Nitrate isotopes (δ^{15}N, δ^{18}O) in precipitation: best practices from an international coordinated research project

Germain Esquivel-Hernández, Ioannis Matiatos, Ricardo Sánchez-Murillo, Yuliya Vystavna, Raffaella Balestrini, Naomi S. Wells, Lucilena R. Monteiro, Somporn Chantara, Wendell Walters & Leonard I. Wassenaar

Published online: 22 Feb 2023.

Submit your article to this journal ⬈

Article views: 221

View related articles ⬈

View Crossmark data ⬈

Taylor & Francis
Taylor & Francis Group

Check for updates

BRIEF REPORT

Nitrate isotopes (δ^{15}N, δ^{18}O) in precipitation: best practices from an international coordinated research project

Germain Esquivel-Hernández[a,j], Ioannis Matiatos[b], Ricardo Sánchez-Murillo [c],
Yuliya Vystavna[d], Raffaella Balestrini[e], Naomi S. Wells[f], Lucilena R. Monteiro [g],
Somporn Chantara[h], Wendell Walters[i] and Leonard I. Wassenaar[d,k]

[a]Stable Isotopes Research Group and Water Resources Management Laboratory, Universidad Nacional Costa Rica, Heredia, Costa Rica; [b]Hellenic Centre for Marine Research, Anavissos Attikis, Greece; [c]Department of Earth and Environmental Sciences, University of Texas, Arlington, TX, USA; [d]International Atomic Energy Agency, Isotope Hydrology Section, Vienna International Centre, Vienna, Austria; [e]Water Research Institute, National Research Council (CNR-IRSA), Brugherio, MB, Italy; [f]Faculty of Environmental Science & Engineering, Southern Cross University, Lismore, NSW, Australia; [g]Instituto de Pesquisas Energéticas e Nucleares, IPEN/CNEN, Cidade Universitária, São Paulo, SP, Brazil; [h]Environmental Science Research Center, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand; [i]Institute at Brown for Environment and Society, Brown University, Providence, RI, USA; [j]Department of Soil and Physical Sciences, Lincoln University, Lincoln, New Zealand; [k]WasserCluster Lunz, Lunz am See, Austria

ABSTRACT

Stable isotope ratios of nitrogen and oxygen (^{15}N/^{14}N and ^{18}O/^{16}O) of nitrate (NO_3^-) are excellent tracers for developing systematic understanding of sources, conversions, and deposition of reactive atmospheric nitrogen (N_r) in the environment. Despite recent analytical advances, standardized sampling of NO_3^- isotopes in precipitation is still lacking. To advance atmospheric studies on N_r species, we propose best-practice guidelines for accurate and precise sampling and analysis of NO_3^- isotopes in precipitation based on the experience obtained from an international research project coordinated by the International Atomic Energy Agency (IAEA). The precipitation sampling and preservation strategies yielded a good agreement between the NO_3^- concentrations measured at the laboratories of 16 countries and at the IAEA. Compared to conventional methods (e.g., bacterial denitrification), we confirmed the accurate performance of the lower cost Ti(III) reduction method for isotope analyses (^{15}N and ^{18}O) of NO_3^- in precipitation samples. These isotopic data depict different origins and oxidation pathways of inorganic nitrogen. This work emphasized the capability of NO_3^- isotopes to assess the origin and atmospheric oxidation of N_r and outlined a pathway to improve laboratory capability and expertise at a global scale. The incorporation of other isotopes like ^{17}O in N_r is recommended in future studies.

CONTACT Germain Esquivel-Hernández ✉ germain.esquivel.hernandez@una.cr 🖥 Stable Isotopes Research Group and Water Resources Management Laboratory, Universidad Nacional Costa Rica, Heredia, Costa Rica

1. Introduction

Human activities have altered the global nitrogen cycle by increasing the rates of atmospheric nitrogen (N) deposition that impact the ecosystem functioning and human health [1–3]. Stable nitrogen and oxygen ($\delta^{15}N$ and $\delta^{18}O$) isotopes of nitrate are reliable tools for tracing nitrogen oxide (NO_x) emission sources and oxidation chemistry [4–8]. Isotopic methods can be applied to better understand how reactive nitrogen (N_r) is introduced and transformed in the atmospheric environment, to determine the input of fixed nitrogen from atmospheric deposition and develop a more comprehensive knowledge of the atmospheric oxidation processes [4,9,10]. Overall, chemical transformations of N compounds in polluted atmospheres are controlled by O_3 and OH during the day via the following general reactions [6]:

$$NO + O_3 \rightarrow NO_2 + O_2 \tag{R1}$$

$$NO_2 + h\upsilon \rightarrow NO + O \tag{R2}$$

$$NO_2 + OH + M \rightarrow HNO_3 + M \tag{R3}$$

and at night by:

$$NO_2 + O_3 \rightarrow NO_3 + O_2 \tag{R4}$$

$$NO_2 + NO_3 + M \rightarrow N_2O_5 + M \tag{R5}$$

$$N_2O_5 + H_2O + aerosol \rightarrow 2HNO_3 \tag{R6}$$

In R3 and R5, M is commonly N_2 or O_2 and stabilizes the product through collisions, whereas hυ in R2 refers to solar radiation with wavelengths < 400 nm. These reactions cause a large range of $\delta^{15}N$ and $\delta^{18}O$ values in oxidized compounds such as nitrate depending on the season, land use, meteorological conditions, geographic location, anthropogenic and natural inputs, and the proximity to pollution sources [7,10,11]. Therefore, regional and global scale investigations of nitrate isotopes in precipitation can improve the evaluation of N influences on the nitrogen cycle. The incorporation of other isotopes like ^{17}O in atmospheric nitrate could also expand the interpretation and comprehension of the fundamental chemical processes related to the mixing of oxygen sources (e.g. O_3, H_2O, O_2) and kinetic and/or equilibrium effects occurring when NO_x is photochemically converted into nitrate. For instance, large $\Delta^{17}O$ values are usually registered in atmospheric nitrate, which suggests ozone plays a central role as an oxidant (and oxygen source). For this same reason, $\delta^{18}O$ values in atmospheric nitrate are typically high, too [12].

