Title: Dynamics of N_2O in groundwater at the aquatic-terrestrial interface.

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Running title: N_2O at the aquatic-terrestrial interface.

Abstract

Few data are available to validate the Intergovernmental Panel on Climate Change's emission factors for indirect emissions of N_2O. In particular the N_2O emissions resulting from nitrogen leaching and the associated groundwater and surface drainage (EF5-g) are particularly poorly characterized. *In situ* push-pull methods have been used to identify the fate of NO_3^- in the groundwater. In this study, we adapted a previously published *in situ* denitrification push-pull method to examine the fate of $^{15}N_2O$ introduced into the subsoil-groundwater matrix. Enriched $^{15}N_2O$ was manufactured, added to groundwater via a closed system in the laboratory, and then introduced into the groundwater-subsoil matrix in an upland-marsh transition zone of a salt marsh and a forested alluvial riparian zone. Conservative tracers (SF_6 and Br^-) and $^{15}N_2O$ were injected into the groundwater and left for 1 to 4 h after which the groundwater was sampled. Added $^{15}N_2O$ behaved in a conservative manner at one site while the other site showed variability with some injections showing significant consumption (3 - 8 μg N_2O-^{15}N kg^{-1} soil d^{-1}) of $^{15}N_2O$. Our results show that the fate and dynamics of N_2O in groundwater are complex and variable and that these dynamics should be considered in the development of improved IPCC inventory calculations.

Introduction

The atmospheric concentration of nitrous oxide (N_2O) has increased since pre-industrial times and continues to do so. The United Nations Framework Convention on Climate Change (UNFCCC) established the Kyoto protocol which requires participating countries to either reduce or take responsibility for their excess greenhouse gas (GHG) emissions. This necessitates signatories of the Kyoto protocol to develop and publish national inventories of anthropogenic GHG emissions on an annual basis. Guidelines for constructing national inventories have been prescribed by the Intergovernmental Panel on Climate Change (IPCC).

In the IPCC methodology, agricultural sources of N_2O are partitioned into three categories: direct emissions from agricultural land, emissions from animal waste systems, and indirect emissions associated with nitrogen (N) that is removed in biomass, volatilized, leached, or exported from agricultural land (Mosier *et al.*, 1998). These categories are thought to contribute an equal 1/3 share of the total estimated agricultural N_2O source but with 2/3 of the uncertainty in the total agricultural source due to the wide range of estimates for indirect emissions (Nevison, 2000). Globally the total agricultural N_2O source equals 6.3 Tg N yr^{-1} (Mosier *et al.*, 1998) with 2.1 Tg N yr^{-1} coming from indirect emissions. The predominate sources of the indirect emissions, over 75%, are associated with N leaching and runoff (Mosier *et al.*, 1998, Nevison, 2000). Nitrogen in leachate and runoff enters groundwater, riparian zones, wetlands, rivers, and oceans.

The IPCC methodology provides estimates of the amount of N leached (NLEACH) based on the amount of N input, assuming a certain fraction

(FRACLEACH) of these inputs are lost to leaching and runoff (Mosier *et al.*, 1998). The emissions of N_2O arising from leached nitrogen i.e. N2O(L) are calculated as follows:

N2O(L) = NLEACH*EF5

The N_2O emissions resulting from NLEACH are assumed to evolve from: 1) groundwater and surface drainage (EF5-g), 2) rivers (EF5-r), and 3) coastal marine areas (EF5-e). Combined these factors represent the emission factor EF5, thus:

EF5 = EF5-g + EF5-r + EF5-e

The default value for EF5 is 0.025 kg N_2O-N kg^{-1} NLEACH (Mosier *et al.*, 1998), with component values of 0.015 for EF5-g, 0.0075 for EF5-r and 0.0025 for EF5-e. The value for EF5- is based on observations of supersaturated concentrations of N_2O in drainage waters due to leaching of N_2O from soil towards ground waters, or production of N_2O in the groundwater via nitrification or denitrification, and the idea that this N_2O eventually degasses to the atmosphere. In contrast with direct emissions, there are very few data available to validate the IPCC emission factors for indirect emissions (Groffman *et al.*, 2002). The IPCC model for estimating N_2O emissions from groundwater is highly uncertain because the controls on both N_2O production and consumption in groundwater are not well understood. Nevison (2000) noted that the default value for EF5-g was based on a literature review of only 6 studies and concluded that the EF5-g default factor should perhaps be reduced to a value of 0.001. More recent studies have also highlighted uncertainties with the magnitude of the default factors that comprise the EF5. (Reay *et al.*, 2003, Clough *et al.*, 2006). We are not aware of any published studies that have tried to determine the potential for

reduction of N_2O in groundwater. It is clear that further studies are needed to examine the fate of N_2O in groundwater.

