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# Improving Nitrite Analysis in Soils: Drawbacks of the Conventional 2 M KCl Extraction

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Soil nitrite ( $\text{NO}_2^-$ ) is an important source of nitrous acid to the atmosphere as well an intermediate in nitrification and denitrification. Few studies, however, have directly linked  $\text{NO}_2^-$  pools with N emissions because  $\text{NO}_2^-$  is reactive and seldom detectable in soils. Here, we test whether the elusiveness of soil  $\text{NO}_2^-$  is due to its reactivity or to problems associated with conventional 2 M KCl extractions. We extracted acidic, neutral, and alkaline soils (pH 5.4– 8.2) in 2 M KCl, pH-8-adjusted 2 M KCl, and deionized water (DIW). Unbuffered KCl consistently underestimated soil  $\text{NO}_2^-$  compared with DIW; soils with lower pH had lower  $\text{NO}_2^-$  in unbuffered KCl than in DIW water. In acidic soils, unbuffered KCl favored the transformation  $\text{NO}_2^-$  to nitric oxide. Because KCl lowers the pH of extracts by ~1 unit, this increase in acidity likely favored the transformation of  $\text{NO}_2^-$  to gaseous N products. Although buffered KCl minimizes  $\text{NO}_2^-$  destruction, it can cause colorimetric interferences when done on small soil samples (4 g). Deionized water extractions offer an alternative for measuring  $\text{NO}_2^-$  in small samples, but filtering beyond the traditionally used 2.5- $\mu\text{m}$  filter paper is necessary to remove suspended solids. Despite its widespread use, unbuffered 2 M KCl should not be used for the analysis of soil  $\text{NO}_2^-$ .

Abbreviations: DIW, deionized water; DTPA, diethylenetriaminepentaacetic acid; EDTA, ethylenediaminetetraacetic acid; NED, N-(1-naphthyl)-ethylenediamine dihydrochloride; SOM, soil organic matter.

**N**itrite ( $\text{NO}_2^-$ ) is an important intermediate in nitrification and denitrification (Robertson and Groffman, 2007) and also participates in abiotic reactions (Nelson and Bremner, 1970???, a, b, or ab???, Venterea et al., 2005; Venterea, 2007). In soils, biologically produced  $\text{NO}_2^-$  can react chemically to produce gaseous N-oxides such as nitrous acid (HONO), nitric oxide (NO), and nitrogen dioxide ( $\text{NO}_2$ ) (Nelson and Bremner, 1970???, a, b, or ab???, Medinets et al., 2015), which all contribute to the oxidative capacity of the lower atmosphere (Oswald et al., 2013). Despite the importance of soil  $\text{NO}_2^-$  to atmospheric chemistry (Su et al., 2011; Oswald et al., 2013)  $\text{NO}_2^-$  is rarely detected in soils, limiting our ability to observe linkages between soil N-cycling processes and atmospheric chemistry.

In spite of the fast reactivity of  $\text{NO}_2^-$  in soils, however, it accumulates under at least two conditions: (i) when high soil ammonium ( $\text{NH}_4^+$ ) concentrations and pH adversely affect the microbes consuming  $\text{NO}_2^-$  (Chapman and Liebig, 1952; Van Cleemput and Abdullahi, 1996), and (ii) in drying soils where solutes concentrate in hydrologically disconnected microsites (Davidson et al., 1991; Xie et al., 2001; Nejidat, 2005; Gelfand and Yakir, 2008; Parker and Schimel, 2011).

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Nitrite is typically measured by extracting soil with 2 M KCl, yielding clear, colorless extracts that facilitate colorimetric analyses, while simultaneously allowing determination of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  (Keeney and Nelson, 1982; Maynard et al., 2007). Several improvements to the colorimetric technique have targeted interferences caused by Fe, either by chelating Fe (Colman, 2010), or by removing Fe from the extract (Yang et al., 2012). However, soil extractions in 2 M KCl can significantly underestimate  $\text{NO}_2^-$  concentrations (Stevens and Laughlin, 1995) because concentrated salt solutions release exchangeable acidity (Ponette et al., 1996), and acid destroys  $\text{NO}_2^-$  (Nelson and Bremner, 1969; Islam et al., 2008). Despite these shortcomings, concentrated salts are still recommended in soil analysis manuals (Keeney and Nelson, 1982; Mulvaney, 1996; Pansu and Gautheyrou, 2006; Maynard et al., 2007) and are routinely used for  $\text{NO}_2^-$  analysis.