This study presents best-practice guidelines based on lessons learned from an international coordinated research project (CRP) organized by the International Atomic Energy Agency (IAEA). This work aims to improve (a) the sampling performance, (b) the analysis and data interpretation of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ in precipitation, and (c) to present validated methods of sampling, preserving, and analyzing nitrate isotopes in precipitation that can be applied worldwide. Proposed methods were tested and validated in sampling sites and laboratories in America, Europe, Asia, and Oceania. This study advances the application of nitrogen and oxygen isotopes in link with chemical

substances to better define the origin of N in precipitation and evaluate the impact of N wet deposition on ecosystems.

2. Methods

2.1. Sampling sites and procedures

From the 16 project participants, nitrate isotope datasets from Costa Rica, Austria, Thailand, and Australia (4 sites, 1 per country) are used here to illustrate a diversity of methods, climates, and pollution sources. However, data on precipitation sampling and preservation from 16 participants were used to evaluate the sample preservation practices. Rainwater samples at the 4 sites (i.e. rural, urban, and coastal) were collected on either an event or daily basis over 12–36 months between 2018 and 2021 (Table 1). The site installed in Australia was classified as rural and coastal, whereas the other three sites were classified as urban sites. No precipitation samples were collected during the dry season in Costa Rica (December to April) and Thailand (December to March). Meteorological data (precipitation type and amount, wind speed and direction, air temperature, and relative humidity) was retrieved from weather stations installed in the vicinity of all sampling sites.

The collection and preservation of the precipitation samples followed the procedure defined by the World Meteorological Organization and the Global Atmospheric Watch Precipitation Chemistry Programme [13]. At the sites operating wet-only precipitation collectors, namely Austria and Thailand, samples were filtered (using 0.45 μm Teflon and cellulose acetate filters, respectively), transferred into polyethylene bottles, and stored at dark and cool conditions until ion analysis. In Costa Rica and Australia, where samples were collected using a bulk precipitation sampler, samples were filtered using 0.2–0.45 μm polytetrafluoroethylene (PTFE) syringe membranes. In Costa Rica and Thailand, a portion of the precipitation samples (30 mL) were stored at dark and frozen conditions (–10 °C) until shipment for nitrate isotope analysis to the IAEA. In Australia, samples were kept frozen until ion and isotope analysis was carried out at Southern Cross University. In Costa Rica, to account for the possible contamination from dry deposition, the collector's funnel and the transfer line to the vessel were rinsed with deionized water every day after the sample collection. In Australia, the sampler and funnel were not covered between rain events, so samples should be considered reflecting bulk nitrogen deposition rather than wet only. Although dry deposition was not within the scope of our study, we acknowledge its importance in the total N deposition.

To investigate a possible degradation of the samples during sample collection, preservation, and shipment to the IAEA before nitrate isotope analysis, we performed a quality control survey that included: (1) differences in the sampling design (i.e. daily or per event); (2) the duration of each sampling campaign; (3) the transportation requirements for samples from Costa Rica and Thailand to Vienna; (4) differences regarding nitrate metrological uncertainties and quantification limits between sites, and (5) the possibility of nitrate chemical degradation at the time of sampling until the final measurement. Nitrate concentration measurements were performed at local laboratories and repeated at IAEA facilities. Replicate analysis was a strategy to evaluate sample preservation

Table 1. Information about the location and the precipitation monitoring programme at each station.

Country	Latitude (decimal degrees)	Longitude (decimal degrees)	Elevation (m asl)	Station type	Type of collector	Frequency of sampling	Period of sampling	Site characteristics
Australia	−28.81	156.26	12.0	Rural/ Coastal	Bulk	Event	2018–2021	It is expected that N_r deposition in this rural site would reflect a combination of marine and soil origins (i.e. biogenic) with fossil fuel combustion emissions mainly from local transportation.
Austria	48.24	16.42	165.0	Urban	Wet-only	Event	2017–2019	Major N_r air pollution sources include municipal incinerators and a wastewater treatment plant, and fossil fuel/gas combustion from industrial and transportation [28].
Costa Rica	10.01	−84.11	1,100	Urban	Bulk	Daily	2018–2019	Major N_r local sources are fossil fuel combustion (i.e. gasoline and diesel) from industrial and transportation. Other N_r sources like biomass burning, soil-related emissions and lightning also contribute to the N emissions [8].
Thailand	18.76	98.93	334	Sub-urban	Wet-only	Daily	2019	Northern Thailand usually suffers from the annual intense smoke haze pollution during hot dry season (February to April) caused by the extensive open burning in Southeast Asia. Other N_r sources are fossil fuel combustion from industrial and transportation.

during storage and transportation to the IAEA [14]. At the IAEA, simulated certified rain-water reference material (ERM-CA408) was used to assess nitrate measurement accuracy.

2.2. Ion analysis

Major ions (chloride, nitrite, nitrate, sulfate, sodium, ammonium, potassium, magnesium, and calcium), electrical conductivity (EC), and hydrogen potential (pH) were analyzed in precipitation samples collected at each site. EC and pH were measured after the sample collection using a glass electrode and a conductivity cell, respectively. The samples collected in Costa Rica, Austria and Thailand were analyzed for major ions using an ion chromatograph (Dionex, CA for samples collected in Costa Rica and Austria and Compact IC plus, Metrohm for samples collected in Thailand). The concentrations of nitrate in the samples collected in Australia were analyzed via flow injection analysis (FIA). In Austria, Costa Rica, and Thailand, where samples were analyzed by ion chromatography, precipitation samples were also screened for nitrite, however, this ion was not detected. In Australia, nitrite was not analyzed. The analysis method for samples collected in Thailand can be found in Chansuebsri et al. [15]. The limits of detection of cations and anions were 0.005–0.027 and 0.001–0.004 mg/L, respectively [15].

