

Video Article

Automated, High-resolution Mobile Collection System for the Nitrogen Isotopic Analysis of NO_x

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Abstract

Nitrogen oxides (NO_x = NO + NO₂) are a family of atmospheric trace gases that have great impact on the environment. NO_x concentrations directly influence the oxidizing capacity of the atmosphere through interactions with ozone and hydroxyl radicals. The main sink of NO_x is the formation and deposition of nitric acid, a component of acid rain and a bioavailable nutrient. NO_x is emitted from a mixture of natural and anthropogenic sources, which vary in space and time. The collocation of multiple sources and the short lifetime of NO_x make it challenging to quantitatively constrain the influence of different emission sources and their impacts on the environment. Nitrogen isotopes of NO_x have been suggested to vary amongst different sources, representing a potentially powerful tool to understand the sources and transport of NO_x. However, previous methods of collecting atmospheric NO_x integrate over long (week to month) time spans and are not validated for the efficient collection of NO_x in relevant, diverse field conditions. We report on a new, highly efficient field-based system that collects atmospheric NO_x for isotope analysis at a time resolution between 30 min and 2 hr. This method collects gaseous NO_x in solution as nitrate with 100% efficiency under a variety of conditions. Protocols are presented for collecting air in urban settings under both stationary and mobile conditions. We detail the advantages and limitations of the method and demonstrate its application in the field. Data from several deployments are shown to 1) evaluate field-based collection efficiency by comparisons with *in situ* NO_x concentration measurements, 2) test the stability of stored solutions before processing, 3) quantify *in situ* reproducibility in a variety of urban settings, and 4) demonstrate the range of N isotopes of NO_x detected in ambient urban air and on heavily traveled roadways.

Video Link

The video component of this article can be found at <https://www.jove.com/video/54962/>

Introduction

Atmospheric nitrogen oxides (NO_x = NO + NO₂) are important species in the global reactive nitrogen cycle^{1,2}. NO_x in the atmosphere is highly reactive and directly contributes to the oxidizing capacity of the atmosphere through its interactions with ozone (O₃) and hydroxyl radical (OH). NO_x is removed from the atmosphere on the scale of hours to days in the lower troposphere via oxidation to nitric acid (HNO₃) or nitrate (NO₃⁻), both of which are highly soluble and can be dry deposited on surfaces in gaseous and particulate aerosol forms or wet deposited by precipitation (e.g., acid rain)². NO_x is emitted from a variety of sources, including fossil fuel combustion, biomass burning, microbial processes in soils, and lightning. Source apportionment is crucial for understanding the impacts of individual sources, but the variety of sources, their variability in space and time, and the relatively short lifetimes of NO_x and HNO₃ make concentration analyses alone an inadequate metric. Stable isotopes may be useful as a way to better track the spatial patterns and temporal trends of sources and the chemistry of NO_x and NO₃⁻ in the environment and to add new constraints on atmospheric models³. To date, the isotopic signatures associated with different NO_x sources remain highly uncertain, particularly because of large uncertainties associated with previous methods⁴.

Previous studies represent a number of different active and passive collection methods and yield large ranges in reported isotopic values, even for the same emission source. Fibiger *et al.* found that previously used methods often varied greatly in terms of their efficiency in capturing NO_x, with changes in conditions greatly influencing field collection (e.g., temperature, humidity, flow rates, age of solution)⁴. The inefficient uptake of previous NO and NO₂ capture methods could lead to fractionations. For example, higher rates of oxidation for ¹⁴N relative to ¹⁵N could yield low biases in δ¹⁵N-NO_x that are not representative of atmospheric values. In addition to methodological issues^{4,17}, a variety of different types of air sampling may also contribute to differences in the reported ranges for isotope values associated with the same source. For example, isotopic signatures associated with vehicle emissions of NO_x have been suggested based on collections at near-road sites⁵, in traffic tunnels⁶, and directly from the tailpipes of vehicles^{7,8}. Furthermore, previous methods have time resolutions of 24 h at best, and significant changes in ambient NO_x concentrations are observed on hourly (or shorter) timescales⁹, potentially limiting the application of isotopic detection for different sources. Many of the NO_x collection methods require very strong oxidizing solutions capable of oxidizing NO_x, but also other collected reactive nitrogen species (e.g., ammonium), to nitrate over time, potentially contributing an isotopic measurement interference. Some previous methods

are also limited to collecting NO_2 in solution, which provides only a limited understanding of NO_x isotopes, as it does not collect NO (the primary emission). Thus, there is a need to capture NO_x from different emissions sources using a consistent, validated method to better constrain whether the variability in isotopes of NO_x (and NO_3^-) in the environment can be used to directly track sources and chemistry.

This paper reports on a field-based NO_x collection technique for isotopic analysis with the requisite time resolution, collection efficiency (100%), and reproducibility ($\leq 1.5\%$) for application in multiple field environments. The method, originally described by Fibiger *et al.*⁴, is further validated through the demonstration of its collection efficiency under changing NO_x and meteorological conditions in the field, the test of solution stability and ammonia interferences, and the substantiation of its reproducibility in urban environments. Spatial and temporal differences in isotopic values are investigated using a single laboratory- and field-verified method that can capture NO_x in solution at high efficiency. This paper demonstrates the application of the method for near-road, on-road, and ambient urban air collections at time resolutions of 30 to 120 min.

In brief, NO_x (NO and NO_2) is collected from the atmosphere in a highly oxidizing solution as NO_3^- . At the same time, ambient NO_x , NO_2 , and CO_2 concentrations and other relevant data, such as GPS location and time of collection, are recorded. After a sample is collected, the solution is processed in the laboratory, which involves reducing the solution to stop the reaction, then neutralizing the solution pH for subsequent NO_3^- concentration and isotopic analyses. The NO_3^- concentration is determined here by an automated spectrophotometric (*i.e.*, colorimetric) process. The nitrogen isotopic composition is determined using the denitrifier method, which quantitatively converts the NO_3^- in solution to gaseous N_2O that is subsequently measured on an isotope ratio mass spectrometer. Laboratory and field blanks are also collected and measured as part of the collections to ensure the sample integrity. Below is a detailed step-by-step protocol.

Protocol

1. Solution Preparation

- Before sampling, prepare the solutions, calibrate the NO_x analyzer (either luminol or chemiluminescence), and check that the system is working properly and that new filters are installed.
- Make sampling solutions using 1 M potassium permanganate (KMnO_4) stock solution and 10 M sodium hydroxide (NaOH)¹⁰, and then dilute the solution with ultrapure water to the correct volume.
NOTE: Purchase premade solutions because they tend to contain lower "blank" NO_3^- concentrations than other forms.⁴
 - Prepare 10 M NaOH .
 - Weigh out 200 g of solid NaOH and pour it into a 500 ml volumetric flask. Add ultrapure water ($18.2 \text{ M}\Omega\cdot\text{cm}$ at 25°C) to the meniscus line of the volumetric flask and allow the NaOH to dissolve.
 - Because this process exudes heat, place the volumetric flask in a room-temperature ($\sim 22^\circ\text{C}$) water bath and allow it to cool as it dissolves, usually taking 1-2 hr. Store 10 M NaOH in 500 ml amber plastic bottles for up to 1 month.
 - Prepare a sampling solution of 0.25 M KMnO_4 and 0.5 M NaOH in a 500 ml graduated cylinder (450 ml solution volume).
 - Add 112.5 ml of 1 M KMnO_4 and then fill with ultrapure water up to 405 ml.
 - Add 22.5 ml of the 10 M NaOH solution prepared in step 1.2.1 to the graduated cylinder and fill to the 450 ml line with ultrapure water.
 - Store the solutions in 500 ml amber glass bottles and label each solution with the date (use letters to distinguish each bottle).
 - Once the solution is made, take a laboratory blank. Remove 25 ml of the solution and record from which solution bottle it came. Store blanks in 60 ml amber glass bottles.
NOTE: Each solution bottle should yield 8-11 samples (35-50 ml each) and one field blank (25 ml) after the laboratory blank is taken.