To improve the recovery of  $\text{NO}_2^-$  in KCl extracts, Stevens and Laughlin (1995) recommended adjusting the pH of 2 M KCl extracts to 8 with 2 M KOH, and discussed the option of using DIW as an extractant. However, their approach uses a substantial amount of soil (200 g) in a 1:1 soil/extractant ratio, and deviating from these recommendations may introduce the risk of pH-induced dispersal of silt and clay particles and/or dissolve organic matter (release humic and fulvic acids; Fujitake et al., 1998), producing yellow- or brown-colored extracts, that may interfere with colorimetric analyses (e.g., Banderis et al., 1976). Similarly, DIW extractions may not yield colorless extracts, potentially influencing absorbance measurements during  $\text{NO}_2^-$  analysis. Here, we tested a series of soil extractions to: (i) validate that unbuffered concentrated salts interfere with  $\text{NO}_2^-$  analyses and should not be used for analyzing  $\text{NO}_2^-$  in soils; and (ii) develop recommendations for analyzing small soil samples (~4 g) in DIW and buffered KCl extractions.

## MATERIALS AND METHODS

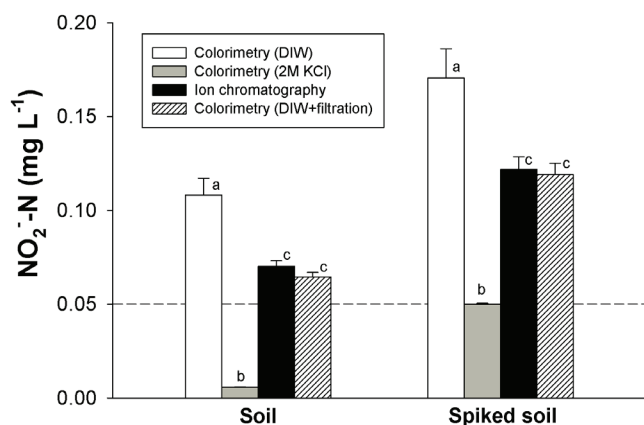
We measured  $\text{NO}_2^-$  concentrations in soils from three sites encompassing a gradient in soil pH (5.4–8.2) to assess whether pH interacts with the extractant (DIW vs. KCl) and analysis approach (colorimetry vs. chromatography) when analyzing  $\text{NO}_2^-$  in soils. All soils were collected in late September (end of dry season; gravimetric water content  $\leq 5\%$ ) from the upper 10 cm (A horizons;  $n = 4$  replicates per site). Acidic (pH 5.4) soil (Entic Cryumbrepts) was obtained from a sub-alpine meadow in the Emerald Lake catchment, located along the western slope of the Sierra Nevada Mountain Range, California, within Sequoia and Kings Canyon National Park. Neutral (pH 6.9) soil (Pachic Argixerolls) was obtained from an annual grassland in the University of California Sedgwick Reserve near Santa Barbara, CA, and alkaline (pH 8.2) soil (Lithic Torriorthents) from Joshua Tree National Park within the Mojave desert, CA (see further descriptions in supplementary materials available online).

We first compared  $\text{NO}_2^-$  concentrations in neutral soils extracted in unbuffered 2 M KCl (extra pure KCl; Acros Organics, Fisher Scientific, Pittsburgh, PA) analyzed colori-

metrically, with soils extracted in DIW and analyzed by both colorimetry and ion chromatography (Dionex ICS-900 ion chromatograph; Bannockburn, IL; method detection limit =  $0.02 \text{ mg NO}_2^- \text{--N L}^{-1}$ ). Extra pure KCl was used to avoid introducing significant  $\text{NO}_2^-$  contamination typically found in reagent-grade KCl. Air-dried soils (4 g;  $n = 4$ ; sieved to 2 mm) were extracted in 30 mL of 2 M KCl or DIW, shaken for 30 min, centrifuged (15 min at  $1600 \times g$ ), and filtered through 2.5  $\mu\text{m}$  Whatman 42 filter paper (Keeney and Nelson, 1982; Maynard et al., 2007). Because DIW extractions yielded turbid extracts, even after filtration (2.5  $\mu\text{m}$ ), we also measured  $\text{NO}_2^-$  in DIW extracts passed through 0.45- $\mu\text{m}$  filters (Millex glass fiber with 0.45- $\mu\text{m}$  hydrophilic PTFE membrane; denoted in figures as DIW+filtration). The recovery of  $\text{NO}_2^-$  was assessed in  $\text{NO}_2^-$ -spiked extracting solutions ( $0.05 \text{ mg NO}_2^- \text{--N L}^{-1}$ ; solution made in DIW or 2 M KCl spiked with  $\text{NO}_2^-$ ). Colorimetric analyses were performed on a discrete analyzer (SEAL AQ-2; Mequon, WI) by  $\text{NO}_2^-$  reaction with sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride (NED;  $\lambda = 520 \text{ nm}$ ; SEAL method EPA-137-A; method detection limit =  $0.0006 \text{ mg NO}_2^- \text{--N L}^{-1}$ ), and by substituting (1:1) ethylenediaminetetraacetic acid (EDTA) with diethylenetriaminepentaacetic acid (DTPA; Colman, 2010). Only DIW extracts were analyzed by ion chromatography because  $\text{Cl}^-$  (from KCl) interferes with  $\text{NO}_2^-$  peaks. During analytical runs, we evaluated accuracy and precision by measuring a sample replicate and a  $0.1 \text{ mg NO}_2^- \text{--N L}^{-1}$  check standard every 10 samples.

