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Technical note: Cavity ring-down spectroscopy is a precise and accurate method for measuring ^{15}N-NO_3^- in soil extracts from ^{15}N tracer experiments

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Abstract. Cavity ring-down spectroscopy (CRDS) provides a low-cost alternative to isotope ratio mass spectrometry (IRMS) for the analysis of ^{15}N-NO_3^- in nitrogen cycle assays, but its performance has not been tested systematically for highly enriched samples. Here we report results from 271 parallel analyses of ^{15}N-NO_3^- in soil extracts after chemical conversion to N_2O, including low (0.36-1.45 at%) and high labelled samples (0.46-8.49 at%). We demonstrate that CRDS measurements deliver comparable accuracy and precision to continuous-flow Gas Bench-IRMS analysis (slope: 0.982 and 0.972, RMSE: 0.0018 and 0.254 at% for low- and high-labelled samples, respectively), thereby offering a robust and affordable alternative for classic ^{15}N-NO_3^- analysis.

Keywords: Gross nitrification rate, soil nitrogen cycle, ^{15}N tracing, isotope pool dilution, ^{15}N-nitrate

Accurate determination of soil nitrogen (N) transformation rates has become increasingly critical due to the global intensification of the N cycle, which stems from mineral fertilizer application, land-use changes, and climate-driven acceleration of N turnover in natural ecosystems (Fowler et al., 2013; Gruber & Galloway, 2008). Within this framework, nitrification represents a pivotal process governing terrestrial N retention and loss, as it directly drives both the emission of gaseous N species to the atmosphere and the leaching of mobile nitrate (NO_3^-) into aquatic systems (Niu et al., 2016). Quantification of gross soil N transformation rates routinely relies on stable isotope assays utilizing ^{15}N. These approaches encompass isotope pool dilution techniques, which measure the dilution of an added ^{15}N label within a specific inorganic pool, and tracer experiments, which track the downstream transformation of a labelled species into another pool (Jia et al., 2022). In both methodologies, analysing the ^{15}N:^{14}N ratio of NO_3^- is fundamental to resolving specific transformation pathways (Jia et al., 2022). Conventionally, this isotope analysis requires the conversion of

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NO_3^- to nitrous oxide (N_2O) via chemical reduction (Altabet et al., 2019; Lachouani et al., 2010) or microbiological denitrification (Casciotti et al., 2002; Sigman et al., 2001). The product N_2O is subsequently separated by gas chromatography, cryogenically preconcentrated, and analysed via isotope ratio mass spectrometry (IRMS).

Despite its widespread application, conventional IRMS analysis is constrained by significant operational and financial bottlenecks. These systems are expensive and complex to maintain. They require specialized experts to manage the continuous use of high-purity helium, manual liquid nitrogen refills, and intricate parts like gas benches and cryogenic traps. In contrast, cavity ring-down spectroscopy (CRDS) is emerging as an accessible alternative for the stable isotope analysis of ^{15}N. The CRDS method offers substantially lower capital and running costs, utilizing small volumes of N_2O free air rather than expensive carrier gases. The simplified architecture of this instrument minimizes maintenance requirements, eliminating the need for cryogenic liquids and complex moving parts. While a recent study has demonstrated the efficiency of CRDS for evaluating NO_3^- stable isotopes at natural abundance levels (McKay et al., 2026), its performance has not yet been systematically cross-evaluated against IRMS using ^{15}N-labelled tracer samples. Furthermore, neither analytical platform is natively optimized to accommodate highly enriched isotope signatures, which can introduce analytical challenges such as memory effects or detector saturation. Therefore, a direct comparison between these two independent methods is essential. This will help establish clear operating limits and ensure accurate data at high enrichment levels typical for gross N transformation rate determination.