In situ push-pull methods have been used to study the fate of nitrate (NO_3^-) and its potential denitrification rate in the subsoil- groundwater matrix (Istok *et al.*, 1997, Addy *et al.*, 2002), yet there has been no analysis of the fate of intermediate denitrification end-products such as N_2O. Addy *et al*. (2002) used a tracer gas (sulphur hexafluoride, SF_6) as a conservative tracer in their push-pull studies, suggesting that gases could be successfully traced with these methods. Few studies have directly measured N_2O consumption rates in soils (Kroeze *et al.*, 1989, Hénault *et al.*, 2001, Mei *et al.*, 2004) let alone the vadose zone. The use of ^{15}N labeled NO_3^- can be used to determine the rate of N_2O production. However, the determination of $^{15}N_2O$ consumption rates is hampered by ongoing production of $^{15}N_2O$ from the $^{15}NO_3^-$ pool. Thus only a net measure of N_2O is achieved i.e. the difference between production and consumption of N_2O. To accurately determine $^{15}N_2O$ consumption rates there is a need to decouple the production of $^{15}N_2O$ from the $^{15}NO_3^-$ pool. Clough *et al*. (2005) recommended the use of $^{15}N_2O$ as a starting substrate in order to better identify the fate of the N_2O molecule. The introduction of $^{15}N_2O$ into the subsoil-groundwater matrix, along with a conservative tracer, could potentially provide data on the fate and production of N_2O below ground. Thus the objectives of this study were to adapt the *in situ* push-pull method of Addy *et al*. (2002) to allow the introduction of dissolved $^{15}N_2O$ into the subsoil-groundwater matrix and to assess the potential for ^{15}N labeled N_2O consumption at two sites with differing denitrification potentials.

Materials and Methods

Study sites and piezometers

Site A was located on the tidally influenced Brushneck Cove of Narragansett Bay, Warwick, RI (41^{o}41' N, 71^{o}24' W) and has been previously described in detail by (Addy *et al.*, 2005). In brief, the site was situated in the transition area between a salt marsh and an upland area with an average slope of 10%. There was no organic horizon present and tidal inundation is rare at this site. The soil was classified as a mixed Mesic Typic Psammequents (Soil Survey Staff, 1998) with vegetation dominated by marsh elder (*Iva frutescens* Pursh var. *oraria*), sea lavender (*Limonium carolinianum*), and seaside goldenrod (*Solidago sempervirens* L.).

A second-order tributary of the Pawcatuck river, 'Meadow Brook', situated in Richmond, RI (41^{o}29' N, 71^{o}41' W) was the location of site B, previously described in detail by Kellogg *et al*. (2005). Briefly, this site was situated on an alluvial soil classified as a coarse-loamy Mesic Fluvaquentic Endoaquept (Soil Survey Staff, 1998), 7 m from the stream, with vegetation dominated by red maple (*Acer rubrum* L.), highbush blueberry (*Vaccinium corymbosum* L.), and summersweet (*Clethra alnifolia*).

Five years prior to this study, mini-piezometers (0.8 cm o.d.; 2 cm screen length [AMS, American Falls, ID.]) attached to Teflon tubing (0.7 cm o.d) were installed at both field sites. One additional piezometer was installed at site B (replicate 1) immediately prior to the study, giving a total of three piezometers at each site. Details of the piezometer installation methodology have been reported on previously (Addy *et al.*, 2002). The piezometers at sites A and B were at depths of 125 and 65 cm respectively,

with respective ground water tables at depths of 90 and 50 cm. Piezometers at site A were 4 m apart while at site B the piezometers were 5 m apart.

Groundwater extraction, $^{15}N_2O$ labeling, and dosing

Groundwater was extracted from the piezometers at sites A and B using a Masterflex L/S portable peristaltic pump (Cole Parmer, Vernon Hills, IL). This water (10 L aliquots) was brought from the field in plastic carboys and stored overnight at 4^{o}C until it was labeled with sulphur hexafluoride (SF_6). Labeling with SF_6 was achieved by purging the water with an SF_6 gas mixture (100 μL L^{-1} SF_6 in He; Med-Tech, Medford MA.) via a sparge stone for 15 minutes. This also served the purpose of lowering dissolved oxygen (DO) concentrations to ambient levels as noted by Addy *et al*. (2002). Immediately after this, 2 L aliquots of the SF_6-dosed water were decanted into 2 L volumetric flasks and sealed with a Suba-seal. A 5 mL volume of potassium bromide (KBr) solution was then injected into the dose-water within each 2 L volumetric flask to achieve a Br^- tracer concentration of 33 mg L^{-1}.

