Supplementary materials

Title: Improving nitrite analysis in soils: Drawbacks of the conventional 2 M KCl extraction
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Number of figures: 4

Sites description

Acidic soil was collected from a sub-alpine meadow in the Emerald Lake catchment (36°35’49”N, 118°40’29”W; 2820 m a.s.l.). The soil is poorly developed, weakly buffered, and well drained, with a bulk density of 1.0 to 1.2 g cm\(^{-3}\) in the upper 10 cm (Huntington and Akeson, 1987). The average soil C content is 3% and N 0.2%. The climate is Mediterranean with warm dry summers averaging 13 °C and cold wet winters with average temperatures of -1.5 °C. The average annual precipitation is 1510 mm, of which approximately 90% falls as snow (Sickman, et al., 2001). The vegetation is sparse and dominated by bluejoint reedgrass (\textit{Calamagrostis Canadensis}) and sedges (\textit{Carex} spp.).

Neutral soil was obtained from an annual grassland (primarily \textit{Bromus} spp.) in the University of California Sedgwick Reserve (43°42’30”N, 120°2’30”W; 370 m a.s.l.). The average soil C content is 2%, N 0.2%, with a bulk density of 1.2 g cm\(^{-3}\) (upper 10 cm). The climate is Mediterranean with average summer daytime temperatures of 32 – 34 °C, and typically above 0 °C during winter. The average annual rainfall is 380 mm, with most of the rain falling between December and March.

Alkaline soil was obtained from the Mojave Desert in Joshua Tree National Park (33°49’45”N, 115°44’48”W; 739 m a.s.l.). The soil has a loamy sand texture with a bulk density
of 1.6 g cm$^{-3}$ (upper 10 cm) (Rao, et al., 2009). The average soil C content is 0.5% and N 0.03%.

The climate is Mediterranean with hot summer temperatures that can exceed 49 °C and winter temperatures that occasionally drop below 0 °C. The average precipitation is 330 mm yr$^{-1}$, mostly occurring between December and March, though monsoon summer rains are common.

The vegetation is dominated by creosote bush (*Larrea tridentata*) and grasses (*S. barbatus* and *Schismus arabicus*) (Rao, et al., 2009).

$NO_2^-$ extraction procedure in deionized water:

1. Weigh 4 g of soil into a 50 mL centrifuge tube. Add 30 mL of deionized water, cap, and shake for 30 minutes.

2. Filter extracts to 0.45 µm. To ease filtering, centrifuge extracts for 15 minutes (1600 $\times$ g or higher), filter extracts through 2.5 µm filter paper (Whatman 42) followed by filtration to 0.45 µm. Samples can be filtered rapidly and efficiently using a ratchet-driven caulking gun equipped with a syringe and syringe-driven filters (Fig. S3).

3. Analyze samples within 24 hours of extraction.

Notes:

For acidic soils (pH$_{DIW}$ < 5.5), we recommend testing whether raising the pH above 5.5 with 2 M KOH can increase the recovery of $NO_2^-$. To adjust the pH, prepare a separate sample containing 4 g of soil in 30 mL of deionized water. Mix the soil for 30 minutes and allow it to settle for another 30 minutes. Using a pH meter, slowly add 2 M KOH while recording the volume necessary to raise the pH above 5.5 (this may be as little as 10 µL) and being careful not to produce brown-colored extracts resulting from organic matter dissolution (Fig. S4). If darkening occurs, it is important to correct for the background absorbance of the solution (Fig. 5).
Fig. S1. Formation of reddish-purple azo dye and flocculation with suspended solids in deionized water soil extract passed through a 2.5 µm filter.
Fig. S2. Clarity of soil extracts using increasingly dilute KCl solutions and deionized water (DIW).
Fig. S3. Modified caulking gun used to facilitate extract filtration through 0.45 µm filters. Soil extract are loaded in syringe, and ratcheting action is used to pass solution through filter.
Fig. S4. Comparison of extract clarity for acidic soils extracted in 2 M KCl and pH-8-adjusted 2 M KCl with 2 M KOH.
57 **Supplementary figure captions**

58 Fig. S1. Formation of reddish-purple azo dye and flocculation with suspended solids in deionized
water soil extract passed through a 2.5 µm filter.

60 Fig. S2. Clarity of soil extracts using increasingly dilute KCl solutions and deionized water
(DIW).

62 Fig. S3. Modified caulking gun used to facilitate extract filtration through 0.45 µm filters. Soil
extract are loaded in syringe, and ratcheting action is used to pass solution through filter.

64 Fig. S4. Comparison of extract clarity for acidic soils extracted in 2 M KCl and pH-8-adjusted 2
M KCl with 2 M KOH.

66 **Literature Cited**

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