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Iron interference in the quantification of nitrate in soil extracts and its effect on hypothesized abiotic immobilization of nitrate

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Abstract Human alteration of the nitrogen cycle has stimulated research on nitrogen cycling in many aquatic and terrestrial ecosystems, where analyses of nitrate (NO_3^-) by standard laboratory methods are common. A recent study by Colman et al. (Biogeochemistry 84:161–169, 2007) identified a potential analytical interference of soluble iron (Fe) with NO_3^- quantification by standard flow-injection analysis of soil extracts, and suggested that this interference may have led Dail et al. (Biogeochemistry 54:131–146, 2001) to make an erroneous assessment of abiotic nitrate immobilization in prior ^{15}N pool dilution studies of Harvard Forest soils. In this paper, we reproduce the Fe interference problem systematically and show that it is likely related to dissolved, complexed-Fe interfering with the colorimetric analysis of NO_2^-. We also show how standard additions of NO_3^- and NO_2^- to soil extracts at native dissolved Fe concentrations reveal when the Fe interference problem occurs, and permit the assessment of its significance for past, present, and future analyses. We demonstrate low soluble Fe concentrations and good recovery of standard additions of NO_3^- and NO_2^- in extracts of sterilized Harvard Forest soils. Hence, we maintain that rapid NO_3^- immobilization occurred in sterilized samples of the Harvard Forest O horizon in the study by Dail et al. (2001). Furthermore, additional evidence is accumulating in the literature for rapid disappearance of NO_3^- added to soils, suggesting that our observations were not the result of an isolated analytical artifact. The conditions for NO_3^- reduction are likely to be highly dependent on microsite properties, both in situ and in the laboratory. The so-called "ferrous wheel hypothesis" (Davidson et al., Glob Chang Biol 9:228–236, 2003) remains an unproven, viable explanation for published observations.

Keywords Iron · Nitrate · Nitrite · Nitrogen · Soil extracts · Abiotic immobilization · Ferrous wheel hypothesis

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Introduction

Nitrogen (N) is among the most commonly measured elements in biogeochemical studies owing to its importance as an essential nutrient for plant, animal, and microbial metabolism. The nitrate ion (NO_3^-) is frequently measured in studies of atmospheric

deposition, agricultural runoff, and wastewater from human sewage systems. Increasing human alteration of the nitrogen cycle (Galloway et al. 2004; UNEP and WHRC 2007) has stimulated research on nitrogen cycling in many aquatic and terrestrial ecosystems, and many studies include analysis of NO_3^- by standard water chemistry methods. Common methods for quantifying NO_3^- concentrations include ion specific electrodes, ion chromatography, and colorimetric analysis of nitrite (NO_2^-) following NO_3^- reduction (Mulvaney 1996). The latter method is widely used, partly because of commercially available flow injection analyzers (FIA) that automate this analysis, allowing large sample numbers to be analyzed quickly with reasonably good accuracy, precision, and sensitivity.

In the process of carrying out an experiment designed to measure abiotic immobilization of NO_3^-, Colman et al. (2007) discovered a potential analytical interference of NO_3^- quantification caused by dissolved iron (Fe) in extracts of thrice-autoclaved soils, leading to an underestimate of NO_3^- concentration. These authors demonstrated that NO_3^- concentrations were underestimated when they used the standard ammonium-chloride–ethylenediaminetetraacetic acid (NH_4Cl–EDTA) buffer in FIA; that the underestimation of NO_3^- was correlated with soluble Fe concentrations; and that this interference did not appear when they substituted an imidazole buffer. The authors concluded that previously published reports of evidence for abiotic immobilization of NO_3^- into a dissolved organic-N (DON) fraction (Dail et al. 2001; Davidson et al. 2003) were due to this analytical artifact, because underestimation of NO_3^- concentrations would cause an underestimation of recovery of experimentally added $^{15}NO_3^-$ label and an over estimation of DON (estimated by the difference between total dissolved N [TDN] and dissolved inorganic-N [DIN]).