Water was then labeled with $^{15}N_2O$ that had been previously prepared in the laboratory by adding ^{15}N labeled ammonium nitrate ($NH_4{}^{15}NO_3$, min 98+ atom % ^{15}N, Sigma Aldrich, Cat No. 366536) to unenriched NH_4NO_3 and distilled water to form a well mixed uniform salt solution containing $NH_4{}^{15}NO_3$ (34 atom % ^{15}N excess relative to N_2 in air). This solution was then dried at 80^{o}C to an $NH_4{}^{15}NO_3$ salt. The $NH_4{}^{15}NO_3$ was then weighed out and placed in glass tubes, sealed and baked at 300^{o}C according to (Friedman & Bigeleisen, 1950) generating N_2O. The mass of $NH_4{}^{15}NO_3$ used was adjusted so that the internal pressure inside the glass tubes did not exceed 2 atmospheres. One glass tube was prepared for each dosing event per peizometer. The resulting N_2O

used in dosing the groundwater was enriched in ^{15}N, 34.0 atom % ^{15}N excess relative to N_2 in air (standard error of the mean (SEM) equaled 0.3, n = 9).

Labeling of the water with $^{15}N_2O$ was achieved by scoring the glass tube that contained the $^{15}N_2O$ with a metal file and placing it inside a silicone tube (12.8 mm ID, 2.4 mm wall thickness, Cole Parmer EW-06411-19) that had been stored in a freezer such that one end of the silicone tube was already plugged with a 3 cm length of previously frozen deionized water. The open end of the silicone tube was then capped with a silicone turn-over flange stopper (12.7 mm diameter; Saint-Gobain Verneret 467013-50). Working rapidly, the Suba-seal was then removed from the 2 L flask, the sealed silicone tube was then bent so that the internal glass tube snapped at the scored mark, releasing $^{15}N_2O$ inside the silicone tube. The silicone tube was then placed into the 2 L flask and the Suba-seal repositioned to seal the flask. Within minutes the ice plug inside the silicone tube melted and released the $^{15}N_2O$ into the dose-water. The flask was gently shaken by hand for 2 to 3 minutes and then left overnight (12 h) at 4^{o}C for gases to equilibrate.

The 2 L flasks containing the dose-water were transported to the field sites in coolers. At the field sites, the water table depth was recorded prior to pumping 1 L of water from the well. This was discarded before a further 150 ml ambient pre-dose-water sample was taken and placed in a nalgene screw-top bottle. These pre-dose samples were transported back to the laboratory in a cooler and stored at 4^{o}C prior to chemical analysis. Dissolved oxygen (DO) in the groundwater was measured on site using a portable DO kit (La Motte, Maryland BA.). The dose-water was then readied to be pumped into the peizometers. This was achieved by quickly swapping the Suba-seal on the flask with a

rubber stopper fitted with stainless steel (SS) (6 mm O.D., 4 mm I.D) and copper (3 mm O.D., 2 mm I.D) tubes. The SS tube was the outlet port for the dose-water and was connected to C-Flex tubing (4.8 mm I.D.; Cole Parmer (HV-06424-15) that was then routed through the peristaltic pump to the air tight SS-sampling apparatus (Addy *et al.*, 2002) that was in turn connected to the Teflon tube of the peizometer head (Fig. 1). The copper tubing was attached to a 10 L Tedlar bag filled with He at atmospheric pressure (Fig. 1). Helium entered the flask as the dose-water was pumped out, thus preventing a vacuum being created inside the flask, which could lead to rapid degassing of the gases of interest from the dose-water and/or the possible implosion of the flask. As the dose-water was pumped (8 L h^{-1}) into the well a sub-sample was taken of the dose-water as follows. The first 20 mL of the dose-water pumped was discarded to waste with the following 20 mL collected in a gas tight syringe fitted with a 2-way stop-cock using the gas tight SS-sampling apparatus. Using this apparatus the pumped water flow could be directed either towards a luer-lok fitting attached to a gas syringe or to the well head. This water sample was then injected into a pre-evacuated (- 0.93 atm.) 160 ml serum bottle sealed with a rubber suba-seal. The serum bottle was inverted so that the water sample covered the septa. Then He gas from a Tedlar bag, was released into the inverted serum bottle via a hypodermic needle until atmospheric pressure was attained, as indicated by the cessation of bubbles coming from the hypodermic needle connected to the He supply. Water samples were then transported back to the laboratory in a cooler and kept at 4^{o}C until analysis, within 24 h.

After the dose-water had been pumped into the soil-groundwater matrix it was left to incubate for a total time of 1.5 and 4.3 h at sites A and B, respectively. These

incubation times were based on previously measured denitrification rates and tracer recoveries at these sites (Addy *et al.*, 2002, Kellogg *et al.*, 2005). Following the incubation period the dose-water was slowly 'pulled' out of each peizometer, using the peristaltic pump (6 L h^{-1}). Samples of the extracted groundwater were taken and treated in an identical manner to the water samples previously described. Six samples were taken from each piezometer when the cumulative volume of water extracted reached 0.22, 0.46, 0.70, 0.94, 1.48, and 2.02 L. Site A was dosed and sampled once, on the 19th August 2005, while site B was dosed and sampled on two occasions, the 22nd and 29th August 2005. These sampling dates at site B are subsequently referred to as site B test-1 and site B test-2, respectively.

