

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

Peter M. Homyak*

Dep. of Ecology
Evolution & Marine Biology
Univ. of California-Santa Barbara
Santa Barbara, CA 93106

Krystal T. Vasquez

James O. Sickman

David R. Parker

Dep. of Environmental Sciences
Univ. of California-Riverside
Riverside, CA 92521

Joshua P. Schimel

Dep. of Ecology, Evolution &
Marine Biology
Univ. of California-Santa Barbara
Santa Barbara, CA 93106

Soil nitrite (NO_2^-) is an important source of nitrous acid to the atmosphere as well as an intermediate in nitrification and denitrification. Few studies, however, have directly linked NO_2^- pools with N emissions because NO_2^- is reactive and seldom detectable in soils. Here, we test whether the elusiveness of soil 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 NO_2^- compared with DIW; soils with lower pH had lower NO_2^- in unbuffered KCl than in DIW water. In acidic soils, unbuffered KCl favored the transformation of NO_2^- to nitric oxide. Because KCl lowers the pH of extracts by ~1 unit, this increase in acidity likely favored the transformation of NO_2^- to gaseous N products. Although buffered KCl minimizes NO_2^- destruction, it can cause colorimetric interferences when done on small soil samples (4 g). Deionized water extractions offer an alternative for measuring NO_2^- in small samples, but filtering beyond the traditionally used 2.5- μm 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 NO_2^- .

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

Nitrite (NO_2^-) is an important intermediate in nitrification and denitrification (Robertson and Groffman, 2007) and also participates in abiotic reactions (Nelson and Bremner, 1970a, 1970b; Venterea et al., 2005; Venterea, 2007). In soils, biologically produced NO_2^- can react chemically to produce gaseous N-oxides such as nitrous acid (HONO), nitric oxide (NO), and nitrogen dioxide (NO_2) (Nelson and Bremner, 1970a, 1970b; Medinets et al., 2015), which all contribute to the oxidative capacity of the lower atmosphere (Oswald et al., 2013). Despite the importance of soil NO_2^- to atmospheric chemistry (Su et al., 2011; Oswald et al., 2013) NO_2^- is rarely detected in soils, limiting our ability to observe linkages between soil N-cycling and atmospheric processes.

In spite of the fast reactivity of NO_2^- in soils, however, it accumulates under at least two conditions: (i) when high soil ammonium (NH_4^+) concentrations and pH adversely affect the microbes consuming NO_2^- (Chapman and Liebig, 1952; Van Cleemput and Abdullahi, 1995), 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).

Nitrite is typically measured by extracting soil with 2 M KCl, yielding clear, colorless extracts that facilitate colorimetric analyses, while simultaneously allow-

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*Corresponding author (peter.homyak@lifesci.ucsb.edu).

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ing determination of NO_3^- and 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 NO_2^- concentrations (Stevens and Laughlin, 1995) because concentrated salt solutions release exchangeable acidity (Ponette et al., 1996), and acid destroys 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 NO_2^- analysis.

To improve the recovery of 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 NO_2^- analysis. Here, we tested a series of soil extractions to: (i) validate that unbuffered concentrated salts interfere with NO_2^- analyses and should not be used for analyzing 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 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 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 NO_2^- concentrations in neutral soils extracted in unbuffered 2 M KCl (extra pure KCl; Acros Organics, Fisher Scientific, Pittsburgh, PA) analyzed colorimetrically, 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 intro-

ducing significant 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 μm Whatman 42 filter paper (Keeney and Nelson, 1982; Maynard et al., 2007). Because DIW extractions yielded turbid extracts, even after filtration (2.5 μm), we also measured NO_2^- in DIW extracts passed through 0.45- μm filters (Millex glass fiber with 0.45- μm hydrophilic PTFE membrane; denoted in figures as DIW+filtration). The recovery of NO_2^- was assessed in 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 NO_2^-). Colorimetric analyses were performed on a discrete analyzer (SEAL AQ-2; Mequon, WI) by 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 Cl^- (from KCl) interferes with 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 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 μm). Soils were extracted as described above, except that 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 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 NO_2^- analyzer (Scintrex; ON, Canada).