Interference of soluble Fe in NO_3^- quantification is a potentially important issue not only for research pertaining to abiotic immobilization of NO_3^-, but also potentially for thousands of other studies that have reported NO_3^- concentrations in soil extracts, rainwater, streamwater, lakes, groundwater, and seawater. We have recently encountered several graduate students who have read the Colman et al. (2007) paper and who were worried about the validity of their NO_3^- concentration data from a variety of different experiments and studies. Hence it is very important to accurately characterize this soluble Fe interference of NO_3^- quantification when employing the widely used NH_4Cl–EDTA buffer methodology.

Ironically, the standard methodology found in the SSSA Methods for Soil Analysis (Bundy and Meisinger 1994; Mulvaney 1996), which follows standard methods published by the U.S. Environmental Protection Agency (US EPA 1983) and by the American Public Health Association (APHA 1989), recommends using the NH_4Cl–EDTA buffer expressly to remove Fe interference for NO_3^- quantification. The reduction of NO_3^- to NO_2^- is typically accomplished in a copper-coated granulized cadmium (Cd) "reduction column", and several potential interferences of this step have been identified, including Fe, copper and other transition metals in excess of 1 mg l^{-1}, which have been reported to alter the efficiency of the Cd reduction column (Alpkem Corporation 1990). The addition of EDTA results in complexation of these metals, thereby removing the problem (Alpkem Corporation 1990). A commonly used method employs 1 M NH_4Cl–EDTA to provide excess complexing ligand (EDTA) to solutions with high metal concentrations, thus enhancing and prolonging the use of the Cd reduction column.

The study by Colman et al. (2007) suggests another, as yet unidentified, mechanism by which dissolved Fe interferes with NO_3^- quantification despite the excess of EDTA in the buffer solution, and they propose the use of an imidazole buffer (0.1 M; pH 7.5) instead. However, switching to the imidazole buffer method, as recommended by Colman et al. (2007), has some potentially serious drawbacks, including possible precipitation of metal hydroxides that cause degradation of the cadmium column for NO_3^- reduction (Nydahl 1976; Patton et al. 2002). A strong rationale and carefully considered balance of pros and cons would be advisable before the current standard procedure for NO_3^- and NO_2^- analysis is abandoned, but several issues remain unresolved. For example, what Fe concentration introduces a significant interference with NO_3^- quantification by the EDTA method such that a change in buffer system (with increased potential for degradation of Cd column efficiency) is justified? Might there be a way to avoid significant Fe interference without introducing new problems associated with use of the imidazole buffer solution?

Here we report the results of systematic studies intended to provide insight into when Fe interference is important and how to test for it. Our primary objective is to convey this new information to those making decisions in the short-term regarding use of data from previous analyses and regarding current methodologies. We also hope that this discussion will help stimulate more in-depth analyses that will provide a longer-term and more definitive solution. A secondary objective is to discuss the recent evidence for and against the existence of abiotic immobilization of NO_3^- in light of this concern about NO_3^- quantification.

Iron in nitrate/nitrite standard solutions

As a first step, we repeated the Fe standard addition experiment of Colman et al. (2007) using a LACHAT Quik Chem FIA+ 8000 Series autoanalyzer (Lachat Instruments, Milwaukee, USA) with the NH_4Cl–EDTA buffer recommended by the manufacturer for nitrate (QuikChem-Method-10-107-04-1-L 1999), ammonium (QuikChem-Method-10-107-06-2-A 1997), and TDN (QuikChem-Method 10-107-04-3-P, 2000) analyses. Iron additions were derived from an atomic adsorption spectrometry standard, wherein Fe is dissolved in a 2% HCl solution in order to maintain the soluble Fe(II) oxidation state. Aliquots of this Fe(II) standard were added to standard solutions of NO_3^- and NO_2^- in deionized H_2O and 0.5 M K_2SO_4 to obtain a range of dissolved Fe concentrations from 0 to 200 mg l^{-1}. The addition of this acidic, Fe(II) solution caused a decrease in the apparent concentrations of 2 mg N l^{-1} NO_3^- and NO_2^- standards (Fig. 1). We observed a small increase in NH_4^+ concentration with increasing Fe(II) addition, consistent with a small amount of reduction of NO_3^- or NO_2^- to NH_4^+ by Fe(II) (Ottley et al. 1997). There was no detectable effect of Fe on total dissolved nitrogen (TDN) quantification by the persulfate digest method (Fig. 1).