2.3. Isotope analysis

The nitrogen and oxygen isotope analyses ($\delta^{15}N$ and $\delta^{18}O$ of NO_3^-) of the samples collected in Costa Rica, Austria and Thailand were performed at the IAEA Isotope Hydrology Laboratory whereas the samples collected in Australia were analyzed at the Southern Cross University, Australia. In both laboratories, samples were analyzed by isotope ratio mass spectrometry (IRMS). The stable isotope ratios were expressed in delta (δ) notation in permil (‰) relative to international standards. Values of $\delta^{15}N$ were reported relative to N_2 in atmospheric air (AIR) and $\delta^{18}O$ values were reported relative to Vienna Standard Mean Ocean Water (VSMOW). The IAEA laboratory uses a Ti(III) reduction method, which involves a one-step chemical conversion employing $TiCl_3$ to reduce NO_3^- to N_2O gas in septum sample vials. Overall, when this reduction method is used with IRMS or laser analyzers, the materials and reagent costs are < $2 USD per sample, given the small amount of reagent and vial septa needed (assuming the availability of reused vials) [16]. At IAEA, the N_2O headspace was measured for ^{15}N and ^{18}O by coupling with a continuous-flow isotope-ratio mass spectrometer (IRMS, Isoprime 100) and a trace gas N_2O purification device. The standards used for the analysis were USGS34 (–1.8 ± 0.1 for $\delta^{15}N_{AIR}$ and +27.9 ± 0.3 ‰ for $\delta^{18}O_{VSMOW-SLAP}$), USGS35 (+2.7 ± 0.1 ‰ for $\delta^{15}N_{AIR}$ and +57.5 ± 0.3 ‰ for $\delta^{18}O_{VSMOW-SLAP}$) and IAEA-NO_3 (+4.7 ± 0.2 ‰ for $\delta^{15}N_{AIR}$ and +25.6 ± 0.4 ‰ for $\delta^{18}O_{VSMOW-SLAP}$). In Australia, the nitrate isotopic composition was measured using the 'denitrifier method' [17]. In this method, a P. aureofaciens culture genetically modified to block N_2O reduction is added to samples to quantitatively convert NO_3^- to N_2O. Each batch for NO_3^- isotope analysis (~20 samples) contained triplicate blanks, a triplicate of three internationally certified standards, namely USGS34, USGS32 (+180 ± 0 ‰ for $\delta^{15}N_{AIR}$ and +25.4 ± 0.2 ‰ for $\delta^{18}O_{VSMOW-SLAP}$), and USGS35, and duplicates of ~1/3 of the samples (to verify method precision for the sample matrix). To run a batch, first, 1 mL of resuspended bacterial media was injected into a

20 mL borosilicate glass crimp top vial (acid washed and pre-combusted between each use) and sealed with a rubber septum. Background air was removed from the vials by purging with N_2 gas for three hours. After, they were injected with the volume of samples, blanks, and standards needed to ensure a consistent ~ 10 nM N peak. Then, vials were inverted (to minimize gas leakage) and placed on a shaker table overnight. In the morning, the reaction was quenched by adding a few drops of 6M NaOH. The produced N_2O in the headspace of each vial was measured on an IRMS fitted with a custom gas bench (Thermo Fisher Gasbench II coupled to a Thermo Fisher Delta V Plus). Detailed information regarding this laboratory method is available in [18–20]. The analytical uncertainties for both methods were ± 0.2–0.3 ‰ for δ^{15}N-NO_3^- and ± 0.4–0.5 ‰ for δ^{18}O-NO_3^-, respectively. Even though no direct method comparison was performed, δ^{15}N-NO_3^- and δ^{18}O-NO_3^- were measured together with IAEA ^{15}N and ^{18}O reference materials to assure the satisfactory performance of isotope measurements comparable to the bacterial method.