We quantified interferences caused by suspended solids on 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 NO_2^- -based colored product. To quantify the size of suspended particles interfering with NO_2^- analysis, we passed DIW extracts through 1- and 0.8- μm 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 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 μm) and DIW (filtered through 2.5 and 0.45 μm), a 0.1- and 0.2- $\text{mg NO}_2^- \text{-N L}^{-1}$ standard made in DIW, a 0.1 $\text{NO}_2^- \text{-N L}^{-1}$ spike added to DIW-extracted soil (filtered through 0.45 μm), and a DIW blank. We used the DIW blank and NO_2^- standards as reference absorbance spectra to which soil extracts were compared. We expected that soil DIW

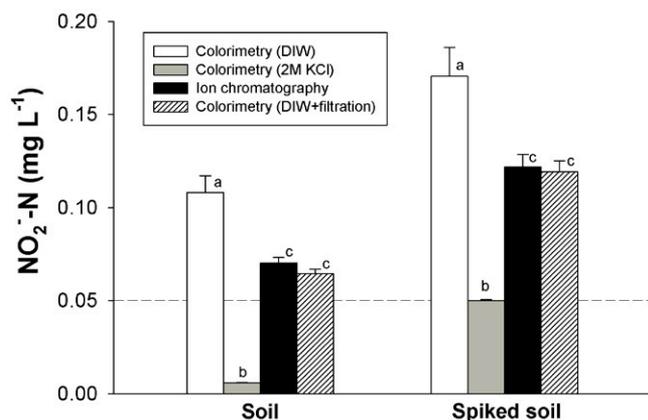


Fig. 1. Comparison of NO_2^- concentrations in Sedgwick soils (pH 6.9) extracted in deionized water (DIW) and 2 M KCl. Soils analyzed by ion chromatography were extracted in DIW. Filtered samples were passed through 0.45- μm filters. Spiked soils were extracted in a 0.05 mg NO_2^- -N L^{-1} solution. Percentage 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 ($\alpha = 0.05$).

extracts filtered through 0.45- μm 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 NO_2^- spike. We used the 2.5- μm 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 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 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 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 NO_2^- concentrations when compared with DIW, and undermeasured the native soil NO_2^- pool in NO_2^- -spiked soils analyzed by colorimetry (Fig. 1). In DIW extracts, the recovery of the NO_2^- spike was 96% for colorimetry and 99% for ion chromatography (Fig. 1). Because 2 M KCl solutions lower soil pH by as much as a full unit (Table 1), likely due to proton release from adsorption of K^+ , increasing ionic strength, and hydrolysis of extractable Al (Ponette et al., 1996), these acidic conditions favor NO_2^- destruction (Stevens and Laughlin, 1995; Van Cleemput and Abdullahi, 1995).

Table 1. Soil pH measured in deionized water (DIW) and 2 M KCl. Soil NO_2^- and NO_3^- concentrations were determined by extraction in DIW followed by filtration (0.45 μm).

Soil	pH		NO_2^- -N	NO_3^- -N
	DIW	2 M KCl		
Alkaline	8.2	7.3	0.11 ± 0.03	1.17 ± 0.09
Neutral	6.9	5.6	0.21 ± 0.01	4.50 ± 0.3
Acidic	5.4	4.2	0.30 ± 0.006	1.46 ± 0.05

When we compared soils, those with lower pH had higher concentrations of 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 NO_2^- . Potassium chloride did not undermeasure 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 NO_2^- (Stevens and Laughlin, 1995, Islam et al., 2008). The recovery of added 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.,