Because the same effect was observed for both NO_3^- and NO_2^- standards, Fe interference evidently does not involve the reduction of NO_3^- to NO_2^- on the Cd column. An explanation could be that NO_2^- reacts with Fe(II) to produce more reduced forms of N, such as NO and N_2O gases and NH_4^+. However, reactions that would result in loss of gaseous forms of N apparently are not occurring instantaneously while

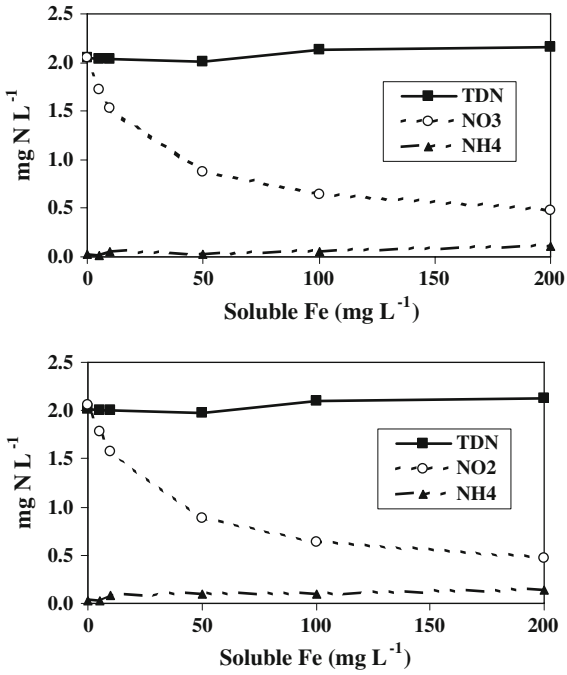

Fig. 1 Recovery of 2 mg N l^{-1} added as nitrate (upper panel) and as added nitrite (lower panel) in the forms of total dissolved nitrogen (TDN), nitrite/nitrate (NO_2^-/NO_3^-), and ammonium (NH_4^+) in solutions with soluble iron ranging from 0 to 200 mg Fe l^{-1}

the sample is in the queue for analysis, because when solutions of Fe(II) and NO_2^- are run through the persulfate digest module of the Lachat instrument, all of the NO_2^--N is recovered as TDN. Therefore, it seems more likely that the EDTA-chelated Fe interferes directly with colorimetric analysis of NO_2^-.

This interference does not occur in samples that have undergone persulfate digestion, probably because the persulfate oxidizes all forms of Fe(II) (including EDTA-chelated-Fe) to Fe(III), and the less soluble Fe(III) precipitates out, preventing its subsequent interference with quantification of NO_2^-. Similarly, by raising the pH to 7.5, the imidazole buffer probably promotes oxidization of Fe(II) to Fe(III) (Herzsprung et al. 2005). Taken together, these data suggest that the NO_3^- and NO_2^- quantification problems shown in Fig. 1 are related to Fe(II) present in NH_4Cl–EDTA buffered solutions interfering with NO_2^- quantification. We speculate that EDTA-chelated Fe passes through the cadmium column and then somehow interferes with the Griess-Ilosvay colorimetric reaction used for NO_2^-

quantification. Reduction of NO_2^- with soluble Fe(II) before NO_2^- reacts with the coloring reagent is also possible, although unlikely at the circumneutral pH values in these analyses (Van Cleemput and Baert 1983). So far, these results are consistent with those of Colman et al. (2007), and the nature of the interference problem has been narrowed somewhat.

Iron in soil extracts

The kinetics of Fe(II) oxidation are very rapid at pH >5 in the presence of atmospheric oxygen (Stumm and Morgan 1996). Therefore, in most oxic soils, Fe is present dominantly in relatively insoluble Fe(III) solids (e.g., Fe(III) oxyhydroxides such as goethite). Any Fe(II) that does get extracted will undergo rapid oxidation to Fe(III) in oxic extracts of soils with solution pH >5. Organically-bound and otherwise chelated forms of Fe in soil extracts could remain soluble in either Fe(II) or Fe(III) states, but we do not know if these forms of soluble Fe have the same effect as the EDTA-chelated Fe that was produced when acidic Fe(II) was added to an EDTA-NH_4Cl buffer solution in the experiments described above.

