Letter

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Characterizing Limits of Precision for Dissolved Organic Nitrogen Calculations

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ABSTRACT

The usefulness of dissolved organic nitrogen (DON) data to environmental science depends on the precision of each calculated concentration. Precision of a calculated DON concentration is based on propagation of error from the measured nitrogen fractions used to calculate the DON concentration. We present an improved approach for calculating DON precision that strengthens conventional error propagation by developing empirical relationships between precision and concentration for each of the measured nitrogen fractions used to calculate DON concentration. Because the concentration and relative importance of each measured nitrogen fraction differs among samples, DON precision and the corresponding detection limit are likely to be different for each sample. Case studies from two different research efforts – a synoptic study of surface water DON and an experimental assessment of DON leached from heat-treated soils – demonstrate how the proposed approach can be tailored to the analytical methods and concentration ranges expected for any research project. In addition, current recommendations for pretreatment (e.g., dialysis) to improve DON precision should be modified to include consideration of analytical precision. Improved characterization of DON precision facilitates a realistic appraisal of sample-specific detection limits, and the results can be generalized to support decisions about pretreatment and statistical analysis of DON data.

INTRODUCTION

Quantification of dissolved organic nitrogen (DON) is important for basic and applied research in many disciplines, including atmospheric science, oceanography, and limnology.[1-5] For example, DON enrichment raises concerns about eutrophication in estuaries,[6] increasing levels of precursors for nitrogenous disinfection byproducts pose risks for drinking water treatment,[7] and there is growing interest in the bioavailability of DON discharged by wastewater treatment facilities.[8-9]

It is not practicable to measure DON concentrations either in aggregate or as the sum of its numerous and diverse components. The components of DON in aquatic systems may include proteins, humic-like substances, urea, peptides, amino sugars, purines, pyrimidines, pteridines, amides, methyl amides, and various other compounds.[10] Instead, it is common practice[3, 11-14] to calculate DON concentration by measuring total dissolved nitrogen (TDN) and subtracting dissolved inorganic nitrogen (DIN), which is the sum of two or three fractions that are easily measured.

Because DON concentrations are calculated and not directly measured, there is no direct method of assessing analytical precision. The precision problem for DON is not new and can be addressed with an error-propagation approach[11] that accumulates the measurement errors contributed by each nitrogen fraction represented in the calculation of DON concentration. For example, when DIN is measured as three separate fractions (i.e., nitrate, nitrite, and ammonia), variance for the calculated DON concentration is the sum of variances for TDN, ammonia, nitrite, and nitrate. Consequently, the calculated DON concentration will be less precise, in absolute terms, than the measured TDN concentration.

Precision has direct bearing on the problem of negative DON concentrations that appear frequently in the literature.[15] If the true DON concentration in a sample is zero, for instance, it is reasonable to expect that half of the calculations from replicate analyses would yield small negative values and half would yield small positive values due to accumulation of error from the measured nitrogen fractions. Consistent application of the error-propagation approach to define DON precision and establish detection limits could do much to simplify reporting and avoid awkward decisions about re-coding[16] or retaining[17-18] negative values. Alternatively, it is possible to calculate DON precision and detection limits based on replicate measurements of the same sample,[13] but the added cost of sufficient replication (e.g., 5 per sample) is rarely warranted.

Previous efforts to estimate DON precision by the error-propagation approach have generally relied on one or the other of two common assumptions concerning the relationship between precision and concentration for the measured nitrogen fractions. One assumption holds that absolute precision (standard deviation) is constant and is independent of concentration, and the other assumption holds that relative precision (relative standard deviation) is constant and is a fixed proportion of concentration. Both assumptions have been applied in the literature (see SI Text S1 for examples).

The assumption of constant relative precision is more common in the literature and has led to insights linking DON precision to the ratio of DIN concentration to TDN concentration. One such study found that DON precision diminished rapidly as the DIN/TDN ratio increased above 0.60 (i.e., DON less than 40% of TDN).[14] Another study placed the threshold DIN/TDN ratio at about 0.85 (i.e., DON less than 15%).[13] For cases where the threshold ratio is exceeded, both studies recommend dialysis pretreatment to improve DON precision. Dialysis, which lowers the DIN/TDN ratio by removing DIN but not DON, can improve DON precision, but only if reduction of DIN concentration yields a commensurate improvement in DIN precision.