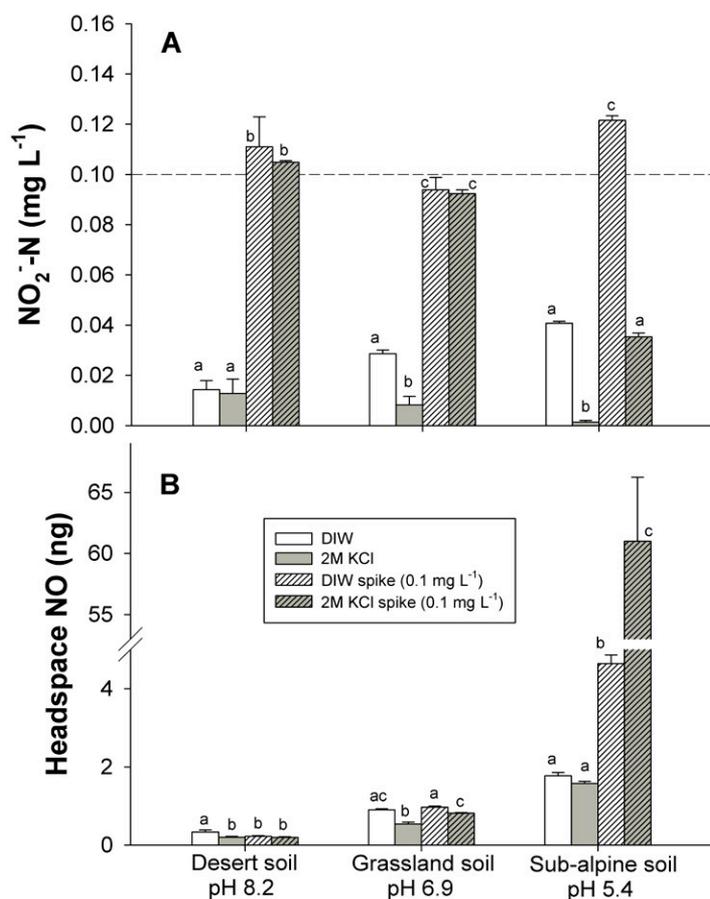


Fig. 2. (A) Comparison of NO_2^- concentrations and (B) headspace nitric oxide among soils extracted in deionized water (DIW) and 2 M KCl in acidic, neutral, and alkaline soils. Soil DIW extracts were passed through 0.45- μm filters. Spiked soils were extracted in a 0.1-mg NO_2^- -N L^{-1} solution. Error bars denote standard errors ($n = 4$). Lowercase letters denote significant differences ($\alpha = 0.05$).

2006), destruction of NO_2^- also occurred when we extracted soils with other salts of equal ionic strength (0.67 M MgCl_2 or Na_2SO_4 ; data not shown). Under acidic conditions, NO_2^- protonates to HNO_2 ($\text{pK}_a = 3.3$ at 25°C), which can break down to gaseous N products (e.g., HONO , NO , NO_2 ; Medinets et al., 2015), and explain the loss of NO_2^- in acidic soils (Smith and Chalk, 1980; Van Cleemput and Abdullahi, 1995; Venterea et al., 2005). Indeed, we measured elevated headspace NO in NO_2^- -spiked KCl-extracted soils (Fig. 2B) representing up to 3% of the NO_2^- measured in acidic soils, which over the length of the extraction and analysis time, can represent an important pathway for NO_2^- loss. Low recovery of NO_2^- in unbuffered 2 M KCl extracts may have also resulted from lower pH and by stimulating NO_2^- adsorption by SOM (Islam et al., 2008; Isobe et al., 2012), via reactions with metal oxides (Nelson and Bremner, 1970b; Bartlett, 1981; Van Cleemput and Abdullahi, 1995), or by stimulating transformation to NO_3^- (Nelson and Bremner, 1969; Nelson and Bremner, 1970a; 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 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 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 2×10^{-3} M KCl can produce noticeably turbid extracts (see supplemental Fig. S2). In DIW soil extracts analyzed colorimetrically, NO_2^- was apparently 35% higher than when measured by ion chromatography (Fig. 1). This pattern occurred even with 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 NO_2^- . In contrast, $0.45\text{-}\mu\text{m}$ filtration yielded NO_2^- concentrations consistent with ion chromatography and with the full recovery of the native soil NO_2^- and the added NO_2^- spike (Fig. 1).

To quantify the effect of suspended solids on 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 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 NO_2^- spike added (Fig. 4). We note that interferences caused by suspended solids, by default, also occur when measuring NO_3^- in DIW, suggesting that proper filtering is essential for analyzing either NO_2^- or 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