The effect of soil pH on  $\text{NO}_2^-$  analysis was assessed by extracting acidic, neutral, and alkaline soils (4 g,  $n = 4$ ) in unbuffered 2 M KCl and DIW (filtered through 0.45  $\mu\text{m}$ ). Soils were extracted as described above, except that  $\text{NO}_2^-$  recovery was assessed with  $0.1 \text{ mg NO}_2^- \text{--N L}^{-1}$  extracting solutions. Nitrite was measured colorimetrically. Soil pH was measured in DIW and 2 M KCl using a pH electrode (Hendershot et al., 2008). To understand potential mechanisms for  $\text{NO}_2^-$  loss, we measured NO in the headspace (25 mL) of the polyethylene centrifuge tubes by injecting a 1-mL sample into a line carrying high purity air to a LMA-3 chemiluminescent  $\text{NO}_2$  analyzer (Scintrex; ON, Canada).

We quantified interferences caused by suspended solids on  $\text{NO}_2^-$  analysis in neutral soils. Soils (3 g,  $n = 4$ ) were extracted in 20 mL of unbuffered KCl solutions varying in concentration (2 to  $2 \times 10^{-5} \text{ M}$ ; with DIW control). We assessed interference by measuring color development in an analysis with NED omitted to avoid developing an actual  $\text{NO}_2^-$ -based colored product. To quantify the size of suspended particles interfering with  $\text{NO}_2^-$  analysis, we passed DIW extracts through 1- and 0.8- $\mu\text{m}$  filter paper, as well as through 100- ( $\sim 0.01 \mu\text{m}$ ) and 50- ( $\sim 7 \text{ nm}$ ) thousand molecular weight cut off membranes. Suspended solids were also separated from the supernatant by centrifugation (20 min at  $30,000 \times g$ ). The efficiency of DIW as an extractant for  $\text{NO}_2^-$  analysis was further assessed by evaluating the absorbance spectrum (400 to 700 nm measured on a Cary UV-Vis spectrophotometer; 1 cm pathway cell) of soils (4 g) extracted in 30 mL of 2 M KCl (filtered through 2.5  $\mu\text{m}$ ) and DIW (filtered through 2.5 and 0.45  $\mu\text{m}$ ), a 0.1- and 0.2-mg  $\text{NO}_2^- \text{--N L}^{-1}$  standard made



**Fig. 1.** Comparison of  $\text{NO}_2^-$  concentrations in Sedgwick soils (pH 6.9) extracted in deionized water (DIW) and 2 M KCl. Soils analyzed by ion chromatography were extracted in DIW. Filtered samples were passed through 0.45- $\mu\text{m}$  filters. Spiked soils were extracted in a 0.05  $\text{mg NO}_2^- \text{--N L}^{-1}$  solution. Percent spike recovery in DIW extractions was 96% for colorimetry and 99% for ion chromatography. Error bars denote standard errors ( $n = 4$ ). Lowercase letters denote significant differences ( $\alpha = 0.05$ ).

in DIW, a 0.1  $\text{NO}_2^- \text{--N L}^{-1}$  spike added to DIW-extracted soil (filtered through 0.45  $\mu\text{m}$ ), and a DIW blank. We used the DIW blank and  $\text{NO}_2^-$  standards as reference absorbance spectra to which soil extracts were compared. We expected that soil DIW extracts filtered through 0.45- $\mu\text{m}$  filters would develop absorbance peaks similar to those of standards, and for the strength of the absorbance signal to increase in proportion to the added  $\text{NO}_2^-$  spike. We used the 2.5- $\mu\text{m}$  filtered DIW extract to understand how suspended solids influenced absorbance spectra and whether changes in absorbance were due to the development of a specific chromophore.