Analytical procedures

The headspaces of the serum bottles containing the water samples were analyzed for N_2O and SF_6 concentrations within 12 h. The bottles were brought to room temperature (22°C) over 1 h which reduced the ability of the water sample to contain dissolved gases and enhanced the concentration in the headspace. The serum bottles were then shaken vigorously for 1 minute to bring water and headspace gases to equilibrium. A 0.1 ml gas sample was then taken and manually injected directly into a Hewlett Packard 5890 gas chromatograph (GC) equipped with an electron capture detector (350°C) fitted with a 5.5 m Poropak Q 50/80 mesh column, N_2 carrier gas and a make up gas consisting of Ar (95%) and CH_4 (5%). Retention times for N_2O and SF_6 were 10.5 and 13.7 minutes respectively**.** Reference gas samples were made from gas bottles of known concentration (Med-tech. Meford MA.). A further 15 ml gas sample was also taken from the headspace

of the serum bottle, using a 20 mL glass syringe equipped with a three-way tap to prevent the ingress of atmospheric air, and placed in a pre-evacuated 12 ml Exetainer® (Labco Ltd, U.K). This sample was used for determining the ^{15}N enrichment, of the N_2O using an automated isotope ratio mass spectrometer (PDZ-Europa Ltd 20-20, Crewe U.K) as described by Stevens *et al*. (1993). These gas samples were brought to ambient pressure prior to analysis by using a double ended needle and a beaker of water. One needle was consistently placed at a constant depth in the water while the other pierced the Exetainer®. Upon the cessation of gas bubbles entering the water, the sample was considered to be at atmospheric pressure. The N_2O reference gas (BOC gases) had a ^{15}N enrichment equal to 0.02122 atom % ^{15}N excess relative to ambient N_2 in air.

Concentrations of the N_2O and SF_6 in the water samples were calculated using the headspace gas concentrations, appropriate Bunsen coefficients (Wilhelm *et al.*, 1977, Weiss & Price, 1980) and the equations of Davidson and Firestone (1988). Once headspace gas samples had been taken, the water samples were returned to the 4^{o}C cooler until the analysis for Br^- was performed as described below.

Water samples were analyzed for dissolved organic carbon (DOC), pH, electrical conductivity (EC), Br^-, sulphate (SO_4^{2-}), chloride (Cl^-) and NO_3^- concentrations. Groundwater pH was measured in the field with a hand held meter (Mettler Toledo, Switzerland). The DOC analyses were performed on a Shimadzu organic carbon analyzer fitted with a Shimadzu ASI-5000A autosampler. Water sample EC was determined with a conductivity meter at 25 ^{o}C (APHA 2510). Ammonium analyses were performed using a flow injection analyzer (Alpkem FS3000 twin channel analyser; application notes P/N

A002380 and P/N A002423). Anion analyses were performed using an ion chromatograph (Dionex DX-120 with an AS50 autosampler).

Reduction rates of $^{15}N_2O$, were calculated using the method described by Addy *et al*. (2002). These calculations utilized data from the water samples pulled at 0.22 and 0.46 L and are based on the difference in N_2O concentration and ^{15}N enrichment between the two water samples. Assumptions included a soil bulk density of 1.65 kg m^{-3} and a porosity of 0.38 m^3 pores m^{-3} soil, as previously reported for this site (Kellogg *et al.*, 2005). Statistical analyses were performed using Minitab® statistical software (Minitab, 2000).

Results

Ambient groundwater concentrations of NO_3^-, NH_4^+, and Br^- were low or undetectable at both sites (Table 1). The groundwater at site B contained more DOC ($p<0.01$) and lower DO ($p<0.01$) than site A. Dissolved SO_4^{2-} concentrations were higher at site A ($p<0.01$) than site B, while Cl^- concentrations did not differ between sites (Table 1). Salinity at site A was four times higher than at site B (Table 1), a reflection of groundwater mixing with sea water that had infiltrated the aquifer in the transition zone, producing groundwater of intermediate salinity (Moore, 1999). Ambient N_2O concentrations in the groundwater averaged 4 μg L^{-1} (Table 1) and were above saturation (0.6 μg L^{-1}), with ambient $^{15}N_2O$ enrichments at site A and B of 0.203 and 0.295 atom % ^{15}N excess (relative to ambient N_2 in air) respectively. Ambient dissolved CH_4 concentrations were significantly higher ($p<0.01$) at site B (Table 1).

When averaged across all nine push-pull tests that were performed the mean (SEM, n = 9) Br^- and SF_6 concentrations in the initial dose solutions were 33.2 (0.6) mg L^{-1} and 1.5 (0.1) μg L^{-1} respectively. Initial concentrations of dissolved N_2O in the dose-water were well in excess of ambient concentrations and averaged (SEM, n = 3) 150 (44), 65 (12), and 70 (44) μg L^{-1} for site A, site B-test 1 and site B-test 2 respectively. The mean ^{15}N enrichment of the N_2O gas in the initial dose solutions was as noted above 34.0 atom % ^{15}N excess relative to N_2 in air.