2. Field Setup

- Choose a sampling location (such as a rooftop) and install the system (if using the stationary system). For the mobile laboratory, pack all instrumentation into a typical passenger vehicle. See **Figure 1** for a diagram of the automated system.
- Change all filters labeled in **Figure 1** before sampling to ensure that they are working most effectively and efficiently.
NOTE: There are three types of filters in the system: a PTFE particle filter ($1.0 \mu\text{m}$; 47 or 25 mm use the larger surface area in more polluted air) to remove particles that may contain NO_3^- , a nylon membrane filter ($1.0 \mu\text{m}$) for removal of HNO_3 gas, and a hydrophobic filter ($10.0 \mu\text{m}$) to protect the vacuum pump and the critical orifice from solution droplets. With new filters at the start of a sampling period, the particulate filter and the NO_3^- filter will not need to be changed for a couple of days, except in highly polluted or dusty conditions. The hydrophobic filter should be changed every 4-6 hr for as long as sampling is continuously done.
- For installation of the system, connect the system and the instruments to polytetrafluoroethylene (PTFE) tubing ($1/4$ -inch outer diameter) and aim the inlet, also PTFE tubing, in the direction of the desired air collection.
NOTE: The mobile laboratory is for taking on-road measurements, whereas the stationary laboratory is for taking ambient urban air and near-road measurements.
- Set up the 'mobile laboratory', consisting of the NO_x collection system, an NO_x box, a CO_2 analyzer, a global positioning system (GPS) unit, and a marine battery.
 - Pack the system and all instrumentation into the car. Power the system with a 12 V marine deep-cycle battery for ~ 12 hr, similar to the maximum duration of one day of mobile measurements. Recharge the battery at the end of the sampling day to prepare for the next day.
NOTE: Use a separate battery so that there is no need to hardwire to the car battery nor to keep the car running to make measurements. Use two batteries if sampling will be close to or more than 12 hr in order to avoid stopping for a few hours to recharge the battery.

2. Connect the instruments to the PTFE inlet tube closer to the inlet than to the collection system, because the vacuum pump for the system operates at flow rates of 3-5 L/min, much larger than the flow rates for the NO_x box (~1.5 L/min) or the CO₂/H₂O analyzer (<1 L/min).
3. Secure the PTFE inlet tube to the front of the car on the roof, pointing towards the front of the car, a position that is the longest possible distance from the exhaust pipe, to avoid capturing self-emissions from the car exhaust pipe. For example, in the mobile laboratory using a midsized sport utility vehicle, the inlet was located on the roof of the car, 2 feet from the driver's side door, placing it 1.6 meters above the road and 2.54 meters from the back bumper.
 1. Alternatively, use an electric or other zero emission vehicle.
4. Record geolocation and vehicle speed data every second using a GPS unit (if this data is of interest). Synchronize the laptop computer recording the NO_x and CO₂ data with the GPS time prior to measurements.
5. Turn on the instrumentation at the beginning of the sampling day and turn them off at the end of the sampling day, even when the collection system is not running (the instruments require warm-up time, so leave it running throughout the day to avoid multiple warm-up times).
6. Load the NO_x box with luminol solution when it is turned on at the beginning of the sampling day, and then flush it with water at the end of the day, before the instrument is turned off, as directed by the manufacturer. Store the luminol and solution samples in a cooler in the mobile laboratory to avoid degradation of the solutions. Store the luminol solution in a refrigerated unit overnight.
7. Calibrate the NO_x box-a luminol-based NO₂/NO analyzer¹¹ and a differential, non-dispersive infrared (NDIR) CO₂/H₂O analyzer using a commercial gas dilution calibrator and following the manufacturer's instructions. The NO_x box has a response time of ~5 sec, which is better equipped to resolve on-road NO_x emission plumes.
5. Set up the 'stationary laboratory', consisting of the NO_x collection system and a chemiluminescence NO_x concentration analyzer.
 1. Fix PTFE tubing to a surface and point it in the direction of the air to be collected.
 2. Split the PTFE tubing at the inlet with a tee fitting to connect both the NO_x analyzer and the automated collection system.
 3. Connect the stationary system to a power outlet (120 V alternating current).
 4. Run the NO_x concentration analyzer continuously throughout the sampling period, even when the collection system is off or switching samples. Use the valve built into the collection system to isolate it during that time so that the NO_x analyzer is sampling ambient air.
 5. Calibrate the chemiluminescence NO_x concentration analyzer. This is used for the stationary measurements, as it has a slower response time (>30 sec), which is better for ambient air measurements.
 1. Calibrate based on the manufacturer's instructions using a gas dilution calibrator. Dilute a standard of 25 ppmv NO in N₂ with zero air to achieve approximately seven calibration points between 0-200 ppbv NO. Using an ozone titrator, calibrate the NO₂ concentrations across the same range (0-200 ppbv NO₂).
 6. If using the mobile laboratory, calibrate the NO_x box-a luminol-based NO₂/NO analyzer¹¹ and a differential, non-dispersive infrared (NDIR) CO₂/H₂O analyzer using a commercial gas dilution calibrator and following the manufacturer's instructions. The NO_x box has a response time of ~5 sec, which is better equipped to resolve on-road NO_x emission plumes.

3. Sample Collection

1. Perform tests on the system to ensure that the flow meter, the syringe pump, the computer software, and the vacuum pump are all working. Turn on each component and check that it is working properly. With the computer software, complete the sampling protocol once or twice to make sure that everything is working properly.
2. Turn on the system so that air is bubbling through the solution and bubbles are visible.

NOTE: The computer program automates the movement of the solutions throughout the system, but the diaphragm pump and the vacuum pump are operated manually. The user must select the amount of time for the collection of a sample (between 30 and 120 min) that collects enough NO_x for the concentration of the sample to be above the blank nitrate concentration of the solution. Atmospheric NO_x concentrations of 50-100 ppbv near sources such as vehicles require only a 30 min collection time. For ambient urban NO_x concentrations (5-30 ppbv), samples should be collected for up to 120 min. Using the equations provided in steps 6.5 and 6.5.1, the user can back-calculate the collection time to achieve the desired sample concentration in solution.