Lastly, we compared  $\text{NO}_2^-$  concentrations in acidic soils (4 g,  $n = 4$ ) extracted in 30 mL of DIW and pH-8-adjusted 2 M KCl (Stevens and Laughlin, 1995) to assess whether raising the pH of 2 M KCl extracts could interfere with the analysis of  $\text{NO}_2^-$ . Because we deviated from the recommended 1:1 soil/extractant ratio used by Stevens and Laughlin (1995), a higher pH could cause dispersion of clays and/or organic matter dissolution (release of humic and fulvic acids) causing interferences during colorimetric analyses.

We used one-way analysis of variance with Tukey post-hoc tests (SAS software, SAS Institute, Cary, NC) on each of the three studied soils to distinguish significant differences ( $\alpha = 0.05$ ) in  $\text{NO}_2^-$  among extracts and among headspace NO (DIW vs. KCl). When necessary, data were log-transformed to meet the assumption of normality.

## RESULTS AND DISCUSSION

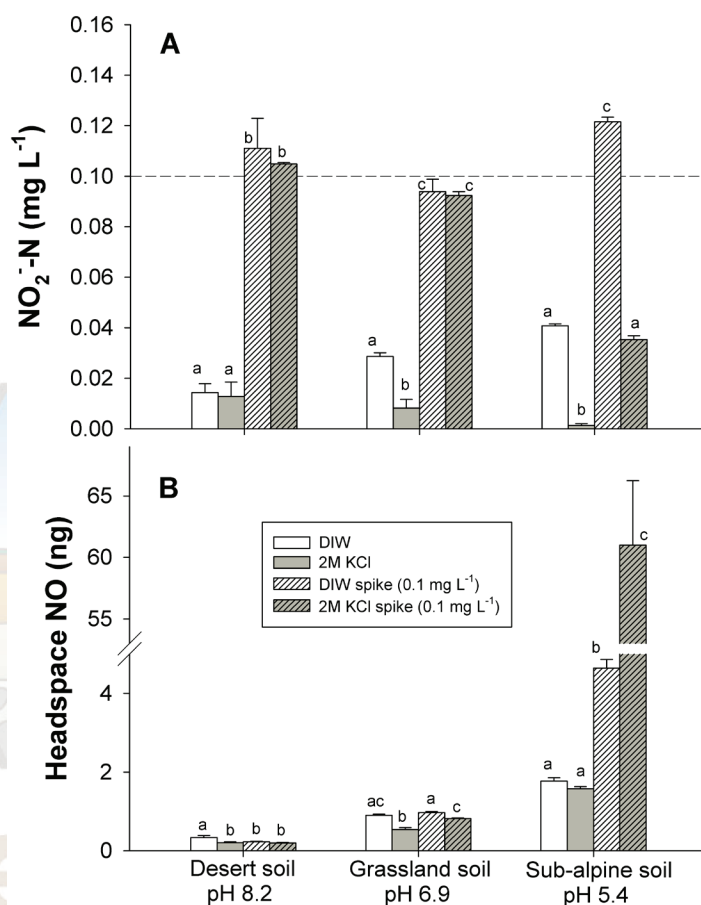
Unbuffered KCl significantly undermeasured soil  $\text{NO}_2^-$  concentrations when compared with DIW, and undermeasured the native soil  $\text{NO}_2^-$  pool in  $\text{NO}_2^-$ -spiked soils analyzed by colorimetry (Fig. 1). In DIW extracts, the recovery of the  $\text{NO}_2^-$  spike was 96% for colorimetry and 99% for ion chromatography (Fig. 1). Because 2 M KCl solutions lower soil pH by

**Table 1.** Soil pH measured in deionized water (DIW) and 2 M KCl. Soil  $\text{NO}_2^-$  and  $\text{NO}_3^-$  concentrations were determined by extraction in DIW followed by filtration (0.45  $\mu\text{m}$ ).