For direct measurements of nitrogen fractions, neither constant absolute precision nor constant relative precision alone is an appropriate assumption for all concentrations. However, both assumptions can be integrated appropriately by a two-component variance model, based on analytical experience.[19-21] This variance model can be developed for the useful range of concentrations for each analytical method used to calculate DON concentrations (Figure 1, Text S2, Equations S1-S4). Evaluation of the two-component variance model shows why each of the two assumptions about precision will fail in the wrong part of the concentration spectrum. Assuming that precision is governed only by a constant standard deviation overestimates precision at high concentrations, and assuming that precision is governed only by a constant relative standard deviation (RSD) overestimates precision at low concentrations (Figure 1). Joining constant standard deviation with constant RSD in a two-component variance model yields a combined standard deviation, as shown over a range of concentrations in Figure 1. Proper characterization of DON precision, therefore, should rely on development of a two-component variance model for each of the measured nitrogen fractions.

In this work, we take a fresh look at DON precision by improving the variance estimates that support implementation of the error-propagation approach. A two-component variance model is used to characterize empirical relationships between precision and concentration for each of the analytical methods supporting calculation of DON concentration. Characterization of sample-specific precision for DON concentration will provide the detection limits necessary for statistical analysis, and it can help decide when pretreatment, such as dialysis, to reduce DIN is likely to improve DON precision.

MATERIALS AND METHODS

Samples of surface water and soil leachate were analyzed for nitrogen fractions by different methods, including the two most common TDN methods – persulfate oxidation and high-temperature catalytic oxidation. Surface water samples collected in and near Rocky Mountain National Park, Colorado, USA were analyzed for nitrate, nitrite, ammonia, and TDN. Soils from the Colorado Front Range, which were thermally altered and leached to simulate the effects of wildfires on nutrient release, were analyzed for nitrate+nitrite, ammonia, and TDN. Sample collection, processing and analytical methods are described in the Supporting Information (Text S3 and S4).

For each analytical method, precision was characterized for a series of concentrations spanning the range expected for each set of samples. At each standard level, the standard deviation and the RSD were determined from replicate measurements of concentration. The RSD was calculated with the average measured concentration as the denominator, rather than the nominal standard concentration, because the average of measured values is "of the same metric" as the standard deviation.[22] The DON concentration for a sample is considered below detection if the RSD for DON in that sample is greater than 1/3 (Text S2). Details of replicates, standards, and precision are presented in the SI (Text S3 and S4).

The relationship between precision and concentration was determined empirically for each analytical method (Figures S2-S3). A region of constant standard deviation was generally evident, but the measured concentrations may or may not have included much of the region of constant RSD. Where the constant RSD could be defined, the two-component variance model was applied; where constant RSD could not be defined, a power function was used to describe the relationship between precision and concentration over the available analytical range (Tables S2 and S5). Ultimately, the aim is practical, rather than theoretical, and a defensible model must be tailored to each method.

Precision of the calculated DON concentration is estimated with the error-propagation approach by applying concentration-specific characterizations of precision for each analytical method to each sample (sample calculations are given in Tables S3 and S6). This approach can be applied to any combination of concentrations for the nitrogen fractions used to calculate the DON concentration, including cases where the measured value of a nitrogen fraction was below detection.

RESULTS AND DISCUSSION

Relationships between precision and concentration derived for each analytical method were used to calculate DON precision for each sample of surface water or soil leachate. Sample-specific calculations are required because the relative and absolute amounts of the inorganic fractions vary among the samples (Tables S3 and S6). Consequently, two samples with the same DON concentration could have different standard deviations.

DON Precision in Surface Water Samples

The error-propagation approach was used to derive a standard deviation and a detection limit for DON in each of 210 surface water samples. Even though high-precision analytical methods were used, 92 of 210 DON concentrations were below detection. A high proportion of non-detects may be common for surface waters, especially where anthropogenic influences are small.