An unambiguous means of determining whether Fe interference has the potential to occur in a given soil extract solution is to conduct NO_3^- and NO_2^- standard additions to those soil extracts with native concentrations of soluble Fe. We conducted standard addition of NO_3^- and NO_2^- to 0.5 M K_2SO_4 extracts (5:1 ratio of solution to soil, shaken for 1 h) of both live (pH = 2.6 in 0.5 M K_2SO_4) and autoclaved (pH = 2.1 in 0.5 M K_2SO_4) soils of the Harvard Forest Oa horizon taken from the same area studied by Dail et al. (2001). The Oa horizon is well humified and C-rich, possibly containing organically-bound Fe. It also contains a small amount of Fe-containing minerals mixed in from the mineral soil layer below it. Furthermore, autoclaving could release Fe to solution from the solid phase because of reductive dissolution. The $NO_2 + NO_3$ concentrations in K_2SO_4 extracts of live and sterile soils were 0.1–0.7 mg N l^{-1} (Table 1). In all cases, when we added a 0.02 ml aliquot of 1,000 mg N l^{-1} KNO_2 or KNO_3 to 10 ml of these extracts, the concentrations increased approximately by the expected 2 mg N l^{-1}. Complete recovery was also obtained when this standard addition was applied to standards in water. These results show that there was not sufficient Fe of any form, or any other constituent in these soil extracts, to interfere with NO_3^- and NO_2^- quantification using our Lachat QuikChem 8000 with the NH_4Cl–EDTA buffer method.

Dissolved Fe concentrations in these solutions (phenanthroline method after boiling in acid and hydroxylamine; APHA 1989) were <1 mg l^{-1} for live soil and 3 mg l^{-1} for sterilized soil. Although these dissolved Fe analyses alone might be used to allay concerns about the likelihood of interference, the standard addition protocol provides conclusive proof that there is insufficient dissolved Fe to be of concern. We recommend this standard addition approach to researchers who have archived extracts

Table 1 Standard additions (2 mg N l^{-1}) of nitrite and nitrate standards to 0.5 M K_2SO_4 extracts of live and sterilized samples of the Oa horizon (5:1 volume to dry mass ratio) of Harvard Forest soils and to 0.4 mg N l^{-1} standards in distilled water

Sample	Replicate	$NO_2 + NO_3$ (mg N l^{-1})		
		Background	Background + NO_2	Background + NO_3
NO_2 standard	A	0.44	2.62	
NO_2 standard	B	0.45	2.65	
NO_2 standard	C	0.45	2.65	
NO_3 standard	A	0.38		2.59
NO_3 standard	B	0.36		2.57
NO_3 standard	C	0.36		2.54
Live soil	A	0.17	2.01	2.44
Live soil	B	0.13	2.48	2.40
Live soil	C	0.19	2.44	2.46
Sterile soil	A	0.50	2.65	2.48
Sterile soil	B	0.51	2.87	2.64
Sterile soil	C	0.36	2.48	2.68

and who wish to determine if their previous NO_3^- analyses were valid. Similarly, new soil samples from the same study site could be extracted and analyzed in this way and this could be a preliminary step to rule out future Fe interference problems before initiating a new study or experiment.

Colman et al. (2007) reported dissolved Fe concentrations ranging up to 216 mg l^{-1} in 0.5 M K_2SO_4 extracts (5:1 ratio) of the thrice autoclaved soils that they studied. The dissolved Fe concentration for an archived Harvard Forest mineral soil provided to them by Bryan Dail was reported as 97.6 mg l^{-1} (Colman et al. 2007). Live and twice-autoclaved sterilized subsamples of this same mineral soil sample were subsequently submitted by us for soluble Fe analysis by the University of Maine State Soils Testing Facility, using ICP-AES (TJA model IRIS 1000) where the concentrations were reported as 0.2 and 1.8 mg Fe l^{-1} in 10:1 KCl extracts of live and autoclaved samples, respectively. It is possible that there are differences in the techniques used among laboratories to quantify dissolved Fe, but some additional explanation is needed for these huge differences in reported concentrations.

