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A new approach for removing iron interference from soil nitrate analysis

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ABSTRACT

The accurate measurement of soil nitrate (NO_3^-) is critical for determining rates of nitrogen (N) cycling and potential N losses from ecosystems. Iron (Fe) can interfere with the colorimetric NO_3^- analysis of soil extracts to cause the appearance of anomalously low NO_3^- concentrations. To resolve the interference, imidazole or NH_4Cl–DTPA has been recommended to replace NH_4Cl–EDTA as the buffer in the analysis. Here we show that phosphate (PO_4^{3-}) filtration can completely remove Fe interference whereas any of these buffers alone may not. Regardless of which buffer was used, 5.5–55 mg Fe L^{-1} ferrous iron (Fe^{2+}) interfered with NO_3^- determination in 0.3 mg N–NO_3^- L^{-1} 2 M KCl solutions. Phosphate filtration removed all detectable Fe^{2+} in 2 M KCl anaerobic soil slurry extracts with high Fe^{2+} concentrations (25.9 ± 1.7 mg Fe^{2+} L^{-1}). With each of the three buffers tested, the measured NO_3^- concentrations in the anaerobic soil slurry extracts were significantly higher with PO_4^{3-} filtration compared to without filtration. After filtration, the measured NO_3^- concentrations were similar across all three buffers, suggesting that NO_3^- concentrations were accurately measured in PO_4^{3-} filtered soil extracts regardless of the buffer used. The Fe:N ratio of Fe interference with NO_3^- determination depended on Fe concentration, NO_3^- concentration, buffer, and cadmium column age, so that the amount of Fe interference that could occur can be difficult to predict. We suggest comparing measured NO_3^- concentrations for unfiltered and PO_4^{3-} filtered soil extracts to determine the potential for Fe interference in colorimetric NO_3^- determination as standard additions may not detect all forms of Fe interference.

1. Introduction

Nitrate (NO_3^-) is a mineral form of nitrogen (N) and a major nutrient for plant and microbial metabolism. Nitrate can become a groundwater pollutant if leached from soil, lead to eutrophication of aquatic and coastal ecosystems, and contribute to air pollution and climate change through the emissions of NO_x and N_2O from nitrification and denitrification (Townsend et al., 2003). The measurement of soil NO_3^- concentrations is both commonplace and critical to biogeochemical, ecological, and environmental research. In iron (Fe) rich soils, NO_3^- can be significantly underestimated due to interference during colorimetric analyses.

Iron interference with colorimetric NO_3^- analysis has long been recognized (Horita et al., 1997; Vaughan et al., 1993). The interference manifests itself as anomalously low measured NO_3^- concentrations (Colman et al., 2007; Davidson et al., 2008) as well as negative peaks, baseline drift, peak shift, calibration check failures, and cadmium (Cd) column poisoning (personal observation). Recently, Colman and Schimel (2010) demonstrated that the likely mechanism for Fe interference is competition by ferrous iron (Fe^{2+}) with *N*-(1-napthyl)-ethylenediamine (NED), the color reagent for the diazo intermediate in the modified Griess–Ilosvay reaction. Ferric iron (Fe^{3+}) can be reduced to Fe^{2+} in the Cd column, so the interference can occur regardless of the Fe redox state in the soil extract. Experiments using pure solutions show that Fe interference occurs with Fe concentrations above 50 mg Fe L^{-1} (Colman et al., 2007; Davidson et al., 2008), but interference at lower Fe concentrations has not been tested.

Various buffers for the modified Griess–Ilosvay reaction have been used to overcome Fe interference. Colman and Schimel (2010) provided a thorough historical review of how ammonium chloride ethylenediaminetetraacetic acid (NH_4Cl–EDTA) became the standard buffer used. However, the NH_4Cl–EDTA buffer creates more Fe interference than it resolves. Imidazole is another widely used buffer (e.g., Hales et al., 2004; Patton et al., 2002), but imidazole may cause Fe to precipitate to form Fe hydroxides that would interfere with photometric analysis and/or coat the Cd column to decrease the surface area available to reduce NO_3^- (Herzsprung et al., 2005; Nydahl, 1976). A NH_4Cl buffer containing 1 g L^{-1} of

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diethylenetriaminepentaacetic acid (DTPA) can prevent Fe interference at Fe^{2+} concentrations up to 200 mg Fe L^{-1}, presumably by the DTPA chelating the Fe so that it cannot react with the diazo intermediate (Colman and Schimel, 2010).