An association between precision and the DIN/TDN ratio is evident for the surface water samples, although it is more complex than has been reported in previous studies (Figure 2). For all samples with a DIN/TDN ratio less than about 0.6, DON concentrations exceeded the sample-specific detection limits. Conversely, for all samples with a DIN/TDN ratio greater than about 0.85, DON concentrations were below detection. That these ratios match ratios reported previously in the literature is misleading; as will be shown later, thresholds also depend on relative precision.

The quantitative basis for calculating sample-specific DON precision developed in this study creates an opportunity to gain further insights regarding the potential benefits of pretreatment, such as dialysis, to improve DON precision by reducing DIN concentrations. In a hypothetical scenario based entirely on calculations, it is assumed that pretreatment removes 90% of the nitrate, which is reasonable for dialysis,[15] and that dialysis does not alter the DON concentration. DON precision can be improved only when reducing concentrations of nitrate (and thus also TDN) also reduces the respective contributions of variance. The precision-concentration relationships developed in this study were used to calculate DON precision before and after hypothetical dialysis treatment (Table S3). Under this scenario, about half of the surface water samples (43 of 92) that were initially below detection would have benefitted from pretreatment to reduce nitrate concentration; DON concentrations for the rest of the samples (49 of 92) would have remained below detection limit. The samples that might benefit, based on this hypothetical pretreatment scenario, occupy a relatively narrow band in the spectrum of DIN/TDN ratios, rising from about 0.6 at low TDN concentrations to more than 0.9 at high TDN concentrations (Figure 2).

The foregoing analysis of surface water samples and exploration of the hypothetical pretreatment scenario illustrate the risk of relying on a single threshold for the DIN/TDN ratio as a criterion for pretreatment. Furthermore, the context provided by sample-specific detection limits shows that pretreatment will not always improve DON precision enough to be worthwhile; if precision cannot be improved to the point that the RSD is less than or equal to one-third of the DON concentration, pretreatment will not be worthwhile. These conclusions reflect the importance of careful characterization of the relationships between precision and concentration for all measured nitrogen fractions.

DON Precision in Soil Leachate Samples

Soil leachate samples, representing four replicates each of 5 treatments (i.e., heating temperature) and a control set (unheated) of soils from six sites (Text S4), were analyzed for TDN and two inorganic nitrogen fractions – nitrate+nitrite and ammonia. DON concentrations were below detection in 24 of 144 samples, primarily those heated to 550 °C. The DON concentrations that were below detection are associated with low TDN concentrations where absolute precision is constant or relatively insensitive to changes in concentration. Consequently, pretreatment to reduce DIN in those samples probably would not be worthwhile because lowering the DIN concentrations would do little to improve DON precision.

Predicting DON Precision

The ability to generalize about opportunities to improve DON precision would be very useful. To some extent, improving analytical precision can help, although the real opportunities lie chiefly with the TDN method.[13] Arguably more useful would be the ability to predict when pretreatment could be beneficial. Generalizing on the basis of published thresholds for the DIN/TDN ratio alone is of limited utility because those thresholds are not currently linked to analytical precision or calculated DON precision. There appears to be a strong connection between a threshold DIN/TDN ratio and the TDN concentration (Figure 2), suggesting that generalizations regarding DON precision must involve both the DIN/TDN ratio and TDN precision.

Establishing a basis for predicting DON precision could help explain why previous studies have reached different conclusions about thresholds for the DIN/TDN ratio. In addition, when DON precision is framed in terms of a detection limit based on the RSD (the DON concentration is below detection if the RSD is greater than 1/3), it becomes possible to assess the need for, and potential benefit of, pretreatment. To simplify matters, the following explanation considers DIN concentration in aggregate (i.e., the sum of nitrite, nitrate, and ammonia), and with the understanding that DIN variance also is aggregated.