 1. Use a collection system with a syringe pump to automatically move the solution from the reservoirs into the gas-washing bottle and from the gas-washing bottle to the waste. The four electronically actuated valves and the syringe pump are controlled by a computer program specifically written for the collection system, which has four modes: 1) dispense new solution, 2) clean the tubing, 3) collect the sample, and 4) clean the gas-washing bottle, as follows:
 2. To automatically dispense new solution, aspirate 35 ml of solution (V_S) into the syringe pump from the solution reservoir and dispense it into the gas-washing bottle. The gas-washing bottle frit causes the solution to bubble when the vacuum pump is turned on and the gas sample is introduced.

NOTE: Choose a solution volume between 25-35 ml based on the NO_x concentrations sampled and the collection times desired.
 3. Clean the tubing between the syringe pump and the gas-washing bottle by automatically pulling the residual solution in the tube back into the syringe and depositing it into the waste container.
 4. Once the sampling solution is in the gas-washing bottle, manually turn on the pump. When the desired amount of time for sampling has been achieved, manually turn off the pump.
 5. After sampling is done, collect the solution by opening the automated valve under the gas-washing bottle to drain the solution into a collection vial and cap via gravity. When the sample is done collecting NO_x, collect the solution in a 60 ml amber glass bottle and manually remove the bottle. The program waits ~2 min for the solution to fully drain and then automatically moves on to the next step.
 6. Once the sample is done draining, automatically close the valve and clean the gas-washing bottle by aspirating ultrapure water into the syringe pump and dispensing it into the gas-washing bottle via a spray nozzle to clean the sides of the gas-washing bottle. Extract this wastewater from the gas-washing bottle by aspirating it into the syringe pump and discard it into the waste reservoir. Store the frit with 25 ml of nanopure water.

7. Repeat steps 3.2.2 to 3.2.6 to dispense the next solution sample.
3. Take field blanks during the collection for each solution bottle (labeled with the letters A-Z before the beginning of collection) that is used by sending 25 ml of solution through the system without turning on the vacuum pump to collect air. Collect the solution immediately after it is put into the system.
4. Record the volumetric flow rate every 5 min using a flow meter during each collection, along with the air temperature (T) and pressure (P) on the flow meter, to derive the standard flow rate. Flow rates of 3-5 L/min are achieved with a diaphragm pump (30 L/min capacity) and a critical orifice to reduce the flow rate.
 1. Set the flow rate at the beginning of sampling to measure the flow approximately every 1 sec. After 5-10 sec, change the flow measurement frequency to 5 min.
 2. Collect the flow-rate data every 5 min for the duration of the sampling period.
NOTE: If the flow rate drops significantly, the sample should be collected for longer than initially expected. Small changes (<25%) in the initial flow rate are to be expected. The hydrophobic filter should be checked to see if it is coated to the point that it is clogged.
 3. Before the sampling is stopped, change back to 1 sec flow-rate measurements and collect flow data for 5-10 sec before turning off the sample.
NOTE: The solutions may be stored for up to (maximum) seven days before step 4 must be performed.

4. Sample Reduction

NOTE: Reduce the samples to remove the KMnO_4 within 7 days of collection. The original method⁴ suggests that this must be done within 24 hr of sample collection. Below are results that suggest that the samples can be stored for up to seven days prior to reduction.

1. The permanganate in solution is a strong oxidizer. Reduce the sample to stop the oxidation reaction of permanganate with ambient NO_x or with other N species that could potentially lead to interference if they are oxidized to nitrate⁴.
2. Label two 400 ml beakers, one for blank solutions and one for samples. Acquire two stir rods, one for each beaker. Also, acquire a 500 to 5,000 μl pipette and pipette tips.
3. Weigh each sample bottle while it contains the solution and record the mass of the full glass bottle. After the solution has been poured into the beaker, weigh the empty glass bottle.
4. Pour the solution from one sample into the sample beaker and one blank solution into the blank beaker.
5. For the sample beaker, slowly introduce 10 ml of hydrogen peroxide (H_2O_2) in 5 ml batches to the sample, stirring vigorously while adding each 5 ml of H_2O_2 . This volume is for sample solutions of 35 ml. For each 25 ml of sampling solution (either blank or sample), add 5 ml of H_2O_2 . Adding the full 5 ml for less than 25 ml of sample solution is recommended to ensure full conversion, and adding more H_2O_2 will only result in the dilution of the solution.
 1. Introduce the first 5 ml of H_2O_2 above the beaker, so that the tip does not touch the beaker, the stir rod, or the solution.
 2. Add the second batch to the side of the beaker and around the side, as more is added, in order to wipe down the sides of the beaker, ensuring that all of the sample solution is reduced. If more than 5 ml of H_2O_2 is added, perform this step on the last 5 ml introduction of H_2O_2 and follow step 4.5.1 for the intermediary introductions of H_2O_2 .
 3. Change the pipette tip after each sample is changed to avoid any cross-contamination.
6. For the blank solution beaker, add only 5 ml of H_2O_2 . Add roughly half of the above the solution and add the other half around the sides of the beaker. Change the tip after each blank.
7. Check to ensure that the solution above the precipitate is clear or pale yellow. If purple or blue colors remain, add more H_2O_2 to verify that it is fully reduced.
8. Pour the entire contents of the beaker, both the liquid and the brown precipitate that forms into 50-ml centrifuge tubes that have been labeled according to the sample or blank number or letter.
9. Once all solutions are reduced (the H_2O_2 is added), load the centrifuge in batches of 20, ensuring that the centrifuge is balanced. A benchtop centrifuge can typically accommodate 20 centrifuge tubes at a time.
 1. Operate the centrifuge at 3,220 x g for 15 min with each batch of centrifuge tubes.
10. While the centrifuge is running, weigh the empty glass bottles and record their masses. Additionally, label 60 ml amber plastic bottles (previously cleaned by leaching in ultrapure water) and weigh the empty plastic bottles. Record their masses as well.
11. Once the centrifugation has finished, pour the supernatant liquid into the amber plastic bottle (with the solid remaining in the tube) and properly dispose of the centrifuge tube.
12. Weigh the sample bottles, now full, and record their masses.

5. Sample Neutralization

NOTE: Perform the neutralization of samples and blanks (reproduced here from Fibiger *et al.*⁴ with updates). Note that this step is required for the colorimetric quantification of the nitrate concentration in the solution; this may not be necessary with other concentration techniques.

1. Perform neutralization manually or with an automatic titrator.
2. For manual neutralization, use 12.1 M hydrochloric acid (HCl) and introduce it into the amber plastic solution bottle with a pipette. Take extreme care (eyewear, lab coat, fume hood, etc.) when handling HCl, particularly at a 12.1 M concentration.
 1. Calculate the volume of 12.1 M HCl that is to be added to the sample solution to neutralize it using the following equation:

$$V_{\text{HCl}} = \frac{0.5 \text{ M} \times (\text{Mass}_{\text{full glass bottle}} - \text{Mass}_{\text{empty glass bottle}})}{12.1 \text{ M}}$$

where V_{HCl} is the volume of HCl added, $\text{mass}_{\text{full glass bottle}}$ is the mass of the glass bottle the solution was collected in with the solution, and $\text{mass}_{\text{empty glass bottle}}$ is the mass of the glass bottle the solution was collected in without the solution. Assume that the densities of the solutions to be 1.00 g/cm^3 , as they are dilute solutions.