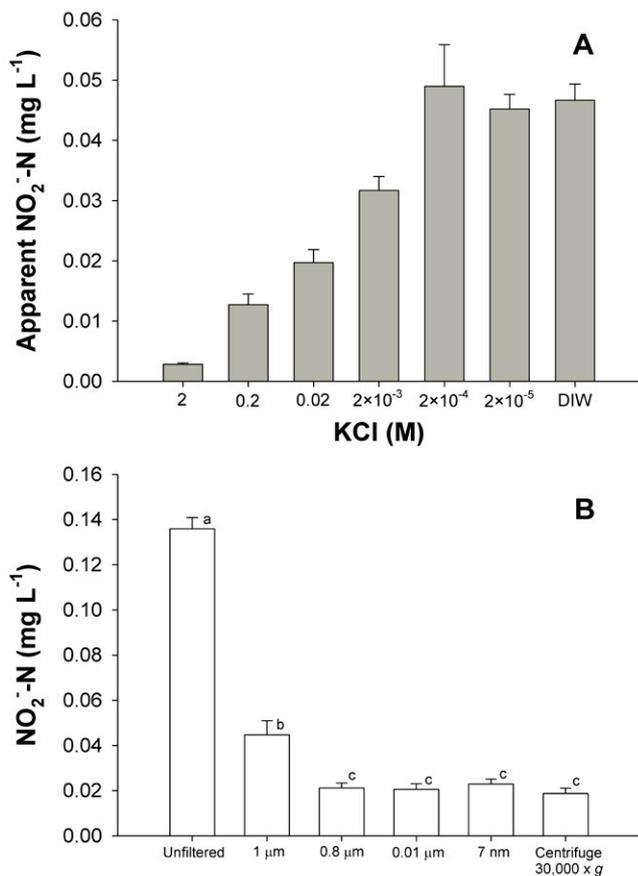


Fig. 3. (A) Apparent NO_2^- concentrations in soils extracted with increasingly dilute KCl solutions and deionized water (DIW). A modified N-(1-naphthyl)-ethylenediamine dihydrochloride-color reagent was used to avoid reactions with NO_2^- and development of a reddish-purple dye. **(B)** Comparison of 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 ($\alpha = 0.05$).

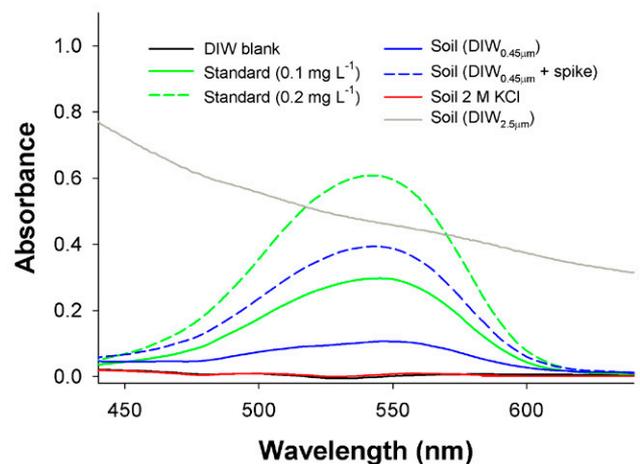


Fig. 4. Nitrite absorbance spectrum for soils extracted in 2 M KCl and deionized water (DIW; passed through $2.5\text{-}\mu\text{m}$ or $0.45\text{-}\mu\text{m}$ filters), a 0.1- and 0.2-mg NO_2^- -N L^{-1} standard made in DIW, a 0.1 NO_2^- -N L^{-1} spike added to DIW-extracted soil, and a DIW blank.

the standard Whatman 42 filter paper (2.5 μm) (Keeney and Nelson, 1982; Maynard et al., 2007) before filtering to 0.45 μm . 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 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 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 to produce more than 4 mL of solution for chemical analyses to produce more than 4 mL of solution for chemical analyses. 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 NO_2^- signal (Fig. 5); correcting for it reduced estimated NO_2^- concentrations to <15% of values measured in 0.45- μm -filtered DIW extracts. Because Fe can catalyze NO_2^- transformations to N gases (Hu et al., 2001), and Fe pools in the acidic soil are substantial (total Fe ~8.5 mg g^{-1} ; Homyak et al., 2014), exchangeable Fe released by KCl may have contributed to 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 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

