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USDA LTAR Common Experiment measurement: Total nitrogen (TN) and total dissolved nitrogen (TDN) concentration

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Abstract

Total dissolved nitrogen (TDN) is composed of dissolved inorganic nitrogen (DIN) and dissolved organic nitrogen (DON). DON is the N-containing component of dissolved organic matter (DOM) and part of the biologically reactive N pool in aquatic ecosystems that can degrade water quality in N-sensitive waters. Evidence suggests that DON may be important in triggering harmful algal blooms, particularly the DON from synthetic urea fertilizers. TDN measurements therefore contribute to an estimate of the N most available to phytoplankton. DIN export from agroecosystems is reasonably well characterized, but the factors that regulate spatial and temporal patterns of DON are not as well understood. Although the export of DON into groundwater has received recognition for more than 100 years and the contribution of DON to total nitrogen (TN) is often significant, leaching losses of N from agricultural systems are often assumed to be dominated by DIN and uninfluenced by DON. The most widely recognized N digestion method is total Kjeldahl N (TKN), whereby organic N is converted to ammonia-N, followed by titrimetric or colorimetric detection. The recommended measurement of TDN at moderate to high concentrations (i.e., >0.5 mg N/L) uses the high-temperature combustion and chemilumniscent detection method.

Before start

Appropriate safety, health, and environmental precautions must be followed based on the selected methods, instrumentation, and workflow. Laboratory supervisors are responsible for knowledge of these precautions and their implementation.

Review the *USDA LTAR Common Experiment measurement: Best practices for collection, handling, and analyses of water quality measurements* protocol (Pisani et al., 2024) prior to implementing this protocol.

Sample collection and filtration

1 Return samples to the laboratory On ice .

2 Measure total N (TN) on unfiltered samples.

3 Measure TDN by filtering samples through a 0.22 or 0.45 µm pore-size filter to minimize interference from particulates before chemical analysis.

> **Note**
>
> - Use a 0.22 µm filter if the more complete removal of the microbial community is desired.
> - Filters can be membrane or glass fiber.
> - Occasional checks of filter blanks by filtering deionized water with the same equipment are always prudent.

Sample storage and preservation

4 For TDN measurements, filter the water samples as soon as possible after sample collection.

5 For TN measurements following TKN digestion, acidify the samples with sulfuric acid to pH < 2 and store them at 4 °C for up to 30 days.

> **Note**
>
> Labs performing alkaline persulfate digestion should not use acid preservation.

6 If necessary, freezing samples at -20 °C is an alternative to acid preservation if storing for more than 30 days (USEPA CBP/TRS 6/87).

Archiving

7 Samples for TDN or TN analyses are stored until data certification (QA/QC verification).

Sample analysis

8 The most widely recognized N digestion method is total Kjeldahl nitrogen (TKN), which involves the conversion of organic nitrogen into ammonia nitrogen using sulfuric acid, potassium sulfate, and a catalyst followed by titrimetric or colorimetric detection.

9 Macro- or micro-TKN methods (EPA 351.2, 0.1-20 mg/L TKN; APHA, 2005) enable this conversion.

10 Obtaining a representative subsample for TKN or TN methods is crucial when particulate matter is present.

11 Stir samples on a stir plate, and once the slurry is homogeneous, withdraw a subsample while stirring using a large bore pipet tip at a consistent depth.

12 In contrast to the Kjeldahl method, Total N oxidation methods convert all N-containing compounds into NO_3^- by oxidation using persulfate digestion followed by colorimetric detection (Water Resources Investigations Report 03-4174; APHA, 2005).

13 To measure TDN, the high-temperature combustion method with chemiluminescence detection (CLD) has gained popularity because it is typically coupled with the simultaneous analysis of dissolved organic carbon (DOC), allowing high sample throughput with reduced hazardous waste generation.

Covariate metrics to be sampled concurrently

14 Additional primary metrics are described in other USDA LTAR Common Experiment protocols:

- Dissolved NO_3^- concentration
- Dissolved NH_3 concentration
- Total dissolved P (TDP) and Total P (TP)
- Total suspended solids (TSS)

Calculations

15 Prepare a calibration curve by plotting the area of each standard peak against its respective N concentration. Correction of standards and samples for the blank is necessary; the reagents are usually the source of most of the blank absorbance, although sometimes the water can be high in ammonia. Only the reagent contribution to the blank should be subtracted from sample absorbances, and it can be estimated by running a 2x reagent blank.

16 Compute the TDN or TN concentration in the sample by comparing the sample peak area to the calibration curve.

17 Report the concentration as mg N per liter (mg/L).

TDN = DIN (NO_3^- + NO_2^- + NH_3) + DON (mg N/L)

TN = total inorganic N (NO_3^- + NO_2^- + NH_3) + total organic N (mg N/L)

18 The analysis of TN following the TKN digestion method results in the measurement of NH_3 plus organic N:

TKN = NH_3 + organic N (mg N/L)

TN = TKN + (NO_3^- + NO_2^-) (mg N/L)

Recommendations for data collection

19 Table 1. Summary of recommendations for the collection and measurement of TKN/TN and TDN concentration.

	A	B	C	D
	Attribute	**Preferred**	**Minimum**	**Comments**
	Spatial scale	Field	Plot	
	Frequency	Event-driven	Event-driven	More frequent (weekly) measurements can be preferential when the flow regime can increase seasonally or after precipitation events. Sampling should be event-driven in this protocol to enable cross-site comparisons
	Covariate metrics	NO3-N, NH3-N, TDP, TSS	NO3-N, NH3-N, TDP	

	A	B	C	D
	Sample preservation and storage TDN	Filter with a 0.45 um pore-size filter and freeze as soon as possible		Omit acid preservation for TDN
	Sample preservation and storage TKN/TN	Acidify to pH <2 with sulfuric acid	Freeze as soon as possible	Avoid acid preservation with the TN method
	Sample analysis TDN	High-temperature combustion followed by CLD	Alkaline persulfate digestion	
	Sample analysis TKN/TN	TKN digestion followed by colorimetric analysis	Acid persulfate digestion followed by colorimetric analysis	Obtaining a representative sample is crucial
	Water quantity	Discharge or flow rate	Discharge or flow rate	Calculate TN or TDN loads by linking this metric to the water quantity metric “flow"

Covariate metrics = other metrics to sample concurrently. NO_3^--N = nitrate-N; NH_3-N = ammonia-N; TDP = total dissolved phosphorus; TSS = total suspended solids.

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