Here we present an alternative solution to the Fe interference problem that has been used successfully in our lab for over a decade. Phosphate (PO_4^{3-}) is added to soil extracts and the resulting Fe precipitate is removed by membrane filtration. We report on a series of four experiments that (1) compare the effectiveness of buffers alone in resolving Fe interference with NO_3^- analysis at low Fe concentrations (<50 mg Fe^{2+} L^{-1}), (2) demonstrate that PO_4^{3-} does not interfere with colorimetric NO_3^- analysis, (3) demonstrate that PO_4^{3-} filtration removes Fe and its interference with colorimetric NO_3^- analysis, and (4) characterize the Fe:N ratio of Fe interference with NO_3^- determination.

2. Materials and methods

2.1. Experimental design

For the first experiment, we used pure solutions to determine if Fe interference occurs at low Fe concentrations (<50 mg Fe^{2+} L^{-1}). We prepared a 0.3 mg N L^{-1} KNO_3 solution in 2 M KCl and added 100 mM Fe(II)Cl_2 in 0.5 N HCl to achieve 0, 5.5, 27.5, or 55 mg Fe^{2+} L^{-1} in replicate aliquots ($n = 5$ per Fe^{2+} concentration). The dilution effect of adding the Fe solutions to the aliquots was within expected measurement error (0.01 mg N L^{-1}) at the concentration of the NO_3^- solution, 0.3 mg N L^{-1}.

For the second experiment to determine if PO_4^{3-} interferes with NO_3^- analysis, we prepared 2 M KCl solutions with a NO_3^- concentration of 0.3 mg N L^{-1} and with PO_4^{3-} concentrations of 0, 10, or 20 mM ($n = 5$ per treatment). We added 100 μL and 200 μL of 1 M Na_2HPO_4 to 5 mL aliquots of the NO_3^- solution to achieve 10 and 20 mM PO_4^{3-}, respectively. The 1 M Na_2HPO_4 solution was made by dissolving 26.8 g Na_2HPO_4 in 100 mL heated deionized water. The solution was returned to room temperature before it was added to the samples.

For the third experiment, we compared measured background NO_3^- concentrations of unfiltered versus filtered soil extracts as well as evaluated the recovery of a 0.3 mg N L^{-1} NO_3^- standard addition in both types of extracts. We used 2 M KCl extracts of soil (0–10 cm depth) from a humid tropical forest in the Luquillo Experimental Forest, Puerto Rico (5:1 ratio of 2 M KCl volume to g oven dry equivalent soil). We used extracts from fresh soil (referred to as "ambient soil") as well as soil slurries (4:1 ratio of DI water to dry soil) that had been incubated in an oxygen-free headspace for three days to increase Fe^{2+} concentrations and decrease NO_3^- concentrations (referred to as "anaerobic soil slurries") to provide a wider range of concentrations for comparison ($n = 5$ per extract type). The extracts were frozen for two months before analysis.

For the PO_4^{3-} filtration procedure, 200 μL of 1 M Na_2HPO_4 solution was added to 10 mL of soil extract contained in a 15 mL polypropylene centrifuge tube to attain a final concentration of 10 mM PO_4^{3-} in the soil extract. Previous work showed that 5 mM PO_4^{3-} was sufficient to prevent Fe interference in our soil extracts, so 10 mM PO_4^{3-} is regularly used to ensure that there is sufficient PO_4^{3-} to remove iron from most, if not all, soil extracts analyzed (data not shown). The tube was capped, and the extract and PO_4^{3-} were shaken vigorously by hand for 10–20 s to precipitate the Fe. The extract was then syringe-filtered through a 0.45 μm membrane filter. Five mL of the filtered extract and 50 μL of 30 mg N L^{-1} KNO_3 were pipetted into a new polypropylene tube. The tube was capped and shaken by hand to mix well. This procedure was repeated with a 10 mL unfiltered aliquot of each soil extract. Nitrate standard addition recovery was calculated as the difference in measured NO_3^- concentration with and without a known quantity of NO_3^- added.