NOTE: Add 85% of this volume first in 0.20 ml increments. Depending on the air that is being sampled, other species that are collected can lower the original solution pH and change how it responds to the addition of acid.

2. Add the 85% volume of HCl to the bottle using a pipette and a disposable tip in 0.2 ml increments. Cap and shake the bottle between each 0.2 ml addition to ensure the acid has mixed with the solution. Check the pH using litmus paper by removing 20 μl of solution and pipetting it onto the litmus paper.
 3. If the pH is between 4 and 10, label the sample as neutralized and record the pH. Repeat steps 4.2.1 and 4.2.2 for all other samples being processed. While the colorimetric concentration analysis can proceed with samples that have a pH as low as 4 or as high as 10, get as close to 7 as possible in order to yield the best results.
 4. If the pH is still above 10 after the 85% volume addition, add HCl in smaller increments than 0.2 ml of HCl (0.10 or 0.05 ml), shake the bottle to homogenize, and check the pH with litmus paper and 20 μl of solution after each addition of HCl.
 5. Once the pH is within the desired range, label the sample as neutralized in the same way as before and set it aside.
 6. If the pH is below 4, use 10 M NaOH to bring the pH up to the correct range. Add increasingly small amounts of NaOH and check the pH after each addition using litmus paper and the same method as before.
 7. Record the amount of HCl (V_{HCl}) and NaOH (if needed) added to each sample bottle, along with the final pH.
3. For the automatic titrator method, use an automatic titrator.
 1. Dilute 12.1 M HCl to 4 M with ultrapure water and introduce it to the titrator (0-25 ml of hydrochloric acid are possible) according to the instructions of the instrument. 4 M allows the titrator to be precise enough without adding large volumes to the samples.
 2. Set the automatic titrator to titrate to a pH of 7.
 3. Record the final pH and the volume of HCl added. Label the sample as neutralized and record the final pH.
 4. Use the same beaker for each titration. In between each sample, wash the beaker, pH probe, and stirrer with ultrapure water at least 3 times and dry.

6. Sample Measurement

1. Measure the concentration of each of the samples (C_B or C_S) using a spectrophotometric nutrient analyzer that utilizes colorimetric chemistry to make concentration measurements.
2. Prepare the samples and put them into the instrument according to the manufacturer's instructions.
3. Generate a standard calibration curve from 0-15 μM nitrate (7 calibration points) from a 30 μM stock KNO_3 solution.
4. Prepare and run quality controls of 8 and 10 μM nitrate along with the samples. Calculate the pooled standard deviation of QCs across multiple runs to estimate the concentration uncertainty. Typical pooled standard deviations of $\pm 0.4 \mu\text{M}$ are observed (across 7 runs, $N = 27$ data points).

1. Convert the NO_x concentration determined by the colorimetric concentration analyzer from μM to ppbv using the following mixing ratio equation:

$$MR_{\text{NO}_x} = \frac{n_{\text{NO}_x} RT}{PV_{\text{air}}}$$

where MR_{NO_x} is the mixing ratio of NO_x (the value reported in ppbv), n_{NO_x} is the number of nmol of NO_x collected, R is the ideal gas constant in $\frac{\text{L} \cdot \text{atm}}{\text{mol} \cdot \text{K}}$, T is the temperature (in Kelvin), P is atmospheric pressure (in atm), and V is the volume of air (in L) collected. The total volume of gas sampled is determined by numerical integration of the flow rate time series (equivalent to the area under the flow rate curve as a function of time).

2. Calculate the number of moles of NO_x using the following set of equations:

$$\mu\text{mol NO}_3(\text{total}) = C_S V_S \frac{1\text{L}}{1000\text{ mL}} \frac{V_{\text{aliquot}} + V_{\text{HCl}}}{V_{\text{aliquot}}}$$

for the sample,

$$\mu\text{mol NO}_3(\text{blank}) = C_B V_B \frac{1\text{L}}{1000\text{ mL}} \frac{V_B}{25\text{ mL}} \frac{V_{\text{aliquot}} + V_{\text{HCl}}}{V_{\text{aliquot}}}$$

for the blank, and

$$\mu\text{mol NO}_x \text{ collected} = \mu\text{mol NO}_3(\text{total}) - \mu\text{mol NO}_3(\text{blank})$$

where C_S is the concentration of the sample measured by the colorimetric concentration analyzer, in μmol ; V_S is the volume of the sample, in ml; C_B is the concentration of the blank, in μmol ; V_B is the volume of the blank, in ml; V_{aliquot} is the volume of what was neutralized, in ml (typically the entire volume of the solution); and V_{HCl} is the volume of HCl added to neutralize the aliquot, in ml.

7. Nitrogen Isotope Ratio Preparation

NOTE: Quantify the nitrogen isotopic composition based upon the denitrifier method. Details of this method are published elsewhere in their entirety, and users should consult these publications for full method instructions^{12,13}. The method utilizes denitrifying bacteria to convert liquid NO_3^- samples into gaseous nitrous oxide (N_2O) for isotopic determination. Users who do not have the denitrifier method readily set up may have samples analyzed for isotopic composition by external facilities. Users should consult these facilities to ensure that the appropriate data corrections are consistent with those in step 8.

- Based upon the concentration determined for each sample and blank, inject the appropriate volumes into pre-prepared, capped vials with bacteria^{12,13}. Target a specific size for injection by dividing the target size, such as 20 nmol N, by the concentration ($\mu\text{mol/L}$) of the sample or blank to determine the number of ml to inject into each vial via a syringe.
 - Inject nitrate reference materials (e.g., IAEA-NO-3 and USGS34) at least in triplicate with each set of samples to be run for isotopes. These reference materials are used to correct the final data to standardized, internationally accepted values¹⁴.
 - Fill two beakers with ultrapure water for rinsing the syringes between the injections of samples and reference materials. Obtain an empty bottle for waste solution.
 - Dip the tip of the syringe being used for injection in the first beaker of water and dry it off. Rinse the full volume of the syringe with ultrapure water and discard the water as waste. Repeat three times.
 - Following a similar procedure to step 3.2.3, fill the syringe with a small amount of sample to pre-rinse the syringe. Discard it as waste. Refill the syringe with sample and gently knock to remove any air bubbles so that an accurate volume is measured.
 - Inject the sample into the sample vial.
 - If there is more than 3 ml being injected into the sample, use a second "vent" needle to relieve pressure in the vial. Push the syringe with the sample into the rubber septa and begin injecting the sample. After 0.5 ml of the sample has been injected, insert the second "vent" needle. Leave the "vent" needle in until 1 ml is left to be injected, and then remove the "vent" needle. Continue injecting the last of the sample.
- Store the vials overnight in a warm ($\sim 24^\circ\text{C}$) area. The following morning, inject 0.1 to 0.2 ml of 10 M NaOH into each sample to lyse the bacteria.

8. Isotope Ratio Determination

NOTE: Once the bacteria are lysed, the samples are ready to be run on the isotope ratio mass spectrometer (IRMS).