Here, we present a method of intercomparison study comprising 271 soil extracts analyzed for the ^{15}N:^{14}N ratios in NO_3^-. The sample material originated from a ^{15}N tracer experiment designed to determine mineral N transformation rates in permafrost sediments from disturbance sites in the West Alaskan Yedoma region. The experimental design included two labelling treatments, where either NH_4^+ or NO_3^- pool was enriched with ^{15}N. The NH_4^+ treatment resulted in a set of samples with lower ^{15}N enrichment in NO_3^- as all the ^{15}N-NO_3^- present in the sample came from the conversion of ^{15}N-NH_4^+ by nitrification. We amended soil with ammonium at either 10 or 25 at% ^{15}N-NH_4^+, targeting a ^{15}N abundance of 1–10 at% in the soil NH_4^+ pool. This resulted in NO_3^- extracts with an at% ranging between 0.36 to 1.45 at% ^{15}N. The second set of samples with higher ^{15}N enrichment originated from the NO_3^- treatment, where we targeted an initial enrichment of 1–10 at% in the soil nitrate pool and subsequent dilution of the label according to the principle of isotope pool dilution experiment as NO_3^- is produced from native non-labeled NH_4^+ (Di et al., 2000). This was achieved by amending the

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soil samples with nitrate at either 10 or 98 at% ^{15}N-NO_3^- and resulted in NO_3^- extracts ranging 0.46-8.49 at%. Samples were incubated in the dark at 5°C for 4, 24, 48, or 72 hours followed by extraction with 1M KCl at a 1:3 (v/v) soil-to-solution ratio. Samples were shaken for 1 hour at 21°C and 175 rpm and subsequently filtered using paper filters (Whatman®-Cytiva, Global Life Sciences, UK).

Nitrate in the extracts was converted to N_2O using the Ti (III) Cl_3 method (Altabet et al., 2019), with a detailed description given in the supplementary methods. Prior to the reaction, NO_3^- concentrations in the KCl extracts (0–6000 µmol NO_3^--N L^{-1}) were equalized to a target concentration of 14.3 µmol NO_3^--N L^{-1} by diluting with 1M KCl. Samples falling below the target concentration were excluded. Titanium reduction reactions were conducted in 12 mL glass exetainer vials (Labco®, Lampeter, UK). Within each reaction batch of 70–80 number of samples, we included standards (STDs, also in 1M KCl) with a target concentration and four different at% levels (natural abundance, 2, 5, and 10 at%) as well as analytical blanks (1M KCl). For isotope analysis, 1 mL of headspace gas was transferred from each reaction vial into a separate secondary exetainer vial, evacuated and prefilled with 25 mL N_2O free synthetic air (5.0 purity). To prevent cross-contamination, a separate syringe was used for each treatment. The secondary exetainer was analyzed via CRDS, while the remainder of the gas in the original reaction exetainer was analyzed by GB-precon-IRMS.

CRDS measurements were performed using a Picarro G5131-i isotopic N_2O analyzer coupled with a custom-built autosampler (Open Autosampler SAM, Frank Krijnen, University of Saskechewan, Wozniak, 2025). To introduce samples to the CRDS analyzer, the transfer line and autosampler needle were flushed with synthetic air for 3 min. The autosampler needle was then lowered into a vial, and the sample was pulled into the analyzer inlet through a moving needle for 8 min. The instrument regulates the cavity pressure through a metered valve placed between cavity and outlet vacuum pump. Placing the needle in a vial therefore means that sample air is pulled into the cavity until the pressure in the vial drops to cavity pressure, at which point the instrument measures the air volume in the cavity until the needle is retracted to ambient air. Isotope ratios were calculated by averaging the measured $\delta^{15}N$ ratios during the last 6 minutes of the measurement. Complementary GB-Precon-IRMS analyses were conducted using a Thermo Delta V Advantage IRMS equipped with Gas Bench II and a PreCon unit. Finally, raw isotope ratios were converted to at% and calibrated against standards using batch-specific linear regressions (Figure 1). This calibration also served to correct the significant blank caused by N contamination present in the sample matrix (1M KCl, Figure S1).

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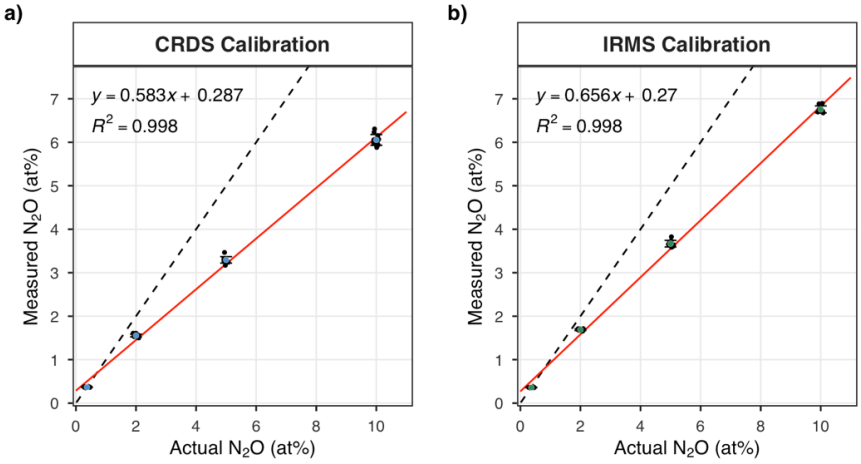