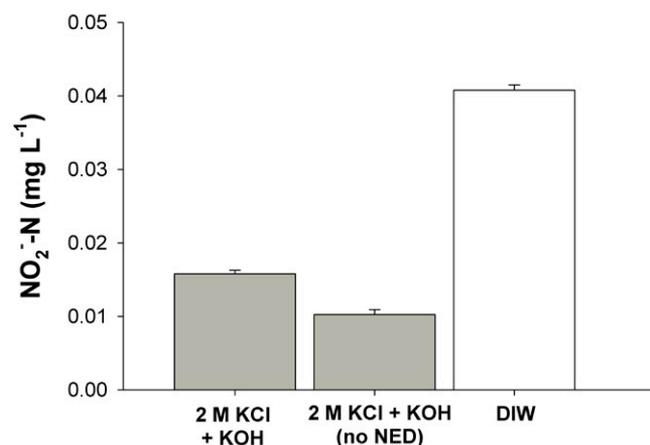


Fig. 5. Nitrite concentrations in deionized water (DIW) and pH-8-adjusted 2 M KCl soil extractions with 2 M KOH. A modified N-(1-naphthyl)-ethylenediamine dihydrochloride-color reagent was used to avoid reactions with NO_2^- and development of a reddish-purple dye.

whether a pH-adjusted DIW extraction with 2 M KOH can maximize the recovery of NO_2^- , and to analyze extracts within a day of extraction. Stevens and Laughlin (1995) found that the stability of 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 NO_2^- may be compromised in the more acidic DIW extracts.

Soil NO_2^- concentrations ranged from 0.11 to 0.30 $\mu\text{g g}^{-1}$ and were up to 21% of the 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 NO_2^- can lead to underestimating nitrification rates (Islam et al., 2008). Assuming that NO_2^- is consistently 21% of the NO_3^- measured in our acidic soil, unbuffered 2 M KCl extractions can underestimate nitrification rates by ~17%. Similarly, accurately measuring 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 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 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 $\mu\text{g g}^{-1}$ 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 NO_2^- . Using 2 M KCl has counterbalancing effects that must be considered in analyzing NO_2^- . In its favor, KCl flocculates suspended solids that can otherwise interfere with colorimetric NO_2^- analysis. However, KCl releases exchangeable acidity that destroys 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 NO_2^- in acidic soils. In contrast, it is more difficult to address interferences that result from 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 NO_2^- in soils when use of large soil amounts (200 g) is impractical, but proper filtering is essential to avoid significant interferences.

Supplementary Material

We include site descriptions and figures illustrating issues associated with suspended solids and dissolution of organic mat-

ter, the filtering set-up used to facilitate extract filtration, and an extraction protocol for analyzing NO_2^- with DIW.