- Inject each sample with three to four drops of antifoam before setting them up to run on the mass spectrometer.
- Determine the isotopic composition by ion ratio mass spectrometry. Use a mass spectrometer interfaced with a modified system for the automated extraction, purification (removal of CO_2 and H_2O), and isotopic analysis of N_2O at m/z 44, 45, and 46^{12,13}.
- Run a blank with only media solution and no sample in the vial at the beginning of each run.
- Calibrate the raw isotope ratios from the mass spectrometer using reference materials (e.g., IAEA-NO-3 and USGS 34) treated in the same manner as the samples, based on the correction scheme in Kaiser *et al.* (2007)¹⁵. This puts them in a form that is usable for data comparisons with other laboratories in the N isotope biogeochemistry community.
- Due to concerns about the linearity of the mass spectrometer values, if the area of the injected sample is outside of $\pm 10\%$ of the target area, adjust the concentration using that percentage and re-inject the sample, following the above procedure.
 - To finalize the sample data for $\delta^{15}\text{N}$ of NO_x (the delta notation is defined using the following equation for $\delta^{15}\text{N}$: ("delta 15-N"): $\delta^{15}\text{N} = [({}^{15}\text{N}/{}^{14}\text{N}_{\text{sample}}/{}^{15}\text{N}/{}^{14}\text{N}_{\text{standard}}) - 1] \times 1,000\text{‰}$ and the standard used for the nitrogen samples is atmospheric N_2 gas), correct the $\delta^{15}\text{N}$ for the contribution of the nitrate blank that is found in the KMnO_4 solution:

$$\delta^{15}\text{N}_{\text{sample,corrected}} = \frac{(\delta^{15}\text{N}_{\text{total,measured}} \cdot [\text{Sample Concentration}]) - (\delta^{15}\text{N}_{\text{blank,measured}} \cdot [\text{Blank Concentration}])}{[\text{Sample Concentration}] - [\text{Blank Concentration}]}$$

where $\delta^{15}\text{N}_{\text{total,measured}}$ is the value determined for the sample from the mass spectrometer run, $\delta^{15}\text{N}_{\text{blank,measured}}$ is the value determined for the blank, and the Sample and Blank Concentrations are the values determined from the colorimetric analyses. This equation removes the effect of the blank from the isotopic ratio, so that the isotopic ratio is now representative of the NO_x collected *in situ*.

NOTE: The isotopic composition and concentration of the blank solution nitrate is measured with every batch of solution used. This blank is different from any potential blank that might be encountered with the denitrifier method alone (which is also quantified with every run). Any blank associated with the denitrifier method is true for all samples and reference materials; however, the permanganate solution blank is only applicable to samples and therefore must be quantified and corrected for (by mass balance) separately.

Representative Results

In the original method development work by Fibiger *et al.*, the NO_x collection method was rigorously tested in the laboratory under a variety of conditions⁴. Here, the focus is on updates to the method and field applications under a variety of environmental conditions. Results are reported on (1) field collection efficiency, (2) sample solution stability in terms of time before sample reduction and sensitivity to high concentrations of ammonium (NH_4^+) in solution, and (3) reproducibility in the field. The versatility of the method is demonstrated in its application for ambient air, near-road, and on-road measurements.

The average concentrations collected in solution were compared with those from 1 min NO_x concentrations from the chemiluminescence NO_x analyzer over a two-day diurnal study in ambient urban air in Providence, RI. **Figure 2** details the collection efficiency during a period when concentrations varied over a large range, from ~ 2.5 -18 ppbv NO_x . **Figure 2A** displays a direct comparison of median NO_x concentrations from the NO_x analyzer compared with concentrations calculated from the solution and flow measurements, indicating that, on average, solution concentrations are 92% of median *in situ* concentrations. This falls within the expected uncertainty range of $\pm 10\%$, but the difference likely reflects varying concentrations during the collection periods (**Figure 2B**). Based upon examination of the percentiles of the distribution of the 1-min NO_x concentration data, the solution-based NO_x concentrations are within the distribution for every collection interval (**Figure 2B**).

It has been recommended to complete the reduction of the samples collected in the field within 1 day after collection is complete (*i.e.*, complete all of step 3). This target was suggested to reduce the potential for interference from the collection of other soluble nitrogen species, such as NH_3 , that could be converted to nitrate in the highly oxidizing $\text{KMnO}_4/\text{NaOH}$ solution over time. To test this more specifically, samples were collected in May and July 2015 in Providence, RI on the campus of Brown University, at a loading dock that is near a regularly traveled local road where diesel delivery trucks are regularly running in idling mode to unload. Samples were collected, and then aliquots of the samples were separated and reduced at different times (1 day, 4-7 days, and 13-15 days) after the sample collection (**Figure 3A**). Samples in **Figure 3B** were also collected during May and July but were prepared by adding 5 ml of 10 mM ammonium chloride to 450 ml of solution. This yielded a concentration of $111 \mu\text{M}$ NH_4^+ in solution, corresponding to collecting 220 ppbv of NH_3 in the air, if only NH_3 was collected. These concentrations are the maximum expected during on-road measurements near vehicle NH_3 sources¹⁶. With or without the added NH_4^+ , samples reduced within 7 days after collection had consistent isotope ratios when compared to the first reduction (within 1 day of collection), all falling within the expected uncertainty range of $\pm 1.5\%$ (**Figure 3A** and **3B**). Note that the $\pm 1.5\%$ uncertainty is representative of isotopic determinations of repeated collections of tank NO_x . The uncertainty associated with repeated measures of isotopic reference materials alone is typically 0.3%. After two weeks, however, samples with or without added NH_4^+ were not necessarily stable. While in some cases the isotope values still appear to be consistent (*e.g.*, **Figure 3A**), samples exhibited small NO_3^- concentration increases ($< 1 \mu\text{M}$) when compared with the first reduction and, in some cases, decreases in NO_3^- concentrations. With the added NH_4^+ , it would have been expected that NO_3^- concentration would *increase* over time above the expected uncertainty range ($\sim 0.8 \mu\text{M}$) for concentration measurements, suggesting that even after two weeks, the NH_4^+ was not the source of interference. Further experiments are needed to better understand the source of this instability, though it is noted that blank solutions left untreated over the same time course consistently showed no change or slight increases in concentrations, and therefore, the instability must be created by the presence of other species found in the ambient urban air. Until this is resolved, it is recommended that sample solutions be reduced within 7 days from the time of collection.