It is possible that the third autoclaving step used by Colman et al. (2007) liberated much more soluble Fe. They also incubated their soil samples in a shaker for 24 h in a saturated solution containing varying concentrations of NO_3^- and then extracted an additional 2 h in 0.5 M K_2SO_4. In contrast, Dail et al. (2001) incubated soils at only 60% water-holding capacity for 24 h, followed by extraction for 20 min in 0.5 M K_2SO_4 and by washings in water. Similarly, the University of Maine extraction was only 20 min. Incubating the soils under saturated conditions for 24 h and extracting in salt for 2 h may have resulted in solubilization of more Fe in the Colman et al. (2007) study. Dail et al. (2001) also used a 0.45 μm nominal pore size filter, whereas Colman et al. (2007) filtered their extracts with a Pall A/E glass fiber filter with a 1 μm nominal pore size, which could have enabled more colloidal Fe to pass through the filters. A more systematic study of the factors that affect dissolved Fe concentrations in soil extracts is clearly needed. A factorial design that includes soil type, ionic strength of extracting solutions, extractant-to-soil ratios, pore sizes of filters used on extracts, number of autoclaving events, and duration of extraction period would help define the source of variation in reported dissolved Fe concentrations.

Until a systematic study of this nature can be accomplished, we can note only that the concentrations of dissolved Fe in Harvard Forest extracts observed under the conditions in our laboratories were substantially less than the >17 mg l^{-1} reported by Colman et al. (2007) to cause a significant Fe interference problem for NO_3^- quantification. This interpretation is consistent with the lack of interference that we found in standard addition experiments with our soil extracts, where our measurements using the NH_4Cl–EDTA buffer accounted for 100% of the NO_3^- and NO_2^- added.

Why wasn't NO_3^- immobilization observed by Colman et al. (2007)?

We have established that dissolved Fe probably did not interfere with quantification of NO_3^- in extracts of organic and mineral horizon soil samples from the Harvard Forest in the study of Dail et al. (2001), because data from repeated manipulations of soils from this site demonstrate that (1) dissolved Fe concentrations are low; and (2) complete recovery occurs of standard additions of NO_3^- and NO_2^- to extracts of both live and sterilized samples of these soils at ambient dissolved Fe and pH. Dail et al. (2001) also reported 100% recovery of NO_3^- added to Harvard Forest mineral soil samples, demonstrating that there was no Fe interference in extracts of those samples at that time. Hence, we maintain that the results of Dail et al. (2001) are indeed consistent with abiotic immobilization of nitrate. If there is evidence for abiotic immobilization of nitrate from our laboratories, then why did Colman et al. (2007) fail to find evidence for this process in any of the 44 soil samples that they investigated? Besides the differences in details of methodologies already noted above (e.g., ionic strength of extracting solutions, extractant-to-soil ratios, pore sizes of filters used on extracts, number of autoclaving events, and duration of extraction period), we offer three additional plausible explanations.

First, Colman et al. (2007) studied primarily mineral soils, although a few of their mineral soils had organic matter contents between 10% and 20%. In the "ferrous wheel hypothesis", Davidson et al.

(2003) suggested that "NO_3^- is reduced to NO_2^- in anaerobic microsites of the humus layer, that NO_2^- reacts with DOC to form DON, and that oxidized forms of Fe and Mn in soil minerals are eventually reduced again by organic acids and phenolic compounds derived from plants and microorganisms." There may have been insufficient organic matter and metabolic activity to reduce Fe to the Fe(II) state in microsites in the mineral soils studied by Colman et al. (2007).

