4. Controls on the production of •OH from iron oxidation in arctic soil waters

5.4.1. The yield of •OH from Fe(II)

Evidence from this study strongly suggests that Fe(II) oxidation is the main source of •OH produced upon aeration of soil waters (Fig. 4). However, the large variability in the amount of •OH produced from soil waters with the same initial concentration of Fe(II), or the same amount of Fe(II) oxidized upon aeration (Fig. 5, S2), suggests that factors other than the initial amount of Fe(II) present can influence the production of •OH. Given that •OH production and oxidation of Fe(II) were each measured over the same time period (24 hours) for all waters, we interpret differences in •OH production per oxidation of Fe(II) to be due to differences in the yield of •OH per mol Fe(II) oxidized. Differences in •OH yield per mol Fe(II) oxidized may be due to the large variability in soil water chemistry (pH, initial Fe(II), DOC) that influenced both the rate of Fe(II) oxidation and the production of specific intermediates and products formed during Fe(II) oxidation (Voelker et al., 1996; Chang et al., 2008; Burns et al., 2010; Fuji et al., 2010). In water containing only dissolved O_2 and iron, the expected (net) stoichiometry is one mole •OH produced for every three moles Fe(II) oxidized (Fig. S2; Remucal and Sedlack, 2011), because the oxidation of iron proceeds by a series of one electron transfer reactions to O_2 producing a suite of reactive oxygen species (Haber and Weiss, 1932). However, in natural waters varying widely in chemistry, the molar yield of •OH per mol Fe(II) oxidized may be substantially different than the expected 1:3 ratio because iron may undergo rapid redox cycling.

Previous work in simulated sediment pore waters reported that iron redox cycles varied widely as a function of pore water chemistry. Burns et al. (2010) reported that the number of Fe(II) / Fe(III) cycles may vary between 10 – 22000, resulting in 3 to 750 mol •OH produced per mol Fe(II). Thus, in natural soil waters it may be possible to generate > 1 mol •OH per 3 mol Fe(II) oxidized if there are many cycles of Fe(II) / Fe(III) (Burns et al., 2010).

The controls on the number of iron redox cycles yielding •OH in natural waters are not yet well understood. In this study, few soil waters exhibited greater than the expected 1 mol •OH produced per 3 mol Fe(II) oxidized (Fig. S2) if there were many cycles of Fe(II) / Fe(III). Most soil waters exhibited less than 1 mol •OH produced per 3 mol Fe(II) oxidized (Fig. S2). We expect in these DOC-rich soil waters for DOC to have the greatest influence on the iron redox cycling (Table 1). DOC can influence the iron redox cycling and thus •OH production by (1) forming complexes with iron and by (2) playing a role in iron oxidation and reduction.

5.4.2. Complexation of Fe(II) with DOC

Complexation of Fe(II) with DOC ligands has been suggested to affect the rate of Fe(II) oxidation (Voelker and Sulzberger, 1996; Miller et al., 2009). In the predominately acidic to mildly acidic soil waters in this study, organic ligands within DOC were most likely to form complexes with Fe(II) given that these waters contain high DOC concentrations and low concentrations of other ligands such as sulfide or carbonate (Table 1; Page et al., 2013). Given that concentrations of Fe(II) and Fe(III) were much higher than expected based on equilibrium with the amount of dissolved oxygen at the specific pH of the soil water (Table 1, Fig. S4), it is likely that interactions between Fe(II) and DOC influenced the stability of Fe(II) (Pham and Waite, 2008; Miller et al., 2009; Jones et al., 2015). Alternatively, DOC may influence iron redox cycling by increasing rates of Fe(II) oxidation. For example, Voelker and Sulzberger (1996) found faster oxidation of Fe(II) by H_2O_2 in the presence of DOC. Fe(II) and DOC concentrations are strongly, positively correlated in soil waters in this study ($p < 0.05$; data not shown), and there is evidence for an association between iron and DOC in soil waters (Sundman et al., 2013; Herndon et al., 2015). However, it is currently not possible to predict the specific effects of DOC on iron redox cycling, and thus on the •OH production during aeration of natural waters, due to lack of information on the identity, abundance, and acidity of DOC ligands for iron.