3. Results and discussion

3.1. Efficiency of practices on precipitation sample preservation

The ion analysis of precipitation is fundamental for at least two aspects: on the one hand, wet deposition gives insights into air pollution from anthropogenic emissions (e.g. NO_x and SO_2 from transportation and industrial activities) and impacts on surface and groundwater systems; on the other hand, it allows the calculation of ion balance for quality control. We evaluated the sample preservation practices using the regression analysis between nitrate concentration measured at the participant laboratories (16 laboratories) and at the IAEA facilities. Overall, if each sample yields an identical result at both laboratories (i.e. at the member state laboratory and the IAEA facility), the regression line will have a zero intercept, a slope, and a correlation coefficient of 1. We found best-fit line slopes between 0.93 and 1.30 and Pearson correlation coefficients (r) between 0.85 and 0.94 ($p < 0.0001$, Figure 1). These significant results suggest that the preservation practices used in the laboratories of the evaluated participants were effective. Small differences are likely attributed to the systematic errors introduced by the analytical instrumentation and metrological traceability chain. However, it is also interesting to account for the possible fractionation shifts that these concentration changes may introduce in the nitrogen and oxygen isotope analyses of nitrate if the implemented preservation method were not applied. In general, transformations between the chemical forms (i.e. NO_3^- and NH_4^+) are often induced by microbes and temperature changes, during which nitrogen and oxygen isotope fractionation may occur, most likely resulting in an enrichment of the heavy isotopes ^{15}N and ^{18}O in the residual N pool (e.g. higher δ^{15}N-NO_3^-) [21]. Thus, the filtration and freezing of the precipitation samples as soon as they are collected are of the highest importance to conserve the nitrogen species in the precipitation samples prior to the isotope analysis. At the laboratory, to avoid the introduction of isotope changes during the sample handling, the preparation time should be as short as possible, and the handling time per sample should be constrained to a few days, including the isotope analysis [16,22]. A systematic bias in the nitrate concentrations measurements observed in multi-site studies, as presented for MS3 in Figure 1, could be corrected by
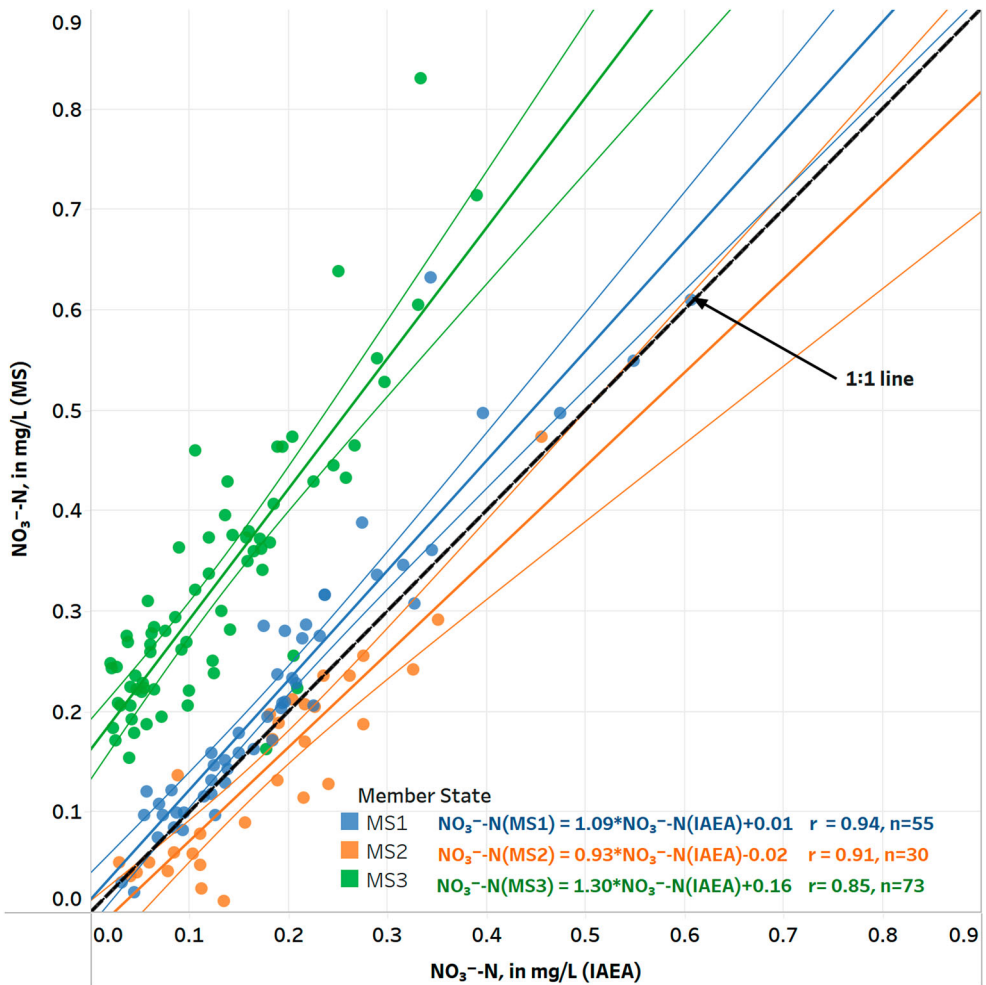
Figure 1. Graph of nitrate concentrations (as N-NO$_3^-$ in mg/L) measured at three member states (MS) vs. the corresponding concentrations measured at the IAEA laboratory. For each MS, the best-fit linear regression, r value, and *p* value is reported.

regular participation in inter-laboratory exercises. It was not possible to organize an inter-laboratory exercise during this project. To our knowledge, such interlaboratory comparisons of nitrate isotopes in atmospheric waters have not been carried out. Overall, these intercomparison exercises should aim at establishing similar analytical standardization and quality control criteria in the laboratories. For instance, the use of different laboratory consumables (e.g. filters, bottles) or preservation (refrigeration vs. freezing) could explain the different slopes and correlations shown in Figure 1. It is worth noting that despite the lack of intercomparison tests, efforts to identify global ^{15}N patterns in precipitation by aggregating literature data produced with different nitrate isotope analytical techniques already exist in the literature (e.g. [10]).

3.2. Spatial and temporal variations of $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^-

Spatial and temporal variations in $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- values in atmospheric precipitation reflect the combined influence of precursor NO_x emissions, land use change, fluctuations in reaction chemistry, and/or fractionation factors that are temperature dependent [11,23,24]. NO_x emissions from fossil fuel combustion by vehicles, electricity generation, and industries typically show higher $\delta^{15}N$-NO_3^- values (from –20 to +26 ‰) compared to other sources such as biogenic/fertilized soil emissions, lightning, and biomass burning (from –60 to +12 ‰). Even though the $\delta^{15}N$ values of NO_x emitted by vehicles and produced in biomass burning largely overlap, the $\delta^{15}N$ of vehicular NO_x emissions measured in urban areas are considerably higher than other sources like agricultural soils [11 and references herein]. We reported nitrate isotope data from 4 participating countries located at different longitudes (between –84°W and 156°E). The $\delta^{15}N$-NO_3^- values in precipitation ranged from –14.8 to +7.1 ‰ (Figure 2, top panel). Austria, Australia, and Thailand showed similar ranges of $\delta^{15}N$-NO_3^- values, indicating multiple common NO_x emission sources (e.g. agricultural areas, biomass burning, vehicles, etc.) but averaging closer to vehicle signatures [25]. Costa Rica showed the highest $\delta^{15}N$-NO_3^- values
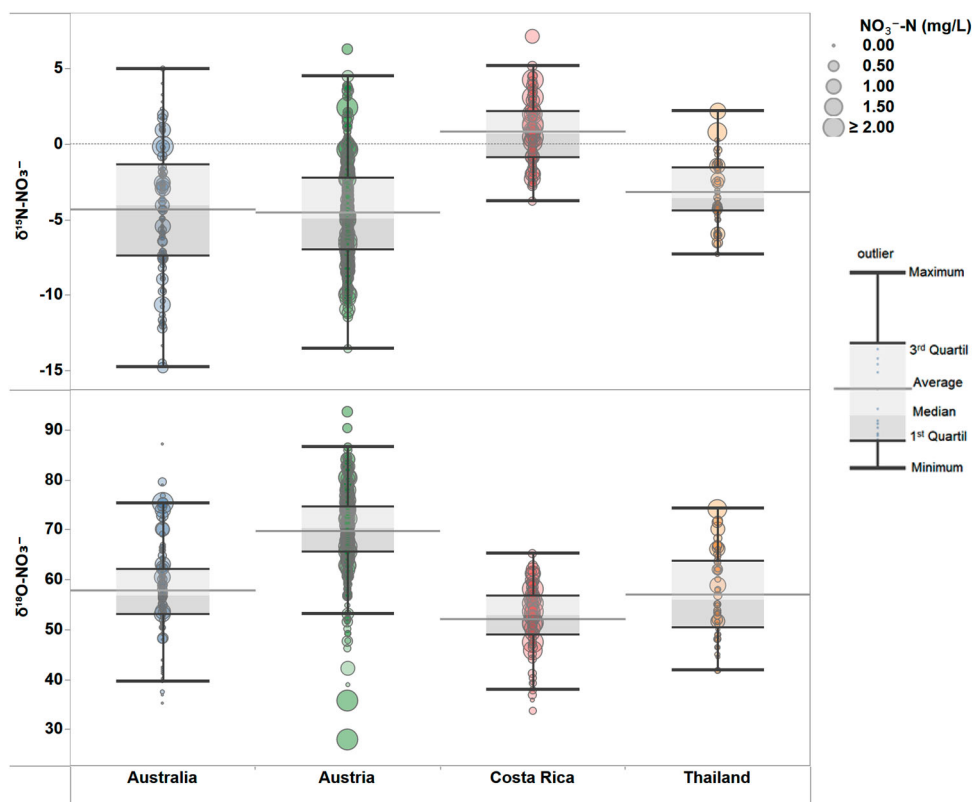