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REFERENCES

- Banderis, A., D.H. Barter, and K. Henderson. 1976. Use of polyacrylamide to replace carbon in the determination of Olsen's extractable phosphate in soil. *J. Soil Sci.* 27:71–74. doi:10.1111/j.1365-2389.1976.tb01977.x
- Bartlett, R.J. 1981. Nonmicrobial nitrite-to-nitrate transformation in soils. *Soil Sci. Soc. Am. J.* 45:1054–1058. doi:10.2136/sssaj1981.03615995004500060009x
- Chapman, H.D., and G.F. Liebig. 1952. Field and laboratory studies of nitrite accumulation in soils. *Soil Sci. Soc. Am. Proc.* 16:276–282. doi:10.2136/sssaj1952.03615995001600030014x
- Colman, B.P. 2010. Understanding and eliminating iron interference in colorimetric nitrate and nitrite analysis. *Environ. Monit. Assess.* 165:633–641. doi:10.1007/s10661-009-0974-x
- Darrouzet-Nardi, A., and M.N. Weintraub. 2014. Evidence for spatially inaccessible labile N from a comparison of soil core extractions and soil pore water lysimetry. *Soil Biol. Biochem.* 73:22–32. doi:10.1016/j.soilbio.2014.02.010
- Davidson, E.A., P.M. Vitousek, P.A. Matson, R. Riley, G. Garcia-Mendez, and M. Maass. 1991. Soil emissions of nitric oxide in a seasonally dry tropical forest of Mexico. *J. Geophys. Res.* 96:15439–15445. doi:10.1029/91JD01476
- Fujitake, N., A. Kusumoto, M. Tsukamoto, M. Kawahigashi, T. Suzuki, and H. Otsuka. 1998. Properties of soil humic substances in fractions obtained by sequential extraction with pyrophosphate solutions at different pHs I. Yield and particle size distribution. *Soil Sci. Plant Nutr.* 44:253–260. doi:10.1080/00380768.1998.10414446
- Gelfand, I., and D. Yakir. 2008. Influence of nitrite accumulation in association with seasonal patterns and mineralization of soil nitrogen in a semi-arid pine forest. *Soil Biol. Biochem.* 40:415–424. doi:10.1016/j.soilbio.2007.09.005
- Heil, J., S. Liu, H. Vereecken, and N. Brüggemann. 2015. Abiotic nitrous oxide production from hydroxylamine in soils and their dependence on soil properties. *Soil Biol. Biochem.* 84:107–115. doi:10.1016/j.soilbio.2015.02.022
- Hendershot, W.H., H. Lalonde, and M. Duquette. 2008. Soil reaction and exchangeable acidity. In: M.R. Carter and E.G. Gregorich, editors, *Soil sampling and methods of analysis*. Canadian Society of Soil Science, Boca Raton, FL.
- Homyak, P.M., J.O. Sickman, and J.M. Melack. 2014. Pools, transformations, and sources of P in high-elevation soils: Implications for nutrient transfer to Sierra Nevada lakes. *Geoderma* 217–218:65–73. doi:10.1016/j.geoderma.2013.11.003
- Hu, H.Y., N. Goto, and K. Fujie. 2001. Effect of pH on the reduction of nitrite in water by metallic iron. *Water Res.* 35:2789–2793. doi:10.1016/S0043-1354(00)00570-4
- Islam, A., D. Chen, R.E. White, and A.J. Weatherley. 2008. Chemical decomposition and fixation of nitrite in acidic pasture soils and implications for measurement of nitrification. *Soil Biol. Biochem.* 40:262–265. doi:10.1016/j.soilbio.2007.07.008
- Isobe, K., K. Koba, Y. Suwa, J. Ikutani, M. Kuroiwa, Y.T. Fang, M. Yohb, J. Mod, S. Otsuka, and K. Senoo. 2012. Nitrite transformations in an N-saturated forest soil. *Soil Biol. Biochem.* 52:61–63. doi:10.1016/j.soilbio.2012.04.006
- Keeney, D.R., and D.W. Nelson. 1982. Nitrogen—Inorganic forms. In: A.L. Page et al., editors, *Methods of soil analysis, Part 2*. 2nd ed. SSSA, Madison, WI, p. 643–698.
- Li, L.P., J.B. Zhang, W.Q. Xing, W. Chen, X.L. Wu, and K. Zhu. 2006. Development and validation of a new soil universal extractant: 0.02 molar strontium chloride. *Commun. Soil Sci. Plant Anal.* 37:1627–1638. doi:10.1080/00103620600710249
- Liu, S., H. Vereecken, and N. Brüggemann. 2014. A highly sensitive method for the determination of hydroxylamine in soils. *Geoderma* 232–234:117–122. doi:10.1016/j.geoderma.2014.05.006
- Maynard, D.G., Y.P. Kalra, and J.A. Crumbaugh. 2007. Nitrate and exchangeable ammonium nitrogen. In: M.R. Carter and E.G. Gregorich, editors, *Soil sampling and methods of analysis*. 2nd ed. CRC Press, Boca Raton, FL, p. 71–80.
- Medinets, S., U. Skiba, H. Rennenberg, and K. Butterbach-Bahl. 2015. A review of soil NO transformation: Associated processes and possible physiological significance on organisms. *Soil Biol. Biochem.* 80:92–117. doi:10.1016/j.soilbio.2014.09.025