For the fourth experiment, we characterized the Fe:N ratio of Fe interference using 2 M KCl solutions containing either 0.3 or 3 mg N L^{-1} KNO_3. We separately added 50 μL of 0, 100, 200, 300, 400, or 500 mM Fe(II)Cl_2 in 0.5 N HCl to 4.95 mL of the NO_3^- solutions to achieve 0, 55, 110, 165, 220, and 275 mg Fe^{2+} L^{-1} in replicate 5 mL aliquots ($n = 5$ per Fe^{2+} concentration). We quantified Fe interference as the difference between the actual and measured NO_3^- concentrations. Thus, when negative peaks were measured, the amount of Fe interference was greater than the actual NO_3^- concentration. We calculated the Fe:N ratio of Fe interference as the Fe molar concentration (mM) divided by the Fe interference expressed in NO_3^- molar concentration (mM).

2.2. Sample analyses

For all experiments the samples were analyzed on a flow-injection autoanalyzer (Lachat Instruments, Milwaukee, WI) in three repeated runs using the following buffers in this sequence: imidazole, NH_4Cl–DTPA, and NH_4Cl–EDTA. The buffers were made according to the recipes described in Colman and Schimel (2010). Each buffer was allowed to flow through the Cd column for 5–10 min before NO_3^- analysis to flush out the previous buffer. For the first three experiments we utilized a well conditioned Cd column (approximately 2000 samples analyzed) because PO_4^{3-} interference with NO_3^- analysis has been documented under these conditions (Olson, 1980). For the fourth experiment we used a Cd column that had previously been used to analyze approximately 1000 samples. The modified Griess–Ilosvay method used for colorimetric NO_3^- analysis measures NO_3^- + nitrite (NO_2^-) concentrations, but we report only on NO_3^- because our pure solutions did not contain NO_2^- and our soil extracts most likely did not contain NO_2^-, which is highly reactive and quickly consumed in soils.

Ferrous iron concentrations for unfiltered and filtered soil extracts were measured using a modified ferrozine assay (Liptzin and Silver, 2009). The samples were analyzed manually on a spectrophotometer (Spectronic 20, Milton Roy, Ivyland, PA for the first three experiments and Genesys 20, Thermo Fisher Scientific, Waltham, MA for the fourth experiment). Standards were prepared using $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ in 0.5 N HCl.

2.3. Statistical analysis

We used SYSTAT Version 10 (SPSS Inc., Evanston, IL) to perform statistical analyses. Analysis of variance and Tukey tests were used to identify statistically significant differences in measured NO_3^- concentrations, NO_3^- standard addition recovery, and Fe:N ratio of Fe interference among buffers. Measured NO_3^- concentrations and NO_3^- standard addition recovery for unfiltered and filtered soil extracts were compared using *t*-tests. Mean values are reported in the text followed by standard errors (±SE). Statistical significance was determined at $p < 0.05$ unless otherwise noted.

3. Results

3.1. Fe interference in pure solutions

We detected Fe interference in colorimetric NO_3^- determination at Fe^{2+} concentrations between 5.5 and 55 mg Fe^{2+} L^{-1} for all buffers tested ($p < 0.05$; Fig. 1a). The imidazole buffer was least sensitive to Fe interference, with a small but statistically significant under-determination of NO_3^- concentration at 27.5 mg Fe^{2+} L^{-1}. In contrast, the measured NO_3^- concentration using the NH_4Cl–DTPA buffer was only half of the actual concentration of 0.3 mg N L^{-1}