Figure 2. Box plots of $\delta^{15}N$-NO_3^- (‰, top panel) and $\delta^{18}O$-NO_3^- (‰, bottom panel) in 4 selected sites. The central box indicates the 25th and 75th percentiles whereas the error bars indicate the minimum and maximum values. The size of each circle is directly proportional to the nitrate concentration in each sample.

that probably reflect contributions from fossil fuel sources combined with natural sources like lightning or biomass burning. Overall, $\delta^{15}N$-NO_3^- values are greatly impacted by land use changes, namely higher population densities (i.e. urbanization) and reduced forest cover (i.e. conversion into agricultural land) [10,26,27]. $\delta^{18}O$-NO_3^- values varied from +27.9 to +93.6 ‰ (Figure 2, bottom panel). These isotope values can give insights into changes in the atmospheric chemistry that converts NO_x to nitrate and the dominant nitrate formation processes, namely the oxygen transfer to NO_x during the oxidation via OH (daytime) and N_2O_5 (nighttime) [12,28]. In Austria and Australia, lower $\delta^{18}O$-NO_3^- can be attributed to summer conditions due to higher OH densities, which results in the dilution of the isotopic O_3 signal in NO_x by the reaction of NO_2 with OH. In turn, the N_2O_5 pathway is more important in winter than in summer due to the longer night-time, which leads to higher $\delta^{18}O$-NO_3^- values [29,30]. In Costa Rica and Thailand, the lower $\delta^{18}O$-NO_3^- values suggest complex oxidation pathways mostly controlled by hydroxyl and peroxy radicals but also the possible influence of organic nitrate chemistry [6,8,31]. In general, the oxidation of NO_x in convective clouds (e.g. cumulus or cumulonimbus) in the tropics is related to strong vertical motions which can efficiently transport OH and RO_2 to the upper troposphere and then react with NO_x by the RO_2 pathway and decreasing the $\delta^{18}O$-NO_3^- [31,32]. We also found significant Pearson correlation coefficients (r) at $p < 0.0001$ between $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- with the latitude of the sampling sites (r = − 0.363 and r = + 0.617, respectively). The observed latitudinal variation in $\delta^{18}O$-NO_3^- values may be associated with changes in the oxygen isotopic composition of atmospheric O_3 with latitude [33]. As the O_3 production rate from UV radiation is highest on average near the tropics, the large-scale atmospheric circulation carries the tropical tropospheric O_3 to the poles, which accumulates the ozone at middle and high latitudes, increasing the total ozone available for atmospheric oxidation [33,34]. As for the $\delta^{15}N$-NO_3^- values, the correlation observed with latitude indicates the higher contribution of non-fossil fuel N sources such as soil emissions and biomass burning in low latitude regions [10].

To investigate temporal variations of nitrate isotopes and ensure consistency, seasons were identified separately for the Northern and Southern Hemispheres using the reported sampling month. The lowest $\delta^{15}N$-NO_3^- values were recorded in Australia and Austria at the beginning of the austral autumn (i.e. March) and during the boreal spring (i.e. May), respectively (Figure 3). At Austria and Australia, the highest $\delta^{15}N$-NO_3^- values were recorded in September and November. In Costa Rica, an increasing trend in $\delta^{15}N$-NO_3^- was registered from May to August, and the highest nitrate isotope values were observed during the rainiest months (i.e. September and October). In Thailand, there was an increasing trend in $\delta^{15}N$-NO_3^- from May to November, but the highest $\delta^{15}N$-NO_3^- values were recorded in April. The temporal variation of $\delta^{15}N$-NO_3^- values showed variability in the emission sources but also possible isotope fractionation effect. For example, higher $\delta^{15}N$-NO_3^- values are favored in lower temperatures and night-time, due to isotope fractionation during chemical transformations of N species in the atmosphere [35]. Austria and Thailand showed a similar temporal pattern with the highest $\delta^{15}N$-NO_3^- values in April and November, compared to Costa Rica and Australia, which showed the opposite. Higher $\delta^{15}N$-NO_3^- values in winter are due to the influence of anthropogenic-related NO_x emissions oxidized via N_2O_5 [4,10].
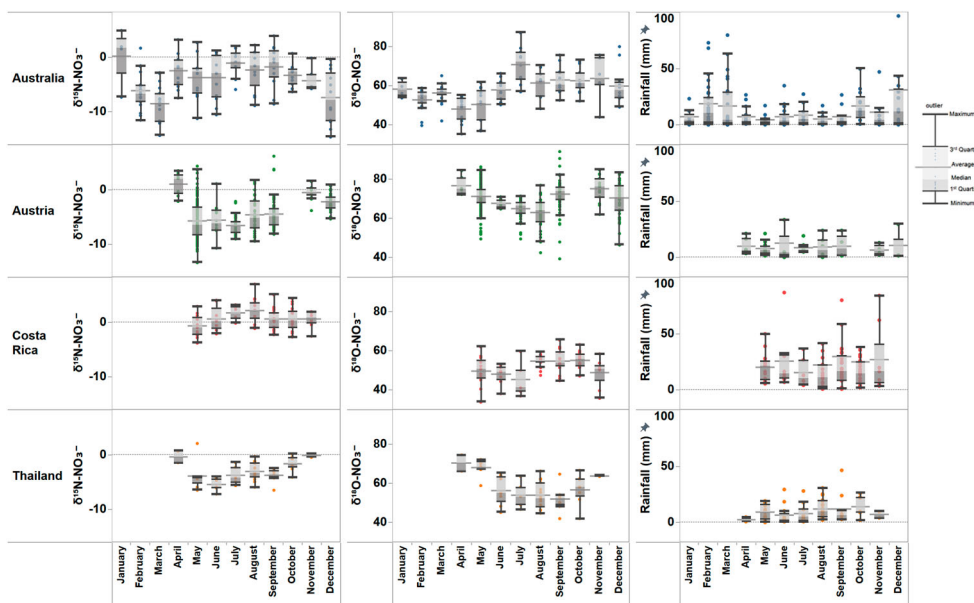
Figure 3. Box plots of monthly δ^{15}N-NO$_3^-$ and δ^{18}O-NO$_3^-$ (‰) and rainfall (mm) measured in Australia (2018–2021; blue circles), Austria (2017–2017; green circles), Costa Rica (2018–2019; pink circles), and Thailand (2019; orange circles). The central box indicates the 25th and 75th percentiles whereas the error bars indicate the minimum and maximum values.