Soil	pH (DIW)	pH (2 M KCl)	$\text{NO}_2^-$ ( $\text{mg g}^{-1}$ )	$\text{NO}_3^-$ ( $\text{mg g}^{-1}$ )
Alkaline	8.2	7.3	$0.11 \pm 0.03$	$1.17 \pm 0.09$
Neutral	6.9	5.6	$0.21 \pm 0.01$	$4.50 \pm 0.3$
Acidic	5.4	4.2	$0.30 \pm 0.006$	$1.46 \pm 0.05$

as much as a full unit (Table 1), likely due to proton release from adsorption of  $\text{K}^+$ , increasing ionic strength, and hydrolysis of extractable Al (Ponette et al., 1996), these acidic conditions favor  $\text{NO}_2^-$  destruction (Stevens and Laughlin, 1995; Van Cleemput and Abdullahi, 1995).

When we compared soils, those with lower pH had higher concentrations of  $\text{NO}_2^-$  in DIW extracts but lower concentrations in unbuffered 2 M KCl extracts (Fig. 2A), further suggesting acidity is driving the under-measurement of  $\text{NO}_2^-$ . Potassium chloride did not undermeasure  $\text{NO}_2^-$  relative to DIW in alkaline soils, but did in neutral and acidic soils ( $p < 0.003$ ; Fig. 2A), reinforcing the conclusion that acidic conditions in the extraction destroyed  $\text{NO}_2^-$  (Stevens and Laughlin, 1995, Islam et al.,



**Fig. 2.** (A) Comparison of  $\text{NO}_2^-$  concentrations and (B) headspace nitric oxide among soils extracted in deionized water (DIW) and 2 M KCl in acidic, neutral, and alkaline soils. Soil DIW extracts were passed through 0.45- $\mu\text{m}$  filters. Spiked soils were extracted in a 0.1-mg  $\text{NO}_2^- \text{--N L}^{-1}$  solution. Error bars denote standard errors ( $n = 4$ ). Lowercase letters denote significant differences ( $\alpha = 0.05$ ).



2008). The recovery of added  $\text{NO}_2^-$  spikes in neutral soils was not affected by 2 M KCl, while in acid soils, spike recovery was reduced to about 40% of the expected value. Although other salts have been used for inorganic N analyses (Li et al., 2006), destruction of  $\text{NO}_2^-$  also occurred when we extracted soils with other salts of equal ionic strength (0.67 M  $\text{MgCl}_2$  or  $\text{Na}_2\text{SO}_4$ ; data not shown). Under acidic conditions,  $\text{NO}_2^-$  protonates to  $\text{HNO}_2$  ( $\text{pK}_a = 3.3$  at  $25^\circ\text{C}$ ), which can break down to gaseous N products (e.g.,  $\text{HONO}$ ,  $\text{NO}$ ,  $\text{NO}_2$ ; Medinets et al., 2015), and explain the loss of  $\text{NO}_2^-$  in acidic soils (Smith and Chalk, 1980; Van Cleemput and Abdullahi, 1996; Venterea et al., 2005). Indeed, we measured elevated headspace  $\text{NO}$  in  $\text{NO}_2^-$ -spiked KCl-extracted soils (Fig. 2B) representing up to 3% of the  $\text{NO}_2^-$  measured in acidic soils, which over the length of the extraction and analysis time, can represent an important pathway for  $\text{NO}_2^-$  loss. Low recovery of  $\text{NO}_2^-$  in unbuffered 2 M KCl extracts may have also resulted from lower pH and by stimulating  $\text{NO}_2^-$  adsorption by SOM (Islam et al., 2008; Isobe et al., 2012), via reactions with metal oxides (Nelson and Bremner, 1970a,b, or ab; Bartlett, 1981; Van Cleemput and Abdullahi, 1995), or by stimulating transformation to  $\text{NO}_3^-$  (Nelson and

Bremner, 1969; Nelson and Bremner, 1970a,b, or ab???; Smith and Chalk, 1980; Bartlett, 1981; Stevens and Laughlin, 1995; Isobe et al., 2012). Irrespective of the mechanism, it is clear that unbuffered 2 M KCl significantly underestimated  $\text{NO}_2^-$  concentrations in neutral to acidic soils (Fig. 1, Fig. 2).