DON precision can be cast in terms of TDN concentration and precision after an assumption is made regarding the variance of DIN relative to that of TDN. With commonly used analytical methods, the variance of the DIN fractions, even in aggregate, is typically much less than the variance of TDN measurements. In this study, DIN variance was typically only 1% of the TDN variance for surface water samples. When the contribution from DIN variance is negligible, the threshold for the DIN/TDN ratio, f, can be defined as follows (see Text S5 for derivation):

$$f = 1 - 3 * RSD_{TDN} \qquad \text{(Eq. 1)}$$

Equation 1 provides an upper bound for the threshold ratio because it assumes that DON precision is determined solely by TDN precision. Increasing the relative importance of DIN variance reduces the threshold ratio (Text S5). For example, in the soil leachate samples, the DIN variance was typically about 20% of the TDN variance. If the DIN variance is as much as 50% of the TDN variance, the equation would be as follows:

$$f = 1 - 3.67 * RSD_{TDN} \qquad \text{(Eq. 2)}$$

The central role for the RSD_{TDN}, which links TDN precision and concentration, can be highlighted by comparing the two analytical methods used in this study. For both of the TDN methods, the threshold DIN/TDN ratio is sensitive to TDN concentration, and it is clear that thresholds for the DIN/TDN ratio can be almost any number between zero and one (Figure 3). Thus, the two previously published thresholds (0.6 and 0.85) do not represent the universe of possibilities largely because they fail to incorporate analytical precision.

Two points made in this study contribute to a better understanding of DON precision. The first point is that the relationship between precision and concentration generally is more complicated than can be captured with a constant standard deviation or a constant RSD; a two-component variance model provides a more realistic foundation. The second point is that the relative precision of TDN measurements, which varies across the concentration spectrum, is the primary determinant of the threshold ratio. High relative precision for TDN, which is more likely at high concentration, makes it easier to resolve DON when the DIN/TDN ratio is high, irrespective of concentration.

It should now be clear that no single threshold for the DIN/TDN ratio can be applied universally. The threshold(s) applicable in a particular study or for a particular set of samples will depend largely on the precision of the methods used to analyze those samples. If a single threshold is chosen, it should be justified on the basis of the methods used and samples analyzed in a particular study.

Environmental Implications

This study yields two unexpected observations related to the precision of DON concentrations, both of which are relevant to studies in environmental sciences. The first observation is the large number of surface water samples in which the DON was below detection despite having used high-precision analytical methods. It is difficult to know how common this problem might be in other studies because detection limits are rarely applied to DON concentrations reported in the literature. However, the numerous reports of negative values suggest that concentrations below detection are common. We recommend general use of a simple detection limit (RSD_{DON} equal to 1/3) for DON concentration and encourage broader use of statistical techniques appropriate when censored data are present.[23-24]

The second observation concerns expectations that pretreatment will necessarily yield better DON results. Many studies in the environmental sciences would benefit from better resolution for DON concentrations, and dialysis pretreatment has been shown to be helpful. However, pretreatment is not a panacea, because it does not always improve

DON precision enough to be worthwhile. As shown above in the hypothetical scenario involving our surface water samples, about half of the candidate samples (i.e., those with DON concentration below detection without pretreatment) would have yielded a meaningful DON concentration (i.e., above detection) following pretreatment. Prediction of the success rate on the basis of a thorough characterization of precision-concentration relationships for the relevant analytical methods creates the basis for an informed decision about allocating resources to pretreatment measures intended to improve DON precision.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at __________. Additional method details, statistical analysis, and example calculations with figures and tables (PDF).