4. Best practices for sampling and analysis of nitrate isotopes in atmospheric waters

4.1. Sampling strategies and monitoring

Systematic precipitation sampling and meteorological monitoring should follow WMO/ GAW precipitation chemistry guidelines [13], which is a key to ensuring dataset applicability for assessing N air pollution using nitrate isotopes (Figure 4). First, the potential sampling sites should be classified based on their location (i.e. urban, industrial, rural, coastal, marine). Site characteristics like climate type, precipitation patterns, seasonality and potential nitrogen emission sources should be registered for future data interpretation. Standardized wet-only and bulk collectors can be used for precipitation sampling, preferably with a 24 h or event-based frequency. Precipitation sampling is recommended using a wet-only collector with a removable lid that protects the collector from contamination during the dry period. However, it has the drawback of being expensive, requiring a power supply and sensors. Bulk precipitation collectors are cheaper but require staff to collect the samples immediately after each event to avoid contamination (e.g. dust, aerosols, and organics). For example, passive collectors typically used for stable water isotopes (^{18}O and ^{2}H) and tritium are proven to be reliable for precipitation sampling as confirmed by the results shown in Figure 1 [36]. HDPE bottles ensure durability and resistance during sampling, transportation, and preservation of sample volume to each laboratory. Samples should be filtered using 0.2–0.45 μm syringe membranes and stored in dark and cool conditions (< 4 °C). A small aliquot to be analyzed for nitrate isotopes should be stored in
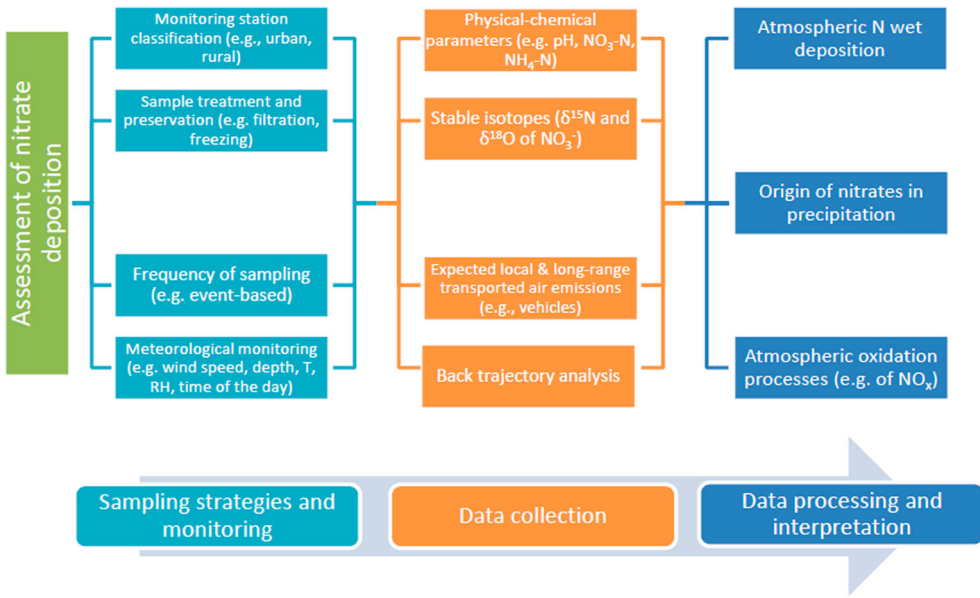
Figure 4. Flow chart of steps for the assessment of nitrate deposition.

frozen conditions until analysis. Systematic recording of meteorological information (e.g. collection time, wind speed and direction, precipitation depth, air temperature, relative humidity) retrieved from weather stations in the vicinity of the sampling sites is highly useful for nitrate isotope data interpretation.