Dilute salt solutions as well as DIW have been used to maximize the recovery of  $\text{NO}_2^-$  and to measure other inorganic soil N pools (Nejidat, 2005; Li et al., 2006; Islam et al., 2008; Darrouzet-Nardi and Weintraub, 2014). However, doing so can produce turbid soil extracts that can interfere with colorimetric analyses; extractants below an ionic strength of  $2 \times 10^{-3}$  M KCl can produce noticeably turbid extracts (see supplemental Fig. S2). In DIW soil extracts analyzed colorimetrically,  $\text{NO}_2^-$  was apparently 35% higher than when measured by ion chromatography (Fig. 1). This pattern occurred even with  $\text{NO}_2^-$ -spiked DIW extracts ( $0.05 \text{ mg NO}_2^- \text{-N L}^{-1}$ , Fig. 1), but not following  $0.45\text{-}\mu\text{m}$  filtration (Fig. 1). Traditionally used  $2.5\text{-}\mu\text{m}$  filter paper did not remove suspended particles; in fact, the color reagent actually flocculated along with suspended solids (see supplemental Fig. S1), elevating absorbance readings, and further overpredicting  $\text{NO}_2^-$ . In contrast,  $0.45\text{-}\mu\text{m}$  filtration yielded  $\text{NO}_2^-$  concentrations consistent with ion chromatography and with the full recovery of the native soil  $\text{NO}_2^-$  and the added  $\text{NO}_2^-$  spike (Fig. 1).

To quantify the effect of suspended solids on  $\text{NO}_2^-$  analysis, we measured absorbance in extracts without the NED to react and develop color. Dilute KCl and DIW extracts produced high absorbance readings that were interpreted as  $\text{NO}_2^-$  (Fig. 3A); the interference was caused by suspended particles  $>0.8 \mu\text{m}$  (Fig. 3B). Although these high absorbances occurred within the measuring wavelength of the azo dye (Fig. 4), they were not due to a specific chromophore—a distinct peak in absorption was not observed. In contrast, filtered ( $0.45 \mu\text{m}$ ) DIW extractions produced sharp absorbance peaks that increased proportionally with the amount of  $\text{NO}_2^-$  spike added (Fig. 4). We note that interferences caused by suspended solids, by default, also occur when measuring  $\text{NO}_3^-$  in DIW, suggesting that proper filter-

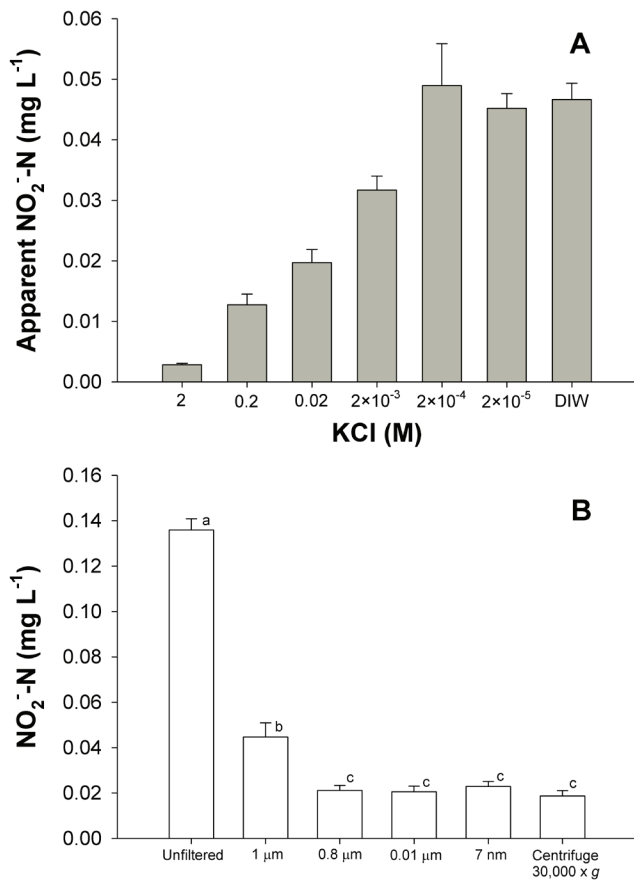


Fig. 3. (A) Apparent  $\text{NO}_2^-$  concentrations in soils extracted with increasingly dilute KCl solutions and deionized water (DIW). A modified N-(1-naphthyl)-ethylenediamine dihydrochloride-color reagent was used to avoid reactions with  $\text{NO}_2^-$  and development of a reddish-purple dye (see section 2.4.4). (B) Comparison of  $\text{NO}_2^-$  concentrations in soils extracted in deionized water following varying degrees of filtration. Error bars denote standard errors ( $n = 4$ ). Lowercase letters denote significant differences ( $\alpha = 0.05$ ).