Figure 1. Calibrations for N_2O atom percent (at%) analysis by **a)** CRDS (Picarro G5131-i) and **b)** IRMS (GB-Precon-IRMS) based on standards with natural abundance, 2 at%, 5 at%, and 10 at% of ^{15}N-N_2O. Solid lines show the linear regression fit, and dashed lines show the 1:1 relationship (perfect agreement between measured and actual values). Small grey circles are individual measurements of each standard; the filled circle (black) is the mean, with error bars representing standard deviation. Regression equations and R^2 values are shown in each panel.

We found strong agreement between IRMS and CRDS measurements across all samples (R^2 = 0.998 for each treatment/both treatments) (Figure 2). For uncorrected at% data, the precision (root mean square error; RMSE) was 0.036 at% for the low labelled samples from the $^{15}NH_4^+$ treatment and 0.745 at% for the highly labelled samples from the $^{15}NO_3^-$ treatment, with an accuracy (slope) of 0.873 ± 0.003 and 0.864 ± 0.004, respectively (**Figure 2a, c**). Applying the at% correction narrowed the data scatter, improving precision to 0.0018 at% (NH_4^+) and 0.254 at% (NO_3^-), while the slopes shifted to 0.982 ± 0.004 and 0.972 ± 0.004 (**Figure 2b, d**). Because neither instrument is optimized for highly labelled samples (>3 at%), this excellent agreement across a wide enrichment range highlights the robustness of both analytical methods.

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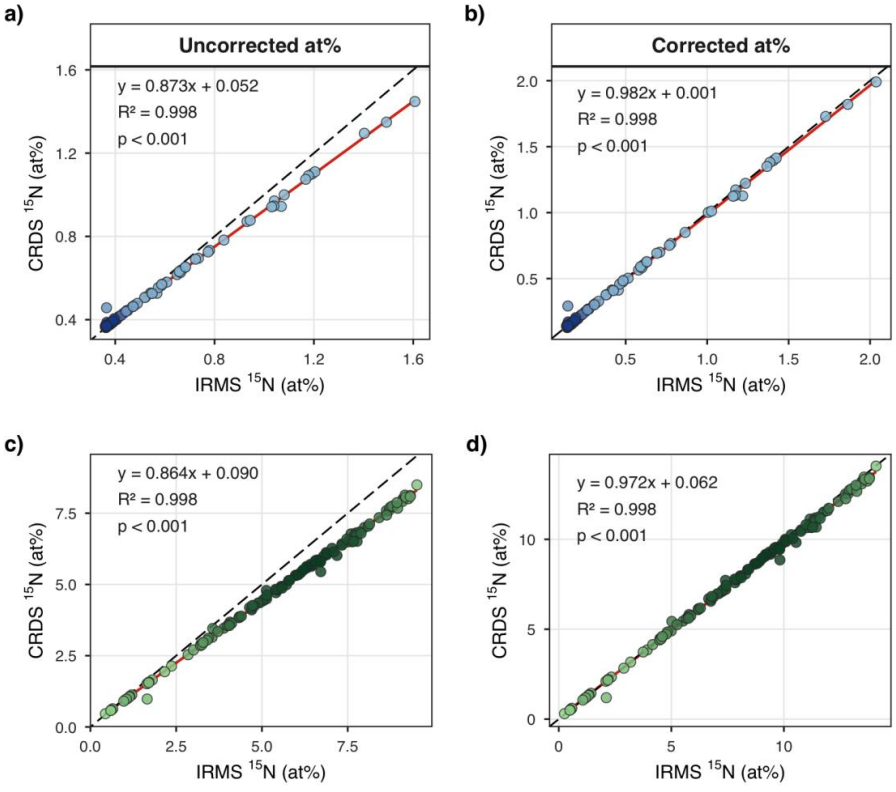