Figure 4 details the collection of samples with the mobile setup over various field campaigns in urban, near-road, and on-road settings. The NO_x concentration range spans three orders of magnitude, and the isotope ratios range from -1 to -13% . This sampling set includes 51 on-road samples taken over 52 hr, covering over 4,000 km, and in a myriad of driving conditions (*e.g.*, heavy stop-and-go traffic to very light traffic at high speeds on the highway). The sampling took place on roads in and between 6 major cities, including Providence, RI, Philadelphia and Pittsburgh, PA, and Cleveland, Columbus, and Cincinnati, OH. Average vehicle speeds ranged from 12.4 km/hr to 119.7 km/hr. The near-roadside samples ($N = 27$) were obtained at a monitoring site at I-95 in Providence, RI. The ambient urban air samples ($N = 44$ samples taken over 117.5 hr) were taken from two rooftop locations in Providence, RI, one near the I-95, I-195 interchange and one 775 meters away from the interchange site. This represents the first steps towards building new capabilities to resolve the ranges of isotopic signatures from NO_x sources, in this case, vehicle emissions and ambient urban sources. The variations in daytime on-road and road-side $\delta^{15}\text{N}-\text{NO}_x$ (**Figure 4**) were not correlated with variations in driving conditions and occurred across relatively constant vehicle fuel-class traffic counts. A more detailed discussion of the variations of isotopic signatures due to vehicle fuel types is the subject of another manuscript (Miller, D.J., *et al.* 2016. *J. Geophys. Atmos.* Submitted).

Finally, **Table 1** details field and laboratory collections where two collection systems were deployed at the same time to test reproducibility. The comparisons show excellent agreement for the isotopic data, quantified here as the absolute deviation between the two data points for each collection period. The data are displayed from urban air collections at a rooftop location in Providence, RI; near-roadside collections in Providence, RI; and from collections in a laboratory-based smog chamber at the University of Massachusetts, Amherst.

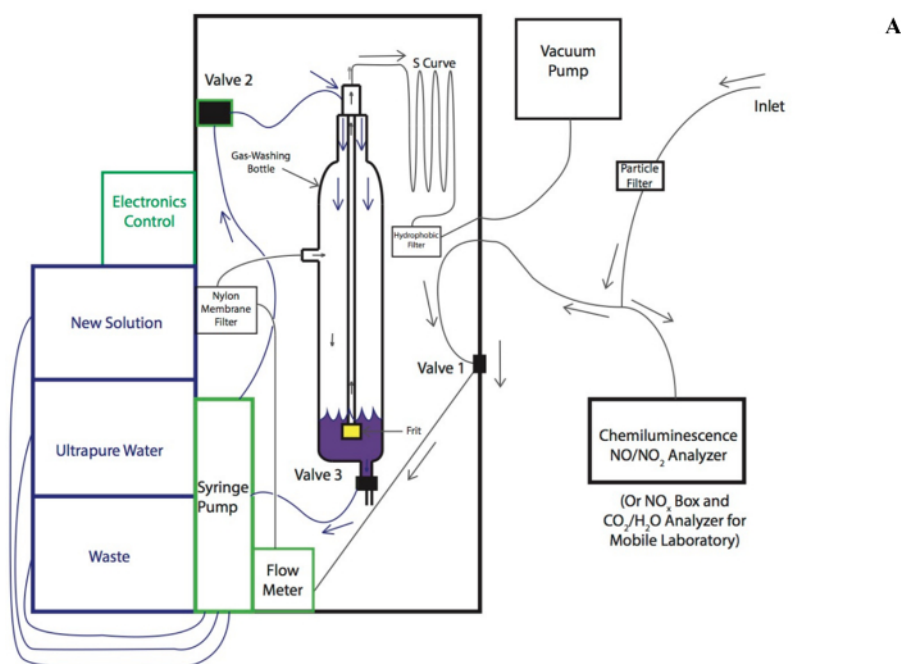


Figure 1: Collection Schematic and Image. (A) Diagram of the Automated NO_x Collection System. Gray is airflow, blue is water/solution flow, green is electronics connections, yellow is the frit, and purple is the permanganate solution. The syringe pump is used to add and remove rinsing solution (ultrapure water) and to add new solution for the start of sample collection (the syringe pump is a commercially available stepper motor syringe pump with a 50 ml syringe, a 5-port distribution valve, and driver/control boards equipped with an RS-232 serial interface). The sample is removed manually via the black valve at the bottom of the gas washing bottle. **(B)** Picture of the NO_x collection system and NO_x box in the mobile laboratory. [Please click here to view a larger version of this figure.](#)

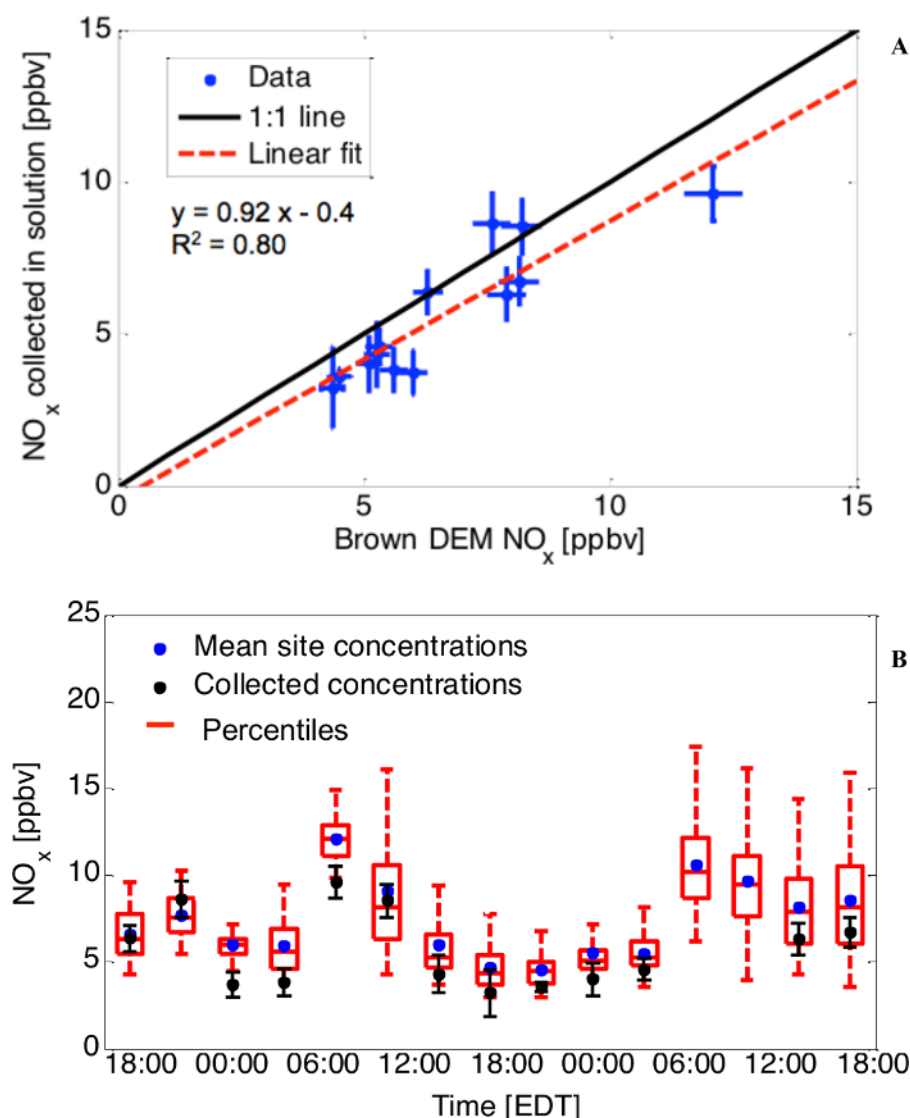


Figure 2: Collection Efficiency of the Automated Collection System. (A) The NO_x concentrations calculated from the NO_3^- concentrations measured in solution and the flow data compared against the median concentration measured by a chemiluminescent NO_x concentration analyzer at a rooftop site in Providence, RI. The error bars are the standard deviation ($\pm 1\sigma$) of the solution-based NO_x mixing ratio estimates derived from the propagated errors of the pooled standard deviations of the quality controls ($0.4 \mu\text{M}$) across colorimetric concentration measurement runs and the flow rate uncertainty ($\pm 1\%$). The NO_x analyzer concentration uncertainties are $\pm 5\%$. (B) The time series of NO_x concentration distributions during diurnal measurements at a rooftop site in Providence, RI. The boxes represent the 25th, 50th, and 75th percentiles. The whiskers represent the extremes without outliers. [Please click here to view a larger version of this figure.](#)