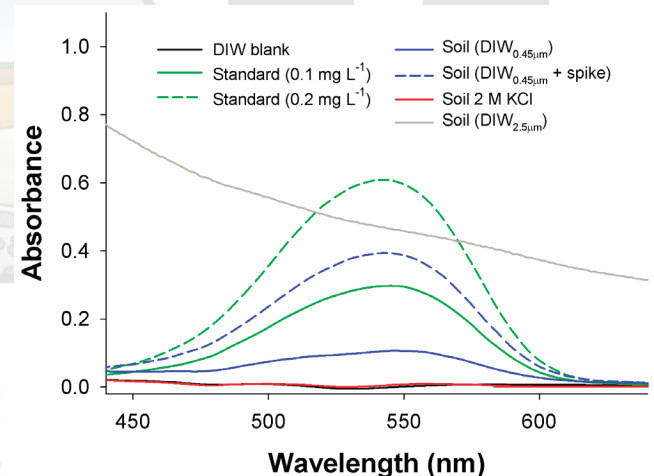


Fig. 4. Nitrite absorbance spectrum for soils extracted in 2 M KCl and deionized water (DIW; passed through 2.5- or  $0.45\text{-}\mu\text{m}$  filters), a 0.1- and 0.2-mg  $\text{NO}_2^-$ -N  $\text{L}^{-1}$  standard made in DIW, a 0.1  $\text{NO}_2^-$ -N  $\text{L}^{-1}$  spike added to DIW-extracted soil, and a DIW blank.

ing is essential for analyzing either  $\text{NO}_2^-$  or  $\text{NO}_3^-$ . As a precaution, we suggest removing suspended solids larger than 0.45  $\mu\text{m}$ . Because filtering to 0.45  $\mu\text{m}$  can be slow, we suggest using the standard Whatman 42 filter paper (2.5  $\mu\text{m}$ ) (Keeney and Nelson, 1982; Maynard et al., 2007) before filtering to 0.45  $\mu\text{m}$ . To ease filtering, we modified a ratchet-driven caulking gun to increase pressure (see supplemental Fig. S3). This system allows samples to be filtered rapidly and efficiently.

To maximize the recovery of  $\text{NO}_2^-$ , several researchers have adjusted the pH of the extracting solution with KOH (Stevens and Laughlin, 1995),  $\text{Ca}(\text{OH})_2$  (Reuss and Smith, 1965), or  $\text{CaCO}_3$  (Nelson and Bremner, 1969). In our case, we were interested in understanding whether the methods developed by Stevens and Laughlin (1995) could be applied to smaller soil samples (4 g instead of 200 g), while deviating from a soil/extractant ratio of 1:1. Being able to analyze small samples is essential in many applications, including remote sites, fine-scale sampling, and microcosm experiments where 200-g samples are impossible. However, using a small sample while lessening the soil/extractant ratio darkened our acidic soil extracts (see supplemental Fig. S4), presumably due to the dissolution of organic matter as soil pH increased (Fujitake et al., 1998). This darker background represented up to ~65% of the apparent  $\text{NO}_2^-$  signal (Fig. 5); correcting for it reduced estimated  $\text{NO}_2^-$  concentrations to <15% of values measured in 0.45- $\mu\text{m}$ -filtered DIW extracts. Because Fe can catalyze  $\text{NO}_2^-$  transformations to N gases (Hu et al., 2001), and Fe pools in the acidic soil are substantial (total Fe ~8.5  $\text{mg g}^{-1}$ ; Homyak et al., 2014), exchangeable Fe released by KCl may have contributed to  $\text{NO}_2^-$  loss.

To facilitate the pH-adjustment procedure, we further developed a pH 7 buffer (0.1 M phosphate), which we expected to minimize the dissolution of organic matter observed at pH 8, but the extracts became darker. Although it is clear that buffering the pH of 2 M KCl can improve  $\text{NO}_2^-$  recoveries in large soil samples using a 1:1 soil/extractant ratio (Stevens and Laughlin, 1995), using smaller samples while lessening the soil/extractant ratio in