Concentrations of CH_4 in the ambient water at sites A, site B-test 1, and site-B test 2 were 5 (1), 415 (120), and 333 (154) μg L^{-1} respectively, (SEM, n=3). At site B the CH_4 concentrations in replicate three were much lower than in replicates one and two, leading to high variability at this site. Immediately prior to 'pushing' the mean (SEM, n=3) dose-water contained CH_4 concentrations of 3 (1), 12 (1), and 10 (4) μg L^{-1} at sites A, site B-test 1, and site-B test 2 respectively.

After incubation of the pushed dose-water in the soil-groundwater matrix the dose-water was withdrawn. There was one particular well where equal volumes of gas were withdrawn along with the water sample during the pull phase of the test. This was at site B, replicate 3, during both tests.

The relationship between $\frac{C}{Co}$, where C is the pulled ground water concentration and Co is the (original pushed ground water concentration), and $\frac{V}{Vt}$, where V is the cumulative volume pulled and Vt is the total volume of water pushed, was plotted for Br^- at site A (Figure 2) and site B (Figure 3a, 3b and 3c). Recovery of the conservative Br^- tracer, averaged (SEM, n=3) over the three peizometers, was 64 (4), 67 (6) and 67 (3) %

for the push-pull tests at site A, site B-test 1 and site B-test 2 respectively (SEM in brackets, *n*=3). The concentration of Br^- in the recovered pull samples had decreased to an average (SEM) 10.7 (0.6) mg L^{-1} in the final aliquot of the pull phase.

The conservative tracer SF_6 behaved in a similar manner to the Br^- anion (Figure 2, Figure 3a, 3b and 3c) but with lower average (SEM, *n*=3) recoveries, 60 (1), 58 (11) and 65 (5) % for the push-pull tests at site A, site B-test 1 and site B-test 2 respectively. Of note however was the divergence between the two conservative tracers at site B, replicates 2 and 3, which was in contrast to site A and replicate 1 at site B. The recovery of N_2O, based upon $\frac{C}{Co}$, was extremely variable and averaged (SEM, *n*=3) 55 (3), 63 (36) and 57 (17) % for the push-pull tests at site A, site B-test 1 and site B-test 2 respectively. This variability was due to both inter-site variability, between sites A and B, and intra-site variability at site B. At site A the mean N_2O $\frac{C}{Co}$ values closely tracked those of the mean $\frac{C}{Co}$ values for the conservative tracers (Figure 2). At site B, replicate 1 behaved in a conservative manner, similar to site A with N_2O $\frac{C}{Co}$ values closely tracking the conservative tracers Br^- and SF_6 (Figure 3a). However, in replicate 2 at site B the N_2O $\frac{C}{Co}$ values decreased rapidly with respect to the conservative tracers during both tests (Figure 3b) while for replicate 3 the N_2O $\frac{C}{Co}$ values increased over and above those of the conservative tracers for both tests. Nitrous oxide concentrations in the final water sample withdrawn averaged (SEM, *n*=3) 46 (13), 25 (15), and 32 (10) μg L^{-1} for sites A, B test-1 and B test-2, respectively.

There was no significant decrease in the ^{15}N enrichment of the N_2O during incubation for all replicates at site A and for replicates 1 and 3 at site B (Figure 4). However, for replicate 2 at site B the ^{15}N enrichment of the N_2O decreased exponentially in both dosings, as $\frac{V}{Vt}$ increased, to a mean of 1.634 atom % ^{15}N excess relative to N_2 in air (Figure 4).

Denitrification rates, i.e. the N_2O reduction rates, were only determined where N_2O concentrations decreased significantly, i.e. for site B, replicate 2, and equated to 8 and 3 μg N_2O-^{15}N kg^{-1} soil d^{-1} for test 1 and 2 respectively.

Dissolved CH_4 concentrations in the pulled samples varied between sites (Figure 5) with concentrations in the final aliquots sampled of 3 (1), 256 (37), and 199 (33) μg L^{-1} for sites A, site B-test 1, and site-B test 2 respectively. Thus there was no significant change in the *C/Co* ratio for dissolved CH_4 at Site A. However, at site B the dissolved CH_4 concentrations increased significantly as the cumulative volume of water pulled increased (Figure 5), although these concentrations were still below ambient levels recorded at the start of the experiment.

Discussion

There are several possible fates for N_2O injected and incubated in the subsoil-groundwater matrix in terms of both concentration and ^{15}N enrichment (Table 2). These fates range from conservation of the added N_2O, i.e. C/C_o behaves in a similar manner as the conservative tracers over time, with constant N_2O ^{15}N enrichment (scenario A, Table 2), to a decrease in both the N_2O C/C_o ratio and its ^{15}N enrichment (scenario E, Table 2).