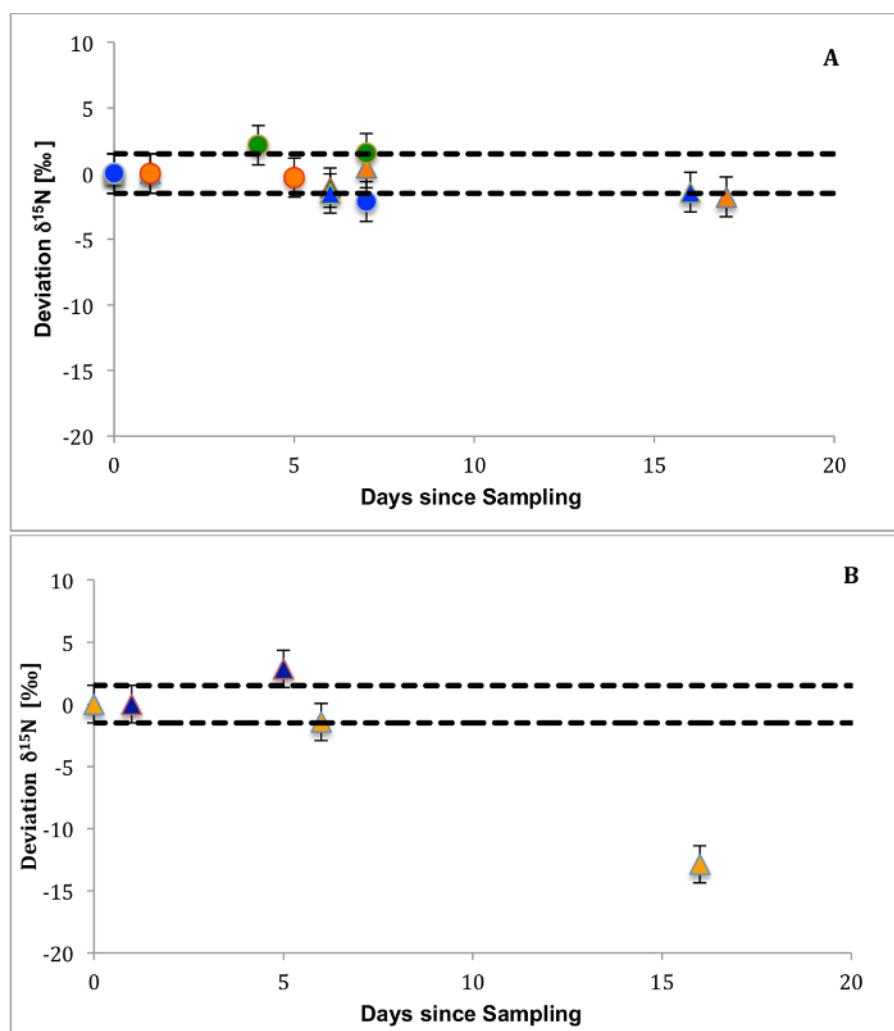


Figure 3: Comparison of the reduction times for NO_x samples collected at Brown University in May and July 2015. (A) The results are recorded as deviations from the first reduction, performed within 1 day of sampling. May samples are displayed as triangles and July samples as circles, with colors denoting different collection periods. Samples in (B) were pre-treated with ammonium chloride prior to air collection to test the interference of NH_4^+ in solution over time. The dashed lines represent the expected overall precision of the isotopic collection method, expressed as a standard deviation of $\pm 1.5\%$. [Please click here to view a larger version of this figure.](#)

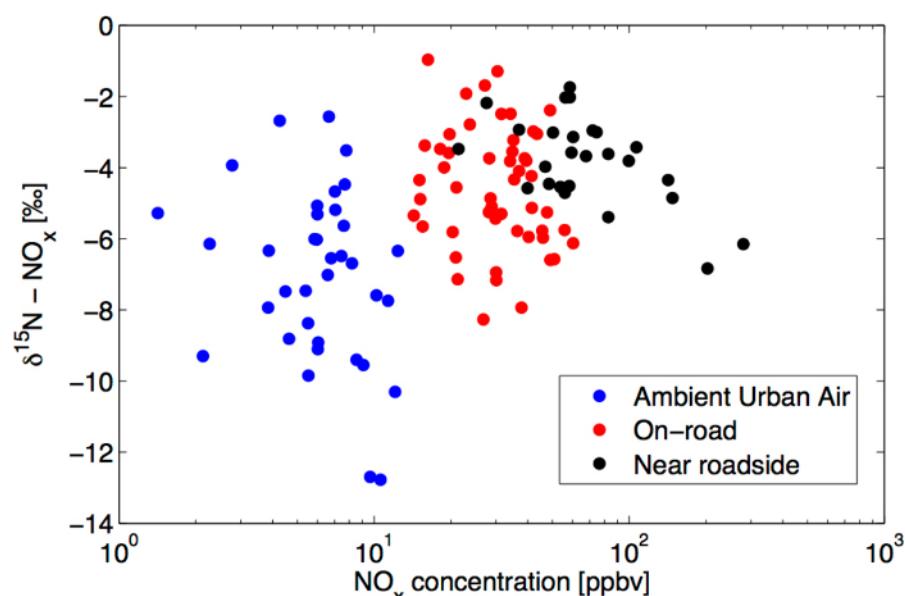


Figure 4: The $\delta^{15}\text{N-NO}_x$ (‰) and NO_x concentration of samples collected in ambient urban air, on-road, and near-road sites. The types of samples are delineated by different colors, and represent a range of conditions (see the text) and NO_x concentrations. [Please click here to view a larger version of this figure.](#)