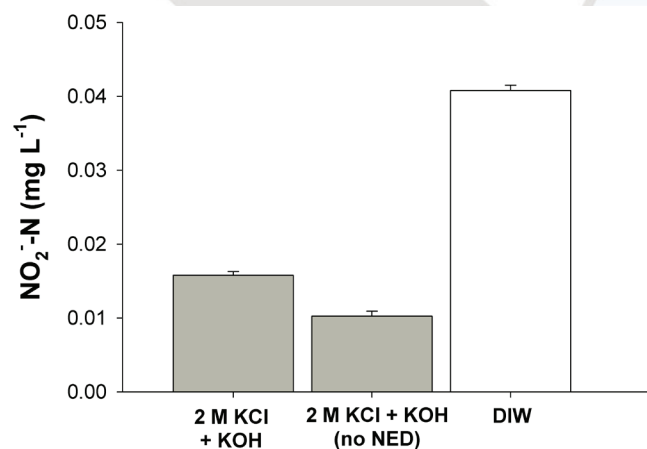
acidic soils may introduce interferences. Nevertheless, we suggest performing a test on acidic soils ( $\text{pH}_{\text{DIW}} < 5.5$ ), to understand whether a pH-adjusted DIW extraction with 2 M KOH can maximize the recovery of  $\text{NO}_2^-$ , and to analyze extracts within a day of extraction. Stevens and Laughlin (1995) found that the stability of  $\text{NO}_2^-$  in stored extracts was higher at pH 5.6 than at pH 8. Thus, we find no reason to suspect the stability of  $\text{NO}_2^-$  may be compromised in the more acidic DIW extracts.

Soil  $\text{NO}_2^-$  concentrations ranged from 0.11 to 0.30  $\mu\text{g g}^{-1}$  and were up to 21% of the  $\text{NO}_3^-$  concentration present in the acidic soil we tested (Table 1). Because rates of soil nitrification are typically determined with unbuffered 2 M KCl (Schmidt and Belser, 1982), under-measuring  $\text{NO}_2^-$  can lead to underestimating nitrification rates (Islam et al., 2008). Assuming that  $\text{NO}_2^-$  is consistently 21% of the  $\text{NO}_3^-$  measured in our acidic soil, unbuffered 2 M KCl extractions can underestimate nitrification rates by ~17%. Similarly, accurately measuring  $\text{NO}_2^-$  is important when determining other soil N pools such as hydroxylamine (Liu et al., 2014), a reactive intermediate during nitrification linked with abiotic mechanisms for gaseous N production (Heil et al., 2015).

Because even low soil  $\text{NO}_2^-$  concentrations (0.001–0.01  $\mu\text{g g}^{-1}$ ) can lead to substantial N losses via emission of HONO (Su et al., 2011), improved estimates of soil  $\text{NO}_2^-$  concentrations may help refine global soil reactive N emission models, while furthering understanding of interactions between soil biogeochemistry and atmospheric chemistry (Venterea et al., 2003). For example, in the sub-alpine acidic soil tested (pH 5.4, 0.3  $\mu\text{g g}^{-1}$   $\text{NO}_2^-$ -N), HONO emissions may range anywhere from 100 to 1000  $\text{ng m}^{-2} \text{s}^{-1}$  (Su et al., 2011), suggesting that gaseous N loss from these high-elevation ecosystems may be substantial. In contrast, predicting N emissions using unbuffered 2 M KCl extractions would have suggested that these ecosystems are a negligible source of HONO to the atmosphere.

## CONCLUSIONS

We compared the efficacy of 2 M KCl and DIW as extractants for determining  $\text{NO}_2^-$ . Using 2 M KCl has counterbalancing effects that must be considered in analyzing  $\text{NO}_2^-$ . In its favor, KCl flocculates suspended solids that can otherwise interfere with colorimetric  $\text{NO}_2^-$  analysis. However, KCl releases exchangeable acidity that destroys  $\text{NO}_2^-$ , particularly in acidic soils if the samples are not buffered with KOH (Stevens and Laughlin, 1995). While interferences caused by suspended solids in DIW extracts can be significant, they can be removed by proper filtration, leading to better recovery of  $\text{NO}_2^-$  in acidic soils. In contrast, it is more difficult to address interferences that result from  $\text{NO}_2^-$  transformations induced by the release of exchangeable acidity in unbuffered KCl extracts. It is equally difficult to address interferences likely caused by the dissolution of organic matter in soils that may be sensitive to pH 8 adjustment. Extractions in DIW offer an alternative for measuring  $\text{NO}_2^-$  in soils when use of large soil amounts (200 g) is impractical, but proper filtering is essential to avoid significant interferences.



**Fig. 5.** Nitrite concentrations in deionized water (DIW) and pH-8-adjusted 2 M KCl soil extractions with 2 M KOH. A modified N-(1-naphthyl)-ethylenediamine dihydrochloride-color reagent was used to avoid reactions with  $\text{NO}_2^-$  and development of a reddish-purple dye (see section 2.4???)

## Supplementary Material

We include site descriptions and figures illustrating issues associated with suspended solids and dissolution of organic matter, the filtering set-up used to facilitate extract filtration, and an extraction protocol for analyzing  $\text{NO}_2^-$  with DIW.

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