The data from site A and site B, replicate 1, show that the N_2O concentration behaved in a conservative manner with no significant decrease in the ^{15}N enrichment of the N_2O (Figure 4), i.e. scenario A in Table 2. We know that the ambient groundwater contained some antecedent N_2O but the N_2O concentration of this groundwater was insignificant when compared with the added ^{15}N labeled N_2O. Therefore hydrodynamic dispersion or advective groundwater flow that occurred, either during the actual dosing event or during the incubation period, did not significantly affect the N_2O ^{15}N enrichment. Had we used a lower concentration of N_2O or ^{15}N enrichment in our original dose-water then we may have observed a significant decrease in ^{15}N enrichment.

For site B, replicate 2, we observed a different result with both the N_2O concentration and ^{15}N enrichment decreasing more rapidly than the conservative tracers. This leads us to consider scenario E in Table 2 where a decrease in ^{15}N enrichment must be due to either an advective influx or *in situ* production of N_2O. While a higher advective flux is possible at this replicate, a comparison of the recovery of the conservative tracers at site B replicates 1 and 2 suggests that groundwater conditions were similar between these two replicates and that *in situ* denitrification was responsible for both the depletion of the $^{15}N_2O$ enrichment as well as the overall decline in the N_2O concentration. Site B, replicate 2, appears to be a relative 'hot-spot' for denitrification. We assume N_2O production did not occur via nitrification due to the low DO concentrations and the lack of any measured NH_4^+ substrate. This assumption could easily be verified by using $^{15}NH_4^+$ as a substrate in future studies.

At site, B replicate 3, we observed marked increases in N_2O concentration, i.e. C/Co, increases, but the ^{15}N enrichment of the N_2O remained constant. The increase in

N_2O concentration would suggest that active net production of N_2O was occurring, but the constant ^{15}N enrichment is difficult to explain. It is possible that production of low enrichment N_2O was balanced by isotopic discrimination in N_2O reduction, resulting in no net change in the ^{15}N enrichment of N_2O in the incubation. A further compounding factor at this peizometer was the occurrence of significant gas bubbles, during the extraction of the dose water after its incubation. This gas had a negligible N_2O content but a considerable CH_4 concentration. In theory it is possible that the gas bubble presence caused an error in the calculations of V, but had this been the case the results for the tracer e.g. Br^- would have been erroneous and this was not the case. We do not believe that this particular result draws the method into question since another eight piezometers were successfully sampled providing data that was interpreted in a sensible and logical fashion. However, further field work is required to fully understand the processes at site B, replicate 3.

Our CH_4 data suggest that conditions are sufficiently anaerobic at site B to support production of this important greenhouse gas. At site A, ambient groundwater concentrations of CH_4 were relatively low and there was no change over the course of the incubation. However, at site B, the process of SF_6 labeling stripped out the high ambient CH_4 concentrations in the dose-water so that when the dose-water was injected at the start of the incubation there was a large differential between the CH_4 concentration in the dose-water and the ambient groundwater. The increase in dissolved CH_4 concentration in the pulled water samples (Figure 5) was likely driven by diffusion of CH_4 from the surrounding groundwater matrix. The differences in CH_4 dynamics between sites A and B are consistent with the differences in ambient dissolved oxygen levels between the sites

and support the results showing that some peizometers at site B are located in denitrification hotspots (Yu & Patrick, 2004).

A previous study in the fringe area of site A recorded a denitrification rate of 2 μg NO_3^-N kg^{-1} soil d^{-1} following the addition of NO_3^- (Addy *et al.*, 2002), while a previous study at site B has measured much higher but more variable rates of NO_3^- denitrification (61-140 μg NO_3^-N kg^{-1} soil d^{-1} at 65 cm depth, (Kellogg *et al.*, 2005). However, these previous studies did not present information on the relative production of N_2O and N_2 production during denitrification. While the relative N_2O reduction rates in the present study are consistent with these previous studies (site B higher than site A), the rate of N_2O reduction that we measured at site B is much lower than the total denitrification rates measured in the previous study. The magnitude of the N_2O reduction rates that we measured are also considerably lower than rates measured in surface soils. Hénault *et al*. (2001) reported a lag phase of 48 h in a gley soil before N_2O was reduced, with measured N_2O reduction rates from a variety of soils that were > 3360 μg N kg^{-1} soil d^{-1} while Blackmer and Bremner (1976) measured N_2O consumption rates > 570 μg N kg^{-1} soil d^{-1}. A possible reason for the low N_2O reduction rates that we observed could be the time required for the denitrifying community to generate N_2O reductase. Although there were ambient levels of N_2O present in our sites, the higher concentrations injected with the dose-water may not have been able to be immediately processed by the denitrifier community. Other studies have shown the denitrification enzymes and communities to be highly responsive to factors such as the temperature and water regime and carbon availability (Chèneby *et al.*, 1998, Hénault *et al.*, 2001) and in some instances denitrification may reduce NO_3^- in preference to N_2O. Previous studies have observed

significant lag times between addition of NO_3^- and denitrification activity (Aelion & Shaw, 2000, Addy *et al.*, 2002). A similar lag may also occur for N_2O. If so, it may be necessary to expose the peizometers to elevated N_2O to condition the microbes to record true potential N_2O reduction rates.