DOC in arctic soil waters is derived mostly from the degradation of plant and soil matter, and thus this DOC pool contains abundant carboxyl and phenolic moieties (Drake et al., 2015; Ward and Cory, 2015). Herndon et al. (2015, 2017) showed that both Fe(II) and Fe(III) present in arctic soil waters were complexed with DOC, and Daugherty et al. (2017) suggested that carboxyl ligands within DOC are most important for complexing Fe(II). These findings are consistent with prior work suggesting that carboxyl and phenolic moieties may serve as ligands to complex with both Fe(II) and Fe(III) (Voelker and Sulzberger, 1996; Miller et al., 2009; Vermilyea and Voelker, 2009; Jones et al., 2015). In carboxyl and phenolic rich DOC soil waters from our study, DOC is expected to complex with Fe(II), but how this complexation may either speed up or slow down Fe(II) oxidation, or influence the number of Fe(II) / Fe(III) redox cycles and thus influence •OH production, remains an open question.

5.4.3. The role of DOC in iron oxidation and reduction

In addition to the role of DOC in complexing with iron, DOC likely influences iron redox cycling and •OH production by acting as a source of reactive oxygen species involved in iron oxidation and reduction, or as a reductant of Fe(III). Oxidation of reduced DOC could produce H_2O_2 (Page et al., 2012), the key reactant in Fenton-mediated Fe(II) oxidation that yields •OH (Figs. 1, 6). Interactions of iron with DOC may also influence the balance of reactive intermediates and products formed during iron redox cycling (Voelker and Sulzberger, 1996; Remucal and Sedlack, 2011). For example, in the presence of terrestrially-derived DOC (e.g., SRFA), Voelker and Sulzberger (1996) found that •OH reacted with DOC to produce an organic radical. The organic radical reduced O_2 to yield superoxide that then oxidized Fe(II) to Fe(III) and regenerated H_2O_2. DOC can also reduce Fe(III) to Fe(II) (Voelker and Sulzberger, 1996; Vermilyea and Voelker, 2009), thereby enhancing iron redox cycling by regenerating Fe(II) that had been oxidized by O_2 or by reactive oxygen species. Thus, by acting as a source of reactive oxygen species or as a reductant of Fe(III), DOC may have influenced the number of iron redox cycles or rate of Fe(II) oxidation, affecting the range of •OH production per mol Fe(II) oxidized in the study waters (Figs. 5, S2).

5.5. •OH oxidation of DOC and CO_2 production

5.5.1. CO_2 production in soil waters

The third main result is that more than half the soil waters tested showed production of CO_2 within 24 hours after addition of H_2O_2. Increasing CO_2 production with increasing H_2O_2 (Fig. 7) and increasing relative •OH (Fig. 9) is consistent with the oxidation of DOC to CO_2 by •OH (Voelker and Sulzberger, 1996; Burns et al., 2010; Hall and Silver, 2013). DOC is likely the main sink for •OH in soil waters due to high DOC concentrations and low concentrations of quenching anions like chloride and bromide (Page et al., 2013, 2014) that can scavenge •OH (Qian et al., 2001). Carbonate can also scavenge •OH to produce low energy radicals at a slower rate than •OH reacts with DOC (Buxton et al., 1988). However, while some of the soil waters or soil leachates contained relatively high DIC (i.e., high carbonate alkalinity), at the pH of these soil waters there were still too few carbonate ions to compete with DOC as a sink for •OH (Table 1; Buxton et al., 1988; Page et al., 2014). Therefore, oxidation of DOC by •OH in the soil waters tested here is expected to be the main source of CO_2 produced.

The linear increase in both relative •OH and CO_2 production with increasing concentrations of H_2O_2 added to soil waters containing Fe(II) strongly supports the model of •OH oxidation of DOC as the source of the CO_2 (Fig. 9). Alternative pathways for CO_2

production from soil waters include aerobic microbial respiration of DOC or anaerobic fermentation. However, soil waters were filtered to remove microbes prior to H_2O_2 addition, thus minimizing CO_2 production from microbes.