4.2. Data collection

Chemical ion concentrations, particularly of N species (NO_3^-, NO_2^- and NH_4^+ as N), and other parameters (e.g. pH and EC), are considered important for N air pollution assessment and should be analyzed as soon as possible after the sample collection. This information could reveal differences in the concentrations of N species like those found in the precipitation collected in the 4 selected sites. For instance, the highest NH_4^+/NO_3^- equivalent ratio was found in Thailand (7.0), where NH_4^+ was also the most prominent ion specie (28% of total ion concentration). The corresponding equivalent ratios in precipitation collected in Austria and Australia were 4.6 and 5.9, whereas in Costa Rica this equivalent ratio was 2.9. These differences may reflect a higher contribution from terrestrial sources (e.g. N soil emissions) in Austria, Australia, and Thailand than in Costa Rica. The analytical method (i.e. ion chromatography and FIA) for nitrate allow precise and accurate measurements in the range of 0.01–5 mg/L, which requires a more sensitive lower limit typically used for surface water monitoring methods. Particularly, for δ^{15}N-NO_3^- and δ^{18}O–NO_3^- analysis, a larger volume ($\sim$ 25 mL) of the sample must be available when the concentrations fall in the range from 0.01–0.1 mg/L as N. The relative standard deviation of the nitrate concentration should be < 10 %. Quality control of the analysis results through ion balance calculations is highly recommended, as by WMO/GAW [13]. A survey to identify all possible local and distal NO_x air pollution sources (e.g. vehicles,

industries, agricultural areas, biomass burning) in the study area and the application of backward trajectories analysis for rain events using existing models (e.g. Hybrid Single Particle Lagrangian Integrated Trajectory – HYSPLIT) are considered complementary, but highly recommended [37,38].

4.3. Data processing and interpretation

Atmospheric wet deposition is a predominant sink of N_r for anthropogenic aerosols and an important pathway that can potentially impact terrestrial and aquatic ecosystems. Therefore, it is highly relevant to calculate N wet deposition rates using standardized methods [13]. Statistical data processing (e.g. regression models, principal component analysis) to identify correlations between variables (e.g. air temperature, rainfall, ion composition) and statistical differences between sample groups is important, particularly for seasonal analysis. The origin of nitrates in precipitation is reflected in nitrate isotopes of rainwater samples [11]. Given the expected overlapping ranges of $\delta^{15}N\text{-}NO_3^-$ values, the statistical processing of the complementary information should be done during the data collection to disentangle the local and distal sources. Nitrate isotopes, and particularly $\delta^{18}O\text{-}NO_3^-$ are important to understand NO_x atmospheric oxidation processes that may be seasonally variable. These processes are also likely to influence $\delta^{15}N\text{-}NO_3^-$ values. Thus, the identification of fractionation factors ε during NO_x oxidation and HNO_3 formation is highly recommended, especially when nitrate isotopes are to be used in source tracking analysis (e.g. MixSIAR) [39].

Overall, this work showed that systematic precipitation sampling and preservation strategies yielded good agreement between the nitrate concentrations measured locally at the collection sites and centrally at the IAEA. This approach can reduce the biases related to the sample collection and storage. These practices in combination with the new Ti(III) reduction method, can also improve the isotope data interpretation and make these results globally comparable. Obtained data can be linked to chemical data to improve N emissions source and trajectory tracing [10,11]. The proposed methods were tested and validated in 4 sampling sites and laboratories. Results were summarized on the flow chart of steps for assessing N air pollution (Figure 4) that will advance the application of nitrogen and oxygen isotopes in link with chemical substances to better define the origin of N in precipitation and evaluate the impact of N wet deposition on ecosystems. Future studies could focus on characterizing the isotope effects (i.e. isotope fractionations of NO_x during HNO_3 production in the atmosphere) or that can occur during sampling (e.g. temperature, dissolved organic matter in precipitation, microbial effects) as both can affect the reliability of isotopic source compositions and the fingerprints of ambient or deposited oxidized nitrogen. Further investigation should also focus on the discrimination of local from distal NO_x emissions sources, by conducting high-frequency precipitation sampling (e.g. with 5 min intervals).

Declaration of interest statement

The authors declare no competing interests.

Disclosure statement

Funding

ORCID

Ricardo Sánchez-Murillo http://orcid.org/0000-0001-8721-8093
Lucilena R. Monteiro http://orcid.org/0000-0002-4457-4925

References

[1] Decina SM, Hutyra LR, Templer PH. Hotspots of nitrogen deposition in the world's urban areas: a global data synthesis. Front Ecol Environ. 2019;18:92–100.

[2] Galloway JN, Dentener FJ, Capone DG, et al. Nitrogen cycles: past, present, and future. Biogeochem. 2004;70:153–226.

[3] Kendall C, Elliott EM, Wankel SD. Tracing anthropogenic inputs of nitrogen to ecosystems. In: Michener R, Lajtha K, editor. Stable isotopes in ecology and environmental science. Malden, MA: Blackwell Publishing Ltd; 2007. p. 375–449.

[4] Elliott EM, Kendall C, Wankel SD, et al. Nitrogen isotopes as indicators of NO_x source contributions to atmospheric nitrate deposition across the Midwestern and Northeastern United States. Environ Sci Technol. 2007;41:7661–7667.

[5] Fang YT, Koba K, Wang XM, et al. Anthropogenic imprints on nitrogen and oxygen isotopic composition of precipitation nitrate in a nitrogen-polluted city in Southern China. Atmos Chem Phys. 2011;11:1313–1325.

[6] Felix JD, Murgulet D. Nitrate isotopic composition of sequential Hurricane Harvey wet deposition: Low latitude NO_x sources and oxidation chemistry. Atmos Environ. 2020;238:117748.

[7] Matiatos I, Wassenaar LI, Monteiro LR, et al. Isotopic composition ($\delta^{15}N$, $\delta^{18}O$) of nitrate in high-frequency precipitation events differentiate atmospheric processes and anthropogenic NO_x emissions. Atmos Res. 2022;267:105971.

[8] Villalobos-Forbes M, Esquivel-Hernández G, Sánchez-Murillo R, et al. Stable isotopic characterization of nitrate wet deposition in the tropical urban atmosphere of Costa Rica. Environ. Sci Pollut Res. 2021;28:67577–67592.

[9] Fibiger DL, Hastings MG. First measurements of the nitrogen isotopic composition of NO_x from biomass burning. Environ Sci Technol. 2016;50:11569–11574.

[10] Song W, Liu XY, Hu CC, et al. Important contributions of non-fossil fuel nitrogen oxides emissions. Nat Commun. 2021;12:243.

[11] Elliot EM, Yu Z, Cole AS, et al. Isotopic advances in understanding reactive nitrogen deposition and atmospheric processing. Sci Total Environ. 2019;662:393–403.

[12] Michalski G, Bhattacharya SK, Mase DF. Oxygen isotope dynamics of atmospheric nitrate and its precursor molecules. In: Baskaran M, editor. Handbook of environmental isotope geochemistry, 1st ed. Berlin, Germany: Springer; 2011. p. 613–635.