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FIGURES

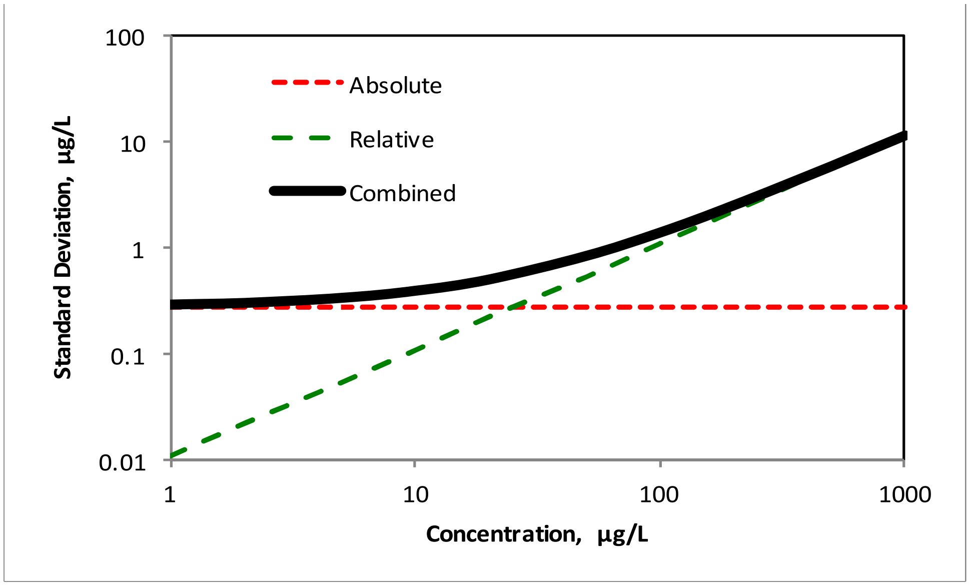


Figure 1. Characterization of analytical precision for a range of nitrate-nitrogen concentrations based on the two-component variance model explained in the text. The model joins a component of constant absolute precision (Absolute) and a component of constant relative precision (Relative) to yield a concentration-specific combined standard deviation (Combined).

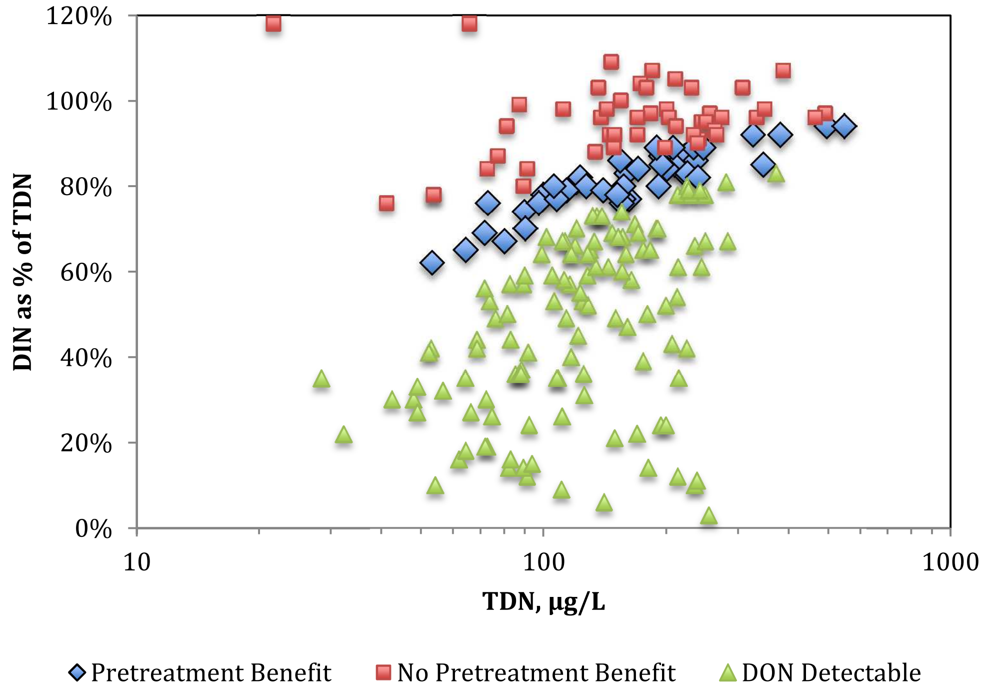


Figure 2. Surface water samples classified according to potential benefit from pretreatment. DON was detectable without pretreatment in 118 samples and below detection in the other 92. Pretreatment under the hypothetical scenario described in the text could have provided benefit in 43 samples, and the DON concentration would have remained below detection in the other 49 samples.