Another line of evidence for oxidation of DOC by •OH as the source of CO_2 is the experiments with SRFA + Fe(II). Previous work showed that addition of H_2O_2 to solutions of SRFA + Fe(II) resulted in production of •OH (Voelker and Sulzberger, 1996), as we observed in this study (Fig. 7). This •OH is expected to oxidize DOC to CO_2, as shown directly here with CO_2 production increasing with increasing relative •OH in SRFA + Fe(II) solutions. Production of CO_2 in microbe-free solutions of SRFA + Fe(II) comparable to production in soil waters suggests that in both cases the source of CO_2 is oxidation of DOC by •OH (Fig. 9).

5.5.2. Variability in CO_2 production

The results suggest that for the same amount of •OH produced there can be large variability in CO_2 production from the oxidation of DOC by •OH. For example, there were differences in the yield of CO_2 produced per relative •OH produced between soil waters amended with a range of H_2O_2 concentrations (Fig. 9). Consistently, there was high variability in yield of CO_2 per relative •OH produced in all soil waters oxidized by H_2O_2 (Fig. S3). Differences in DOC composition between the soil waters studied could influence whether CO_2 versus other products are formed from the oxidation of DOC by •OH (Zepp et al., 1992; Brezonik and Fulkerson-Brekken, 1998; Westerhoff et al., 1999). It is expected that within the broad range of aromatic and aliphatic fractions of DOC shown to react with •OH, the abundance of the moieties that react most rapidly with •OH are expected to control the rate of DOC oxidation and thus the rate of CO_2 production (Westerhoff et al., 1999; Waggoner et al., 2015). In addition to DOC composition, other factors can influence the amount of CO_2 produced during the series of iron oxidation reactions that yield •OH, including the production of alternate oxidants (i.e., ferryl iron; Vermilyea and Voelker, 2009; Remucal and Sedlack, 2011). Therefore, the range in the concentrations of CO_2 produced by DOC in the presence of •OH (Fig. 9) is consistent with the variable chemistry and DOC composition between the soil waters tested (Table 1; Ward and Cory, 2015).

5.5.3. Underestimation of •OH production

Greater yield of CO_2 produced from oxidation of DOC by •OH in this study than a previously measured laboratory yield (Goldstone et al., 2002) may be evidence of underestimation of •OH produced in our study. Two methodological constraints may have contributed to an underestimation of •OH. First, as described in the *methods* and *results*, CO_2 production was quantified from undiluted soil water exposed to the same concentration of H_2O_2 as diluted soil water used to quantify •OH production. Due to differences in the methodological constraints for detection of CO_2 versus •OH, there was a larger ratio of Fe(II) and DOC to H_2O_2 present in the subset of (undiluted) soil waters used to quantify CO_2 production versus the subset of (diluted) soil waters used to quantify •OH. Higher concentrations of Fe(II) and DOC in undiluted soil waters could lead to a greater number of Fe(II) / Fe(III) redox cycles (Burns et al., 2010), and thus more •OH and CO_2 produced compared to diluted waters. In contrast to undiluted soil waters, in diluted soil waters containing low Fe(II) and DOC relative to the H_2O_2 present, Fe(II) may be unable to complete the redox cycle to produce •OH, or •OH may react with excess H_2O_2 (instead of DOC) to produce less reactive radicals (Ciotti et al., 2009). Thus, the values of relative •OH produced reported here may be conservative, and there may have been more •OH produced in the undiluted soil water used to quantify CO_2 produced versus the same diluted soil water used to quantify •OH produced.

Second, assumptions about the yield for reaction of the TPA probe used to quantify •OH produced may lead to an underestimation of •OH produced from soil waters. Specifically, TPA reacts with •OH to produce hTPA, with a yield of 35% demonstrated in simulated natural waters (Page et al., 2010). However, Charbouillot et al. (2011) reported that the yield of hTPA produced per mol •OH in aqueous solutions decreased with decreasing pH between pH 7.5 to 3.9. Because the pH range of the soil waters studied here ranged from 7.6 to 4.5, applying a constant yield of 35% could underestimate the concentrations of •OH produced if the yield of hTPA produced per mol •OH present was lower. Although it is not possible to determine controls on the yield of •OH across the range of water chemistries studied here, a yield of 0.35 mol hTPA per 1 mol •OH has been proposed to be an upper limit (Fang et al., 1996; Charbouillot et al., 2011). Assuming the yield varies from 0.10 to 0.35 mol hTPA per 1 mol •OH (Charbouillot et al., 2011), concentrations of •OH could be almost four times greater in some soil waters than reported here. Therefore, the multiple methodological limitations in •OH detection suggest that •OH produced from soil waters is likely a conservative estimate. While it was not possible to know with confidence the absolute •OH produced from oxidation of soil waters by O_2 or H_2O_2, the findings from this study strongly suggest that when •OH is produced, it oxidizes DOC to CO_2 (Fig. 9).