Sample Name	System Number	Collection Date	Hours of Collection	Temperature (°C)	[NO ₃] (μM)	Blank/total N	$\delta^{15}\text{N}$ (‰)	Deviation $\delta^{15}\text{N}$ (‰)
Urban Air PVD 1	1	10/8/2013 - 10/9/2013	6.75	15.8	14.43	0.3	-0.6	0.7
	2				16.78	0.26	-1.3	
Urban Air PVD 2	1	11/6/2013 - 11/7/2013	2.5*	17.1	30.86	0.2	-7.7	1
	2		5.25		37.05	0.17	-6.7	
Urban Air PVD 3	1	11/20/2013 - 11/21/2013	8.9	3.28	44.29	0.14	-7.1	0.4
	2				29.66	0.21	-6.7	
Near Roadside 1	1	8/14/2014 - 8/15/2014	29	19.2	13.3	0.37	-9.47	0.69
	2				16.4	0.3	-10.16	
Near Roadside 2	1	8/17/2014 - 8/18/2014	30	21.85	9.4	0.68	-8.95	1.56
	2				11.6	0.55	-7.39	
Near Roadside 3	1	5/25/2015	3.5	20	6.86	0.51	-7.67	0.86
	2				9.49	0.42	-8.53	
Near Roadside 4	1	5/26/2015	2.75	25.56	6.07	0.656	-8.7	1.57
	2				6.49	0.61	-7.13	
Smog Chamber 1	1	8/26/2014 - 8/27/2014	24.4	21	24.392	0.27	-12.28	0.33
	2				33.2	0.2	-12.61	
Smog Chamber 2	1	8/27/2014 - 8/28/2014	19.8	21	10.96	0.54	-10.22	1.25
	1				14.245	0.41	-11.47	
Smog Chamber 3	1	8/28/2014 - 8/29/2014	24.2	21	7.476	0.8	-5.86	1.27

Table 1: Reproducibility of samples collected at the same time using two identical collection systems. *The collection had to be stopped due to a clogged filter. Urban Air PVD (PVD = Providence, RI) 1-3 were previously published⁴. Near Roadside represents roadside collections in Providence, RI; Smog chamber represents samples collected from air inside a smog chamber at the University of Massachusetts, Amherst⁴.

Discussion

The protocol above details the steps involved, from the field collection of air samples in solution to the laboratory processing of these solutions, to yield concentration and isotopic results. The critical steps in this protocol include comparing NO_x analyzer measurements, minimizing the time before the reduction of solutions, and maintaining stable flow rates. If directly comparing solutions with *in situ* measurements of NO_x concentrations, it is very important that an NO_x analyzer is calibrated for ranges relevant for the chosen environment and that short-term variability in NO_x concentrations be understood in the context of the longer time collections for the solutions. The accurate determination of solution NO_3^- concentrations is also important, both for calculation of the airborne NO_x concentrations and for determining accurate injection volumes for the isotopic denitrifier method. The time period of solution stability before sample reduction is important to ensure consistent isotope ratios. As a result of the oxidizing potential of the solution, it is possible to oxidize in solution other reactive nitrogen species, most notably NH_3 , as it can be in high enough concentrations in certain areas to potentially affect the concentration of NO_3^- in solution. The oxidation of NH_4^+ to NO_3^- is expected to take longer than the oxidation of NO_x to NO_3^- , so it had been recommended to reduce the samples (and thus stop the reaction) within 1 day of sample collection. Given that field conditions may result in the requirement of longer solution storage times, the stability of solutions was tested by examining solutions with and without added ammonium. With and without the addition of ammonium chloride, concentration and isotope values were stable within the 1σ uncertainty range (1.5‰) for up to one week (**Figure 3**). At two weeks after sampling, solutions with or without added NH_4^+ were not stable, in that NO_3^- concentration decreases were observed in some cases and blank corrections were no longer robust. Although it was expected that NO_3^- might increase over time due to NH_4^+ oxidation, decreases in concentration were actually observed in some cases, suggesting that even after two weeks, NH_4^+ interference is not causing the instability. As such, solutions should be reduced within one week, particularly if sampling is done in an environment with high NH_3 concentrations (e.g., >200 ppbv). Finally, it is also critical to record the flow rate during field collections. The flow rate measured at the inlet was found to vary considerably and is difficult to control, even with a critical orifice in the system, since it can be influenced by the clogging of the hydrophobic filters and/or the frit. It is recommended to record the flow rate periodically (e.g., at 5 min intervals) throughout collections periods, such that the volume of air collected over time for each sample can be accurately determined (see step 5).

There are several alternatives or possible modifications of the protocols presented. For example, an important advantage of the denitrifier method is the low sample size requirement^{12,13}. However, other isotopic methods may be used. Similarly, we use colorimetric determination of concentration, but other methods can yield accurate NO_3^- concentration results.

Collection efficiency in the field, as detailed in **Figure 2**, is $92 \pm 10\%$. This is critical to ensure that there is no fractionation during the collection process. With collection efficiency less than 100%, fractionation in the collection process can occur, biasing the resulting isotopic ratios measured. The efficacy of this new collection method across a range of conditions in urban-influenced air has been shown. **Table 1** outlines the multiple tests that were done under ambient-air, near-roadside, and smog-chamber sampling conditions to determine the reproducibility of the method. All isotope ratio differences between systems are <1.57‰. This demonstrates the reproducibility of this method over a range of different sampling conditions. The field-based method has a precision and reproducibility significantly better than the ~12‰ isotopic ratio variations observed in the environment (**Figure 4**).

The most significant limitation of the method is the NO_3^- blank or background associated with the KMnO_4 solution. A variety of KMnO_4 types have been tested (e.g., crystals, powders, and stock solutions)⁴, and all contained NO_3^- before being exposed to NO_x in the air. As a result, it is necessary to collect enough NO_x as NO_3^- in solution to achieve a concentration above the blank. Further studies are currently underway to quantify the level at which the sample should exceed the blank concentration for the most accurate results. Under very low ambient NO_x concentrations, it may be necessary to modify the collection conditions to maximize the sample concentration. For instance, the flow rate could be increased to collect more air in a shorter timeframe or the solution volume could be reduced to increase the air-to-solution volume and to concentrate the air collection. In any case, the solution must remain above the frit in the collection vessel to maintain the bubbling of air through the solution.

This method of NO_x collection for isotopic analysis is unique among existing methods (e.g., passive samplers^{6,17} and sulfuric acid and hydrogen peroxide solution⁸) in that it has been laboratory- and field-verified with respect to field applicability, reproducibility, sample solution stability, and efficiency of collection under a range of field conditions. This novel method is unique in its capabilities to actively collect NO_x in field environments for isotopic analysis at ambient concentrations at a 30-120 min time resolution. It collects NO_x at near 100% efficiency and has been demonstrated repeatedly to be reproducible within the range of uncertainty of the method. Sample solutions collected in the field remain stable for up to 1 week before needing to be reduced. The method can collect samples over a range of concentrations and isotope ratios, and it is shown to be reproducible from collection to collection. This technique can be used for sampling under a variety of different conditions, including on-road, using the mobile laboratory approach outlined in the protocol. The interpretation of the spatiotemporal variability in vehicular emissions of NO_x is the subject of a separate manuscript, in preparation (Miller, D.J., *et al.* 2016. *J. Geophys. Atmos.* Submitted).

Future sampling includes the application of this method to other types of NO_x emissions (e.g., microbially produced emissions in soils and biomass fires). Isotopes are a potential way to track NO_x sources, but only if different source signatures can be quantified and understood. Our new method makes it possible to quantify the isotopic composition of NO_x from a variety of NO_x emission sources and to directly test whether the impacts of emissions in the environment can be directly and quantitatively tracked.

Disclosures

The authors declare that they have no competing financial interests.

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