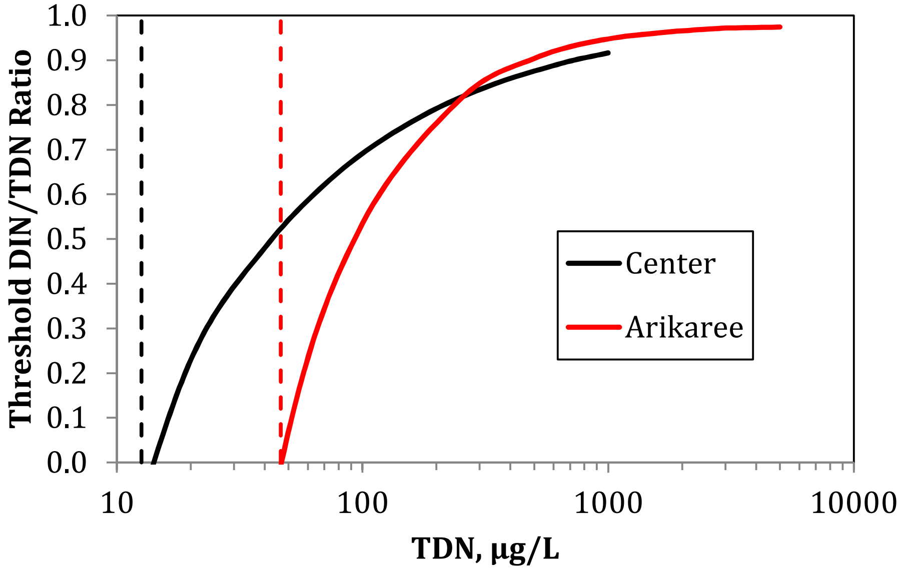
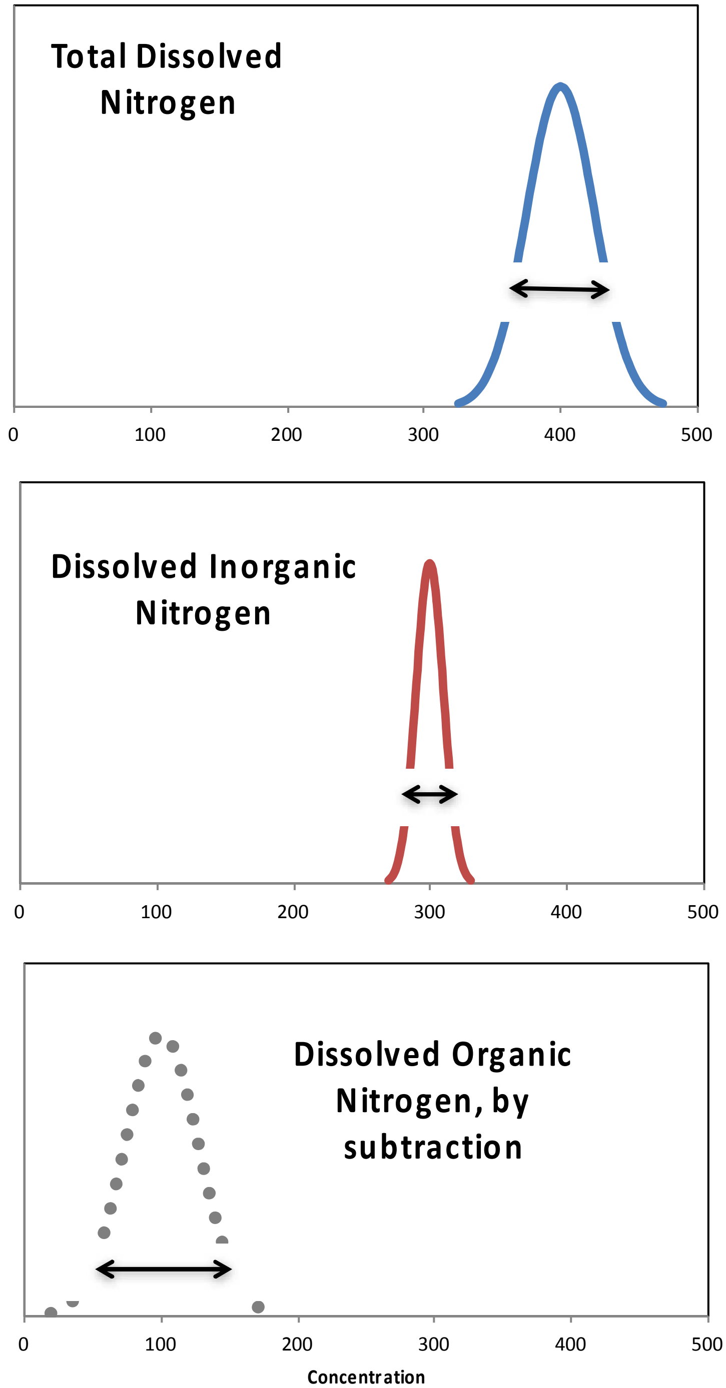
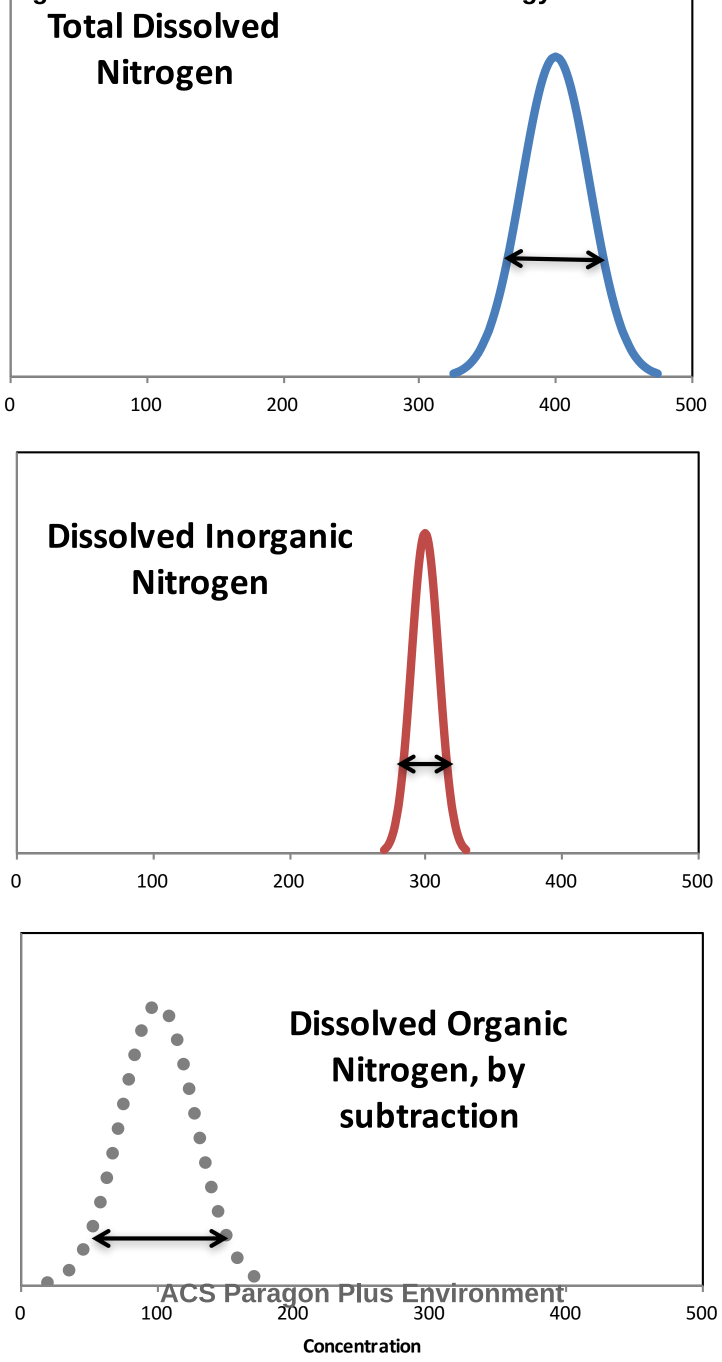


Figure 3. Threshold values for the DIN/TDN ratio based on analytical results obtained by the Center for Limnology Lab (persulfate oxidation) and the Arikaree Lab (high-temperature catalytic oxidation). Thresholds are calculated using *Equation 1*, which assumes that variance for the inorganic fraction is negligible compared to that of TDN. The dashed lines show detection limits for each method.


Total Dissolved Nitrogen
0
100
200
300
400
500
Dissolved Inorganic Nitrogen
0
100
200
300
400
500
Dissolved Organic Nitrogen, by subtraction
0
100
200
300
400
500
Concentration


Total Dissolved Nitrogen
0
100
200
300
400
500
Dissolved Inorganic Nitrogen
0
100
200
300
400
500
Dissolved Organic Nitrogen, by subtraction
0
100
200
300
400
500
Concentration