The divergence of the conservative tracers at site B, replicates 2 and 3, could possibly have been due to the relative physical states of the tracers and their respective interactions with the soil-groundwater matrix. It is possible that the peizometers at replicates 2 and 3 were in a soil matrix that was less dense or denser than the other peizometers. Thus the resulting physical turbulence or mixing of the dose water with the groundwater may have resulted in the gas tracer behaving differently to the anion tracer as a result of varying pressure during injection.

Further modifications of this method are possible to facilitate measurement of N_2O dynamics at multiple sites. While we used highly enriched $^{15}N_2O$, the use of N_2O that is closer to levels of natural abundance ^{15}N enrichment could be used if there was a sufficient difference between the ^{15}N enrichment of any dissolved ambient N_2O and the N_2O supplied in the dose water. When ambient N_2O concentrations are low, it may even be possible to use commercially manufactured N_2O, with a sufficiently different ^{15}N signature from that of the ambient dissolved N_2O, so that the dose-water could be simultaneously labeled with SF_6 and N_2O by bubbling a tank-gas mixture of these gases through the dose-water for a suitable period. While we have used a 2 L dose water volume there is the potential for a greater volume of N_2O labeled dose-water to be used e.g. 10 L as used by Addy *et al*. (1999). This would allow the integration of N_2O dynamics to occur over a greater volume of soil and reduce the potential impact of

ambient ground water diffusing into the dose plume. This could be achieved by replacing the flask holding the dose water with a gas impermeable bag. Then there would also be no requirement for the He gas, as the gas impermeable bag would deflate as the dose-water was injected into the groundwater. A further modification could be the inclusion of a pressure gauge to note the pressure of the dose water as it is injected into the groundwater. This could indicate the relative densities of the soil matrix. Modified methods that allow for collection of data at multiple sites could allow for information on N_2O dynamics that could be scaled to address questions about the importance of these dynamics to IPCC inventories.

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Table 1. Site characteristics and ambient chemical conditions at sites A (a transition area between a salt marsh and the upland area with tidal inundation a rare event with the soil classified as a mixed Mesic Typic Psammequents (Soil Survey Staff, 1998)) and B (situated on a forested alluvial soil classified as a coarse-loamy Mesic Fluvaquentic Endoaquept (Soil Survey Staff, 1998), 7 m from a stream). Further site details are presented in the text.

Site characteristics	Site A: Warwick	Site B: Meadow Brook
Depth of mini-peizometers (cm)	125	65
Water-table depth (cm)	90	50
Ground water temperature (^{o}C)	12	13
Dissolved oxygen (mg L^{-1})	3.2	<1
Dissolved organic carbon (mg L^{-1})	2.2 (0.2)	9.1 (1.4)
CH_4 μg L^{-1}	5 (1)	333 (154) - 415 (120)
N_2O μg L^{-1}	4.0 (0.4)	4.1 (0.2)
NO_3-N (mg L^{-1})	0	0
NH_4-N (mg L^{-1})	0.07 (0.02)	0.06 (0.03)
Br^- (mg L^{-1})	0.07 (0.03)	0.27 (0.11)
Cl^- (mg L^{-1})	7.1 (0.5)	6.3 (0.5)
SO_4^{2-}-S (mg L^{-1})	3.8 (0.2)	0.8 (0.2)
PO_4^{2-}-P (mg L^{-1})	< 0.1	< 0.1
Electrical conductivity (mS m^{-1})	20.3	5.6
Soil texture	fine sand	coarse-loamy alluvium

Table 2. Possible scenarios for changes in the N_2O concentration and its ^{15}N enrichment, in the recovered dose-water. The assumption is made that the associated tracers (SF_6 and Br^-) shows good recovery in all cases, behaving in a conservative manner.

Scenario	*Initial ^{15}N enrichment*	*C/Co for N_2O relative to tracer*	*Potential reason(s)*
A	Maintained	Conservative	• No $^{15}N_2O$ reduction occurring. • No advective influx of ambient N_2O or *in situ* N_2O production.
B	Maintained	Decreasing over time	• $^{15}N_2O$ reduction occurring. • No advective influx of ambient N_2O or *in situ* N_2O production.
C	Decreasing with time	Increase over time	• Ambient N_2O input rate(s) (advective influx or *in situ* production) > reduction rate of incubated $^{15}N_2O$.
D	Decreasing with time	Conservative	• Ambient N_2O input rate(s) (advective influx or *in situ* production) equal reduction rate of incubated $^{15}N_2O$.
E	Decreasing with time	Decreasing over time	• Ambient N_2O input rate(s) (advective influx or *in situ* production) < reduction rate of incubated $^{15}N_2O$.