Figure 2. Comparison of CRDS and IRMS measurements for ^{15}N-$^{15}NO_3^-$ at% in soil extracts. Left column shows uncorrected measurements for samples originating from (**a**) $^{15}NH_4^+$ and (**c**) $^{15}NO_3^-$ labelled treatments. The right column shows blank and at% corrected measurements for (**b**) $^{15}NH_4^+$ labelled samples and (**d**) $^{15}NO_3^-$ labelled samples. Solid red lines represent the linear regression fit; dashed lines represent the perfect 1:1 instrumental agreement.

To validate even further the efficacy of CRDS for gross N transformation assays, we compared gross nitrification rates calculated using the Kirkham and Bartholomew (1955) model (Figure 3). This comparison was performed on samples from four randomly selected isotope pool dilution assays. Again, we find a high degree of agreement between the two methods. The integrated average rate for 68-hour interval was identical between both CRDS and IRMS (1.18 $\mu g\ Ng^{-1}d^{-1}$), which is commonly reported in N cycling. The convergence of these values demonstrates that CRDS provides a robust, high-throughput alternative to traditional IRMS for measuring gross N transformation rates in biogeochemical ^{15}N field studies utilizing ^{15}N-NO_3^- measurements.

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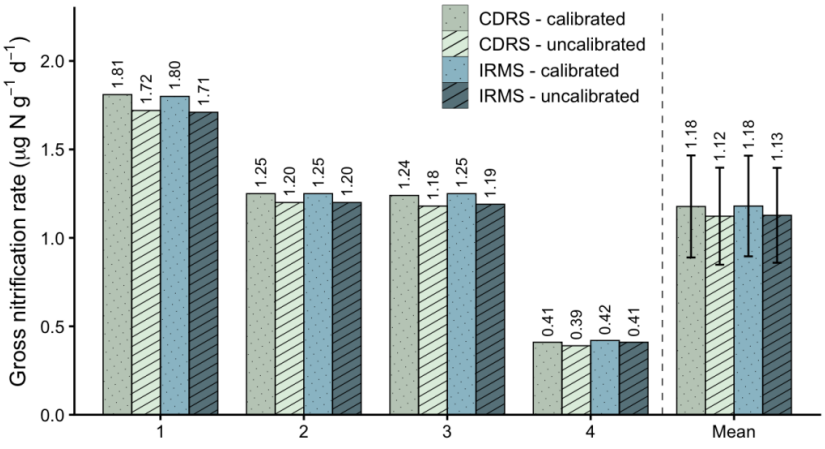

Figure 3. Comparison of gross nitrification rates (µg N $g^{-1}d^{-1}$) across four individual assays (1–4) and their overall mean. Rates were measured using both Cavity Ring-Down Spectroscopy (CDRS) and Isotope Ratio Mass Spectrometry (IRMS). Dotted bars represent calibrated rates, while patterned bars represent uncalibrated rates. Values above each bar indicate the calculated rate. Error bars on the mean bars represent the standard error (SE, n = 4).

As demonstrated above, CRDS provides precision and accuracy comparable to conventional IRMS for determining ^{15}N at% in soil NO_3^- from highly enriched samples. Furthermore, CRDS operates effectively at lower N_2O concentrations than IRMS. Consequently, future studies should evaluate the performance of this method across different soil types and NO_3^- concentrations below the 14.3 µmol N L^{-1} targeted here. Given its reduced consumable costs and lower maintenance requirements, CRDS represents an attractive alternative to IRMS, potentially facilitating the broader adoption of ^{15}N tracer techniques in N cycle research.

Author contribution. MEM and LK conceptualized the study, supervised and acquired funding. WH conducted the experiment and laboratory analysis with the help of PMRM, JG, MEM and LK. WH, MEM, PMRM, JG, LK, processed the data. WH, MEM and LK drafted the manuscript after which WH finalize it. All authors reviewed the manuscript.

Code and data availability. The code and data that support the findings of this study is openly available at https://doi.org/10.6084/m9.figshare.33328221

Competing interests. The authors declare that they have no conflict of interest.

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