[13] Allan MA. Manual for the GAW Precipitation Chemistry Programme: Guidelines, Data Quality Objectives and Standard Operating Procedures. Geneva: World Meteorological Organization; 2004.

[14] Ricci M, de Vos E, Oostra A, et al. Certification Report: Certification of the mass concentrations of ammonium, chloride, fluoride, magnesium, nitrate, ortho-phosphate, sulfate, and of pH and conductivity in simulated rainwater. Certified Reference Material ERM®-CA408. Geel: European

Commission, Joint Research Centre Institute for Reference Materials and Measurements (IRMM); 2010.

[15] Chansuebsri S, Kraisitnitikul P, Wiriya W, et al. Fresh and aged PM2.5 and their ion composition in rural and urban atmospheres of Northern Thailand in relation to source identification. Chemosphere. 2022;286:131803.

[16] Altabet MA, Wassenaar LI, Douence C, et al. A Ti(III) reduction method for one-step conversion of seawater and freshwater nitrate into N_2O for stable isotopic analysis of $^{15}N/^{14}N$, $^{18}O/^{16}O$ and $^{17}O/^{16}O$. Rapid Commun Mass Spectrom. 2019;33:1227–1239.

[17] McIlvin MR, Casciotti KL. Technical updates to the bacterial method for nitrate isotopic analyses. Anal Chem. 2011;83:1850–1856.

[18] Erler DV, Santos IR, Zhang Y, et al. Nitrogen transformations within a tropical subterranean estuary. Mar Chem. 2014;164:38–47.

[19] Murray R, Erler D, Rosentreter J, et al. A seasonal source and sink of nitrous oxide in mangroves: Insights from concentration, isotope, and isotopomer measurements. Geochim Cosmochim Acta. 2018;238:169–192.

[20] Wells NS, Eyre BD. Flow regulates biological NO_3^- and N_2O production in a turbid sub-tropical stream. Geochim Cosmochim Acta. 2021;306:124–142.

[21] Xu S, Kang P, Sun Y. Stable isotope approach and its application for identifying nitrate source and transformation process in water. Environ Sci Pollut Res. 2016;23:1133–1148.

[22] Silva SR, Kendall C, Wilkison DH, et al. A new method for collection of nitrate from fresh water and the analysis of nitrogen and oxygen isotope ratios. J Hydrol. 2000;228:22–36.

[23] Felix JD, Elliott EM, Shaw SL. Nitrogen isotopic composition of coal-fired power plant NO_x: Influence of emission controls and implications for global emission inventories. Environ Sci Technol. 2012;46:3528–3535.

[24] Hastings MG, Jarvis JC, Steig EJ. Anthropogenic impacts on nitrogen isotopes of ice-core nitrate. Science. 2019;324:1288–1288.

[25] Miller DJ, Wojtal PK, Clark SC, et al. Vehicle NO_x emission plume isotopic signatures: Spatial variability across the eastern United States. J Geophys Res Atmos. 2017;122:4698–4717.

[26] Almaraz M, Bai E, Wang C, et al. Agriculture is a major source of NO_x pollution in California. Sci Adv. 2018;4:eaao3477.

[27] Hastings MG, Casciotti KL, Elliott EM. Stable isotopes as tracers of anthropogenic nitrogen sources,: deposition, and impacts. Elements. 2013;9:339–344.

[28] Altieri KE, Hastings MG, Gobel AR, et al. Isotopic composition of rainwater nitrate at Bermuda: the influence of air mass source and chemistry in the marine boundary layer. J Geophys Res Atmos. 2013;118:11,304–11,316.

[29] Savarino J, Morin S, Erbland J, et al. Isotopic composition of atmospheric nitrate in a tropical marine boundary layer. Proc Natl Acad Sci USA. 2013;110:17668–17673.

[30] Xiao HW, Xie LH, Long AM, et al. Use of isotopic compositions of nitrate in TSP to identify sources and chemistry in South China Sea. Atmos. Environ. 2015;109:70–78.

[31] Alexander B, Sherwen T, Holmes CD, et al. Global inorganic nitrate production mechanisms: comparison of a global model with nitrate isotope observations and Prasad Kasibhatla. Atmos Chem Phys. 2020;20:3859–3877.

[32] Aggarwal PK, Romatschke U, Araguas-Araguas L, et al. Proportions of convective and stratiform precipitation revealed in water isotope ratios. Nat Geosci. 2016;9:624–629.

[33] Hauglustaine DA, Brasseur GP. Evolution of tropospheric ozone under anthropogenic activities and associated radiative forcing of climate. J Geophys Res. 2001;106:32337–32360.

[34] Saud S, Fahad S, Hassan S. Developments in the investigation of nitrogen and oxygen stable isotopes in atmospheric nitrate. Sustain Chem Clim Action. 2022;1:100003.

[35] Walters WW, Michalski G. Theoretical calculation of oxygen equilibrium isotope fractionation factors involving various NO_y molecules, OH, and H_2O and its implications for isotope variations in atmospheric nitrate. Geochim Cosmochim Acta. 2016;191:89–101.

[36] Gröning MH, Lutz O, Roller-Lutz Z, et al. A simple rain collector preventing water re-evaporation dedicated for $\delta^{18}O$ and δ^2H analysis of cumulative precipitation. Samples. J Hydrol. 2012;448:195–200.

[37] Stein AF, Draxler RR, Rolph GD, et al. NOAA's HYSPLIT atmospheric transport and dispersion modeling system. Bull Am Meteorol Soc. 2015;96:2059–2077.
[38] Monteiro LR, Terzer-Wassmuth S, Matiatos I, et al. Distinguishing in-cloud and below-cloud short and distal N-sources from high-temporal resolution seasonal nitrate and ammonium deposition in Vienna, Austria. Atmos Environ. 2021;266:118740.
[39] Parnell A, Inger R. Simmr: a stable isotope mixing model. 2016. [Accessed 10 February 2021]. Available from https://cran.r-project.org/web/packages/simmr/vignettes/simmr.html.