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Figure 4. The relationship between the N_2O ^{15}N enrichment (atom % ^{15}N excess relative to ambient N_2 in air) of the sampled waters and V/Vt is shown for each of the nine dosings that were performed, where V is the cumulative volume pulled and Vt is the total volume of the pushed water. Data points are from individual replicates. Legend shows site (A or B) with the following numeral indicating the replicate while the numeral in brackets indicates either test-1 or test-2 at site B.

Figure 5. Relationship between C/Co and V/Vt, for dissolved methane (CH_4) from site A and sites B during test 1 and 2. C is the pulled ground water CH_4 concentration, Co is the original pushed dose-water CH_4 concentration, V is the cumulative volume pulled and Vt is the total volume of the pushed water. Data points are the average of 3 replicates with error bars the standard error of the mean.

Figure 1

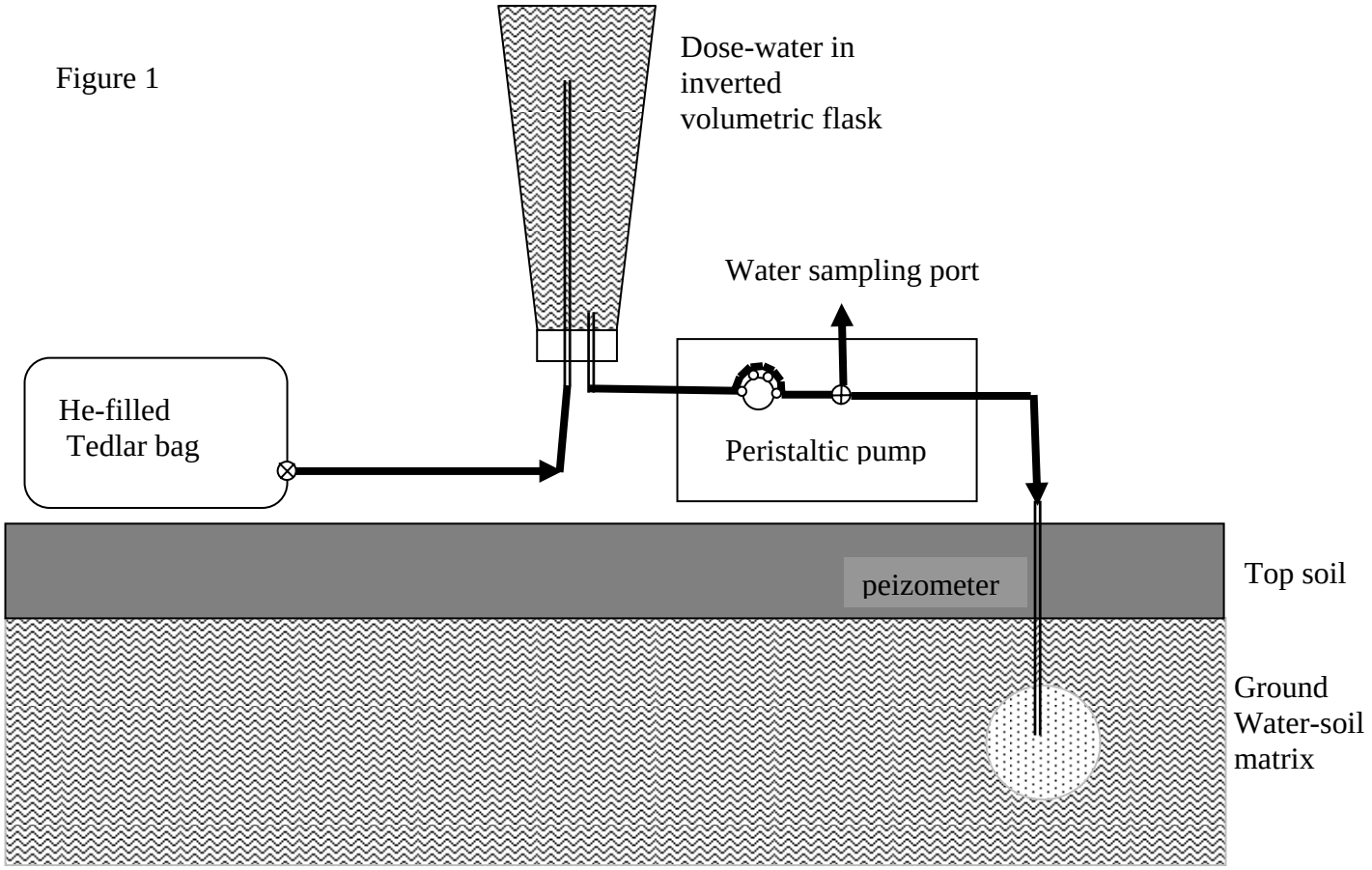