6. Conclusions and implications

Results from this study show for the first time that Fe(II) was the main electron donor upon aeration of soil waters, and that H_2O_2 is likely produced from aeration of natural waters with reduced species such as iron and DOC. Prior work and our results strongly indicate Fe(II) oxidation as the predominant pathway for •OH production when O_2 is introduced to arctic soil waters. This study also is the first to directly demonstrate that CO_2 is produced from natural soil waters in proportion to •OH produced, likely due to the oxidation of DOC by •OH. Thus, this study demonstrates that the dark, chemical oxidation of DOC by •OH may be an important source of CO_2 produced in arctic soils. Direct evidence for CO_2 from •OH oxidation of DOC in this study supports prior work in tropical soils, where a correlation between CO_2 production and Fe(II) oxidation was suggested to be due in part to oxidation of DOC by •OH (Hall and Silver, 2013).

However, the quantitative importance of •OH in soil carbon cycling depends on the *in-situ* •OH production as redox constituents in soil waters cycle between reducing and oxidizing conditions. Waterlogged soils result in the accumulation of Fe(II) (Lipson et al., 2010) that can be oxidized by the introduction of O_2. O_2 can be introduced through a change in the water table depth, slow diffusion to the oxic-anoxic interface, rain events, or downslope flow of anoxic soil waters into oxic surface streams. Introduction of O_2 by any of these pathways to Fe(II) rich soil waters could trigger the oxidation of DOC by •OH to CO_2 or to low molecular weight organic compounds (Goldstone et al., 2002) at these redox interfaces. Interestingly, Herndon et al. (2015) reported the presence of low molecular weight compounds like acetate at redox interfaces in arctic soils. Therefore, understanding (1) the frequency of oxygenation events, (2) the rates of production of reduced species after oxygenation, (3) the variability in Fe(II) concentrations and *in-situ* •OH production, and (4) the controls on the production of CO_2 from oxidation of DOC by •OH, are the next steps needed to understand the role of dark •OH in soil carbon cycling.

Understanding iron-mediated •OH production is important because increased thaw depth in a warming Arctic may increase the abundance of Fe(II) in arctic and boreal soils (Keller et al., 2007; Pokrovsky et al., 2013; Herndon et al., 2015; Reyes and Lougheed, 2015; Vonk et al., 2015). For example, Barker et al. (2014) reported the highest concentrations of total dissolved iron in arctic streams in late fall, which they attributed to deeper thaw into the mineral layer of the soils. Previous work has shown that minerals in permafrost soils contain leachable iron (Keller et al., 2007), but information from broad geographic settings is limited.

In addition to greater iron availability with increasing thaw depth, thawed permafrost soils contain tremendous stores of soil carbon (Ping et al., 2008) susceptible to oxidation by •OH. Oxidation of DOC by •OH may be less selective than microbial oxidation and degradation of DOC (Ward and Cory, 2015), suggesting that even if annual rates of DOC oxidation by •OH to CO_2 are much less than microbial production of CO_2 from arctic and boreal soils, •OH oxidation of DOC may influence microbial respiration of DOC. For example, •OH may oxidize a fraction of DOC that would otherwise be relatively resistant to microbial degradation, or produce low molecular weight acids that are more labile to microbes (Goldstone et al., 2002). Thus, iron-mediated •OH production and its oxidation of organic carbon could influence the conversion of the vast stores of organic carbon in permafrost soils to CO_2 on relatively short time scales, and potentially contribute to an accelerating feedback to global warming (e.g., MacDougall, 2012).

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