Second, the laboratory incubation procedures use by Colman et al. (2007) likely destroyed anaerobic microsites. They used only 4 g of well-mixed, sieved soil in each incubation vial, which would have allowed oxygen to penetrate throughout the sample. It is not surprising that reduction processes did not occur under these conditions. In most laboratory studies of anaerobic processes, such as methanogenesis, denitrification, and N_2-fixation, care is taken to mimic undisturbed soil conditions to the extent possible (Davidson and Schimel 1995). In the Dail et al. (2001) study of abiotic immobilization of nitrate, large batches (several kilograms) of moist soil were autoclaved and then moderate amounts (the wet weight equivalent of 30 g dry soil) were aseptically transferred to incubation jars. Subsamples of only 4-g were removed periodically from incubation jars for several analyses conducted at various time points during soil incubation. Hence, these small subsamples were isolated only at the moment of analysis. While autoclaving creates a variety of artifacts, including alteration of the oxidation of some organic soil constituents, it may also promote reductive dissolution of metals, and the oxidation state of metals in solution may then depend on how the soil is subsequently handled while setting up and carrying out incubations (Lotrario et al. 1995). In any case, each study can only claim whether or not abiotic NO_3^- immobilization was observed under the particular experimental conditions employed during that study. The conditions employed in the Colman et al. (2007) study were not conducive to maintaining microsites of nitrate reduction, which are a necessary component of the ferrous wheel hypothesis.

Third, Colman et al. (2007) employed a dose-response method that assumes that abiotic immobilization of NO_3^- is a first-order reaction with respect to nitrate. It was assumed that for each dose of NO_3^- (0, 0.2, 0.4, 0.6, 1, 2, 3, 4, and 5 mg NO_3-N l^{-1}), the abiotic immobilization process should remove a constant fraction. If, for example, this first-order process removed 10% of the NO_3^- during the incubation period, then the expected recovery would be 0.18, 0.36, 0, 54, 0.9, 1.8, 2.7, 3.6, and 4.5 mg NO_3-N l^{-1} for each of the respective solutions listed above. The slope of the regression line for this recovery versus the amount added would be 0.9, with an intercept of zero, indicating that 10% was immobilized by the assumed first-order process (Fig. 2). However, the limiting factor is more likely the amount of reactive Fe(II) present in the sample. Most of the Fe in well-aerated soils (especially 4 g of mixed and sieved soil) is in the Fe(III) state, and reduced forms of Fe would occur only in a few microsites. For this process to be quantitatively important at the ecosystem scale, there must be a way that the small number of microsites containing reduced Fe can be regenerated, which is why an abundant supply of organic-C is necessary and why we refer to the hypothesis as a "wheel." In the Colman et al. (2007) study, if there were still any microsites of reduced Fe in the sieved and aerated

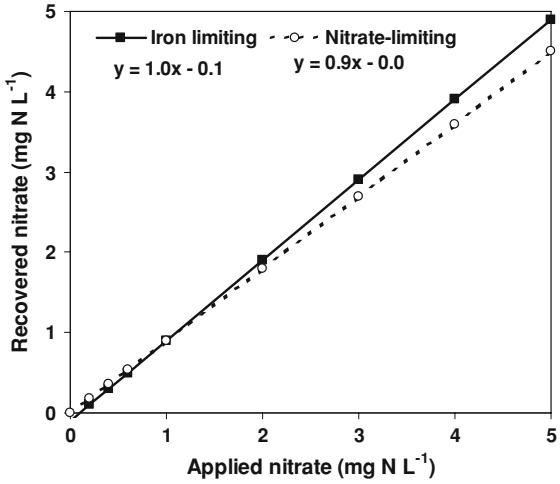

Fig. 2 Expected results of a dose response experiment when nitrate reduction is limited by either nitrate concentrations or by concentrations of reduced iron. When the reaction is nitrate limited, the reaction is first order with respect to nitrate, and the slope of the line (0.9 in this example) indicates the fraction of added nitrate (10% in this example) that was reduced. When the reaction is limited by microsites of reduced iron in the soil, the slope of nitrate recovery is unity (zero order with respect to nitrate) and the offset (Y-intercept of −0.1 in this example) is a measure of the amount of iron that was available to reduce the nitrate