- Mulvaney, R.L. 1996. Nitrogen—Inorganic forms. In: D.L. Sparks, editor, *Methods of soil analysis, Part 3*. SSSA, Madison, WI, p. 1123–1184.
- Nejidat, A. 2005. Nitrification and occurrence of salt-tolerant nitrifying bacteria in the Negev desert soils. *FEMS Microbiol. Ecol.* 52:21–29. doi:10.1016/j.femsec.2004.10.011
- Nelson, D.W., and J.M. Bremner. 1969. Factors affecting chemical transformations of nitrite in soils. *Soil Biol. Biochem.* 1:229–239. doi:10.1016/0038-0717(69)90023-6
- Nelson, D.W., and J.M. Bremner. 1970a. Gaseous products of nitrite decomposition in soils. *Soil Biol. Biochem.* 2:203–215. doi:10.1016/0038-0717(70)90008-8
- Nelson, D.W., and J.M. Bremner. 1970b. Role of soil minerals and metallic cations in nitrite decomposition and chemodenitrification. *Soil Biol. Biochem.* 2:1–8. doi:10.1016/0038-0717(70)90019-2
- Oswald, R., T. Behrendt, M. Ermel, D. Wu, H. Su, Y. Cheng, C. Breuninger, A. Moravek, E. Mougin, C. Delon, B. Loubet, A. Pommerening-Röser, M. Sörgel, U. Pöschl, T. Hoffmann, M.O. Andreae, F.X. Meixner, and I. Trebs. 2013. HONO emissions from soil bacteria as a major source of atmospheric reactive nitrogen. *Science* 341:1233–1235. doi:10.1126/science.1242266
- Pansu, M., and J. Gautheyrou. 2006. *Handbook of soil analysis: Mineralogical, organic and inorganic methods*. Springer, New York.
- Parker, S.S., and J.P. Schimel. 2011. Soil nitrogen availability and transformations differ between the summer and the growing season in a California grassland. *Appl. Soil Ecol.* 48:185–192. doi:10.1016/j.apsoil.2011.03.007
- Ponette, Q., D. Andre, and J.E. Dufey. 1996. Chemical significance of aluminium extracted from three horizons of an acid forest soil, using chloride salt solutions. *Eur. J. Soil Sci.* 47:89–95. doi:10.1111/j.1365-2389.1996.tb01375.x
- Reuss, J.O., and R.L. Smith. 1965. Chemical reactions of nitrites in acid soils. *Soil Sci. Soc. Am. Proc.* 29:267–270. doi:10.2136/sssaj1965.03615995002900030014x
- Robertson, G.P., and P.M. Groffman. 2007. Nitrogen transformations. In: E.A. Paul, editor, *Soil microbiology, ecology, and biochemistry*. Academic Press, New York, p. 341–364.
- Schmidt, E.L., and L.W. Belsler. 1982. Nitrifying bacteria. In: A.L. Page et al., editors, *Methods of soil analysis, Part 2*. 2nd ed. SSSA, Madison, WI, p. 1027–1042.
- Smith, C.J., and P.M. Chalk. 1980. Fixation and loss of nitrogen during transformations of nitrite in soils. *Soil Sci. Soc. Am. J.* 44:288–291. doi:10.2136/sssaj1980.03615995004400020017x
- Stevens, R.J., and R.J. Laughlin. 1995. Nitrite transformations during soil extraction with potassium chloride. *Soil Sci. Soc. Am. J.* 59:933–938. doi:10.2136/sssaj1995.03615995005900030044x
- Su, H., Y.F. Cheng, R. Oswald, T. Behrendt, I. Trebs, F.X. Meixner, M.O. Andreae, P. Cheng, Y. Zhang, and U. Pöschl. 2011. Soil nitrite as a source of atmospheric HONO and OH radicals. *Science* 333:1616–1618. doi:10.1126/science.1207687
- Van Cleemput, O., and S.H. Abdullahi. 1995. Nitrite in soils: Accumulation and role in the formation of gaseous N compounds. *Fert. Res.* 45:81–89. doi:10.1007/BF00749884
- Venterea, R.T. 2007. Nitrite-driven nitrous oxide production under aerobic soil conditions: Kinetics and biochemical controls. *Glob. Change Biol.* 13:1798–1809. doi:10.1111/j.1365-2486.2007.01389.x
- Venterea, R.T., P.M. Groffman, L.V. Verchot, A.H. Magill, J.D. Aber, and P.A. Steudler. 2003. Nitrogen oxide gas emissions from temperate forest soils receiving long-term nitrogen inputs. *Glob. Change Biol.* 9:346–357. doi:10.1046/j.1365-2486.2003.00591.x
- Venterea, R.T., D.E. Rolston, and Z.G. Cardon. 2005. Effects of soil moisture, physical, and chemical characteristics on abiotic nitric oxide production. *Nutr. Cycl. Agroecosyst.* 72:27–40. doi:10.1007/s10705-004-7351-5
- Xie, G.H., I. Lahav, G. Barness, and Y. Steinberger. 2001. Dynamics of soil nitrogen along a topoclimatic gradient in the Judean desert. *Arid Land Res. Manage.* 15:135–146. doi:10.1080/15324980151062760
- Yang, W.H., D. Herman, D. Liptzin, and W.L. Silver. 2012. A new approach for removing iron interference from soil nitrate analysis. *Soil Biol. Biochem.* 46:123–128. doi:10.1016/j.soilbio.2011.12.003