soil samples when they were treated with the NO_3^- solution, they would have been quickly saturated even at the lower end of the NO_3^- concentration range; no additional immobilization would be expected with higher NO_3^- application rates. If the concentration of Fe(II), rather than NO_3^-, is the limiting factor, then one would expect that the slope of recovery in the dose-response relationship should be very near unity, but with a modest offset (a negative intercept) that is equivalent to the NO_3^- reduction afforded by the limited number of Fe(II)-containing microsites (Fig. 2). In other words, abiotic immobilization of NO_3^- is probably zero-order with respect to NO_3^- concentration and perhaps first order with respect to Fe(II) concentration. A small negative Y-intercept (i.e., a small and identical change in NO_3^- concentration in all NO_3^--amendment treatments) expected from a zero-order process would be difficult to detect and may not have occurred if all of the reduced Fe in the soil sample had been oxidized during sample preparation. Regardless of whether Fe(II) was present, the method used by Colman et al. (2007) to infer NO_3^- immobilization rates based on analysis of slopes in dose-response treatments was flawed by an inappropriate assumption that NO_3^- was the limiting factor and that the process is first-order with respect to NO_3^-.

Status of the ferrous wheel hypothesis and nitrate quantification

This hypothesized mechanism for abiotic immobilization of NO_3^-—that NO_3^- is reduced to NO_2^- by reduced metals [e.g., Fe(II)] in soil microsites, followed by reaction of NO_2^- with organic matter to produce organic-N, and subsequent regeneration of reducing microsites by heterotrophic activity in a C-rich medium (Davidson et al. 2003)—remains an unconfirmed hypothesis. It may not be the correct mechanism to explain rapid disappearance of NO_3^- in live and sterile soils, but, as far as we are aware, it is the only working hypothesis currently under consideration. Meanwhile, evidence for rapid disappearance of NO_3^- in soils and inferred abiotic immobilization continues to accumulate in the literature (Berntson and Aber 2000; Dail et al. 2001; Davidson et al. 1991; Compton and Boone 2002; Corre et al. 2003, 2006, 2007; Hall and Matson 2003; Huygens et al. 2008; Magill et al. 2004; Micks et al. 2004; Perakis and Hedin 2001; Sotta et al. 2008; Zogg et al. 2000). While it is possible that some estimates of abiotic immobilization may have been compromised by Fe interference in NO_3^- quantification as described by Colman et al. (2007), we have shown that this was not the case in the study of Dail et al. (2001), nor would it have occurred where the imidazole buffer method was used (Huygens et al. 2008), and it is unlikely that all of the other studies contained sufficiently high dissolved Fe concentrations to account for the reported rapid disappearance of NO_3^-.

Conclusions

We have reproduced the effect of high concentrations of dissolved Fe on NO_3^- and NO_2^- quantification in an NH_4Cl–EDTA buffer that was reported by Colman et al. (2007), but we have also demonstrated that such high Fe concentrations were not present in the extracts of the Harvard Forest soils under the experimental conditions in our laboratories. Therefore, we stand by our published results on rapid NO_3^- disappearance in live and sterilized samples of the O horizon of this forest (Dail et al. 2001). The nature of the Fe interference problem is likely related to EDTA-complexed Fe(II) interfering with the colorimetric analysis of NO_2^-, but more work is needed to clarify the mechanism and to refine estimates of the amount of soluble Fe that causes significant error in NO_3^- quantification by this analytical method. Such concerns about analytical error certainly merit caution and testing, such as the standard addition of NO_3^- described here.

Until this issue can be clarified further, we recommend that before switching buffers in FIA analysis and before discarding old data that were derived from analysis with an NH_4Cl–EDTA buffer, researchers should assess the recovery of NO_3^- standard additions on a subset of their soil extracts or water samples to test for possible interferences at native Fe concentrations using the standard NH_4Cl–EDTA buffer in FIA analysis. This approach provides an effective means of determining if Fe interference is potentially important at ambient levels of soluble Fe and pH in any kind of soil extract or water sample. Using the imidazole buffer or some other approach to

precipitate ferric hydroxide, perhaps followed by a method such as dialysis to remove the precipitate, may be necessary for waters and extracts where soluble Fe concentrations are sufficiently high to cause detectable interference of nitrate/nitrite analysis. At present, the NH_4Cl–EDTA buffer remains the standard method for FIA of nitrite/nitrate and may still be appropriate for many or most circumstances, but checking for potential Fe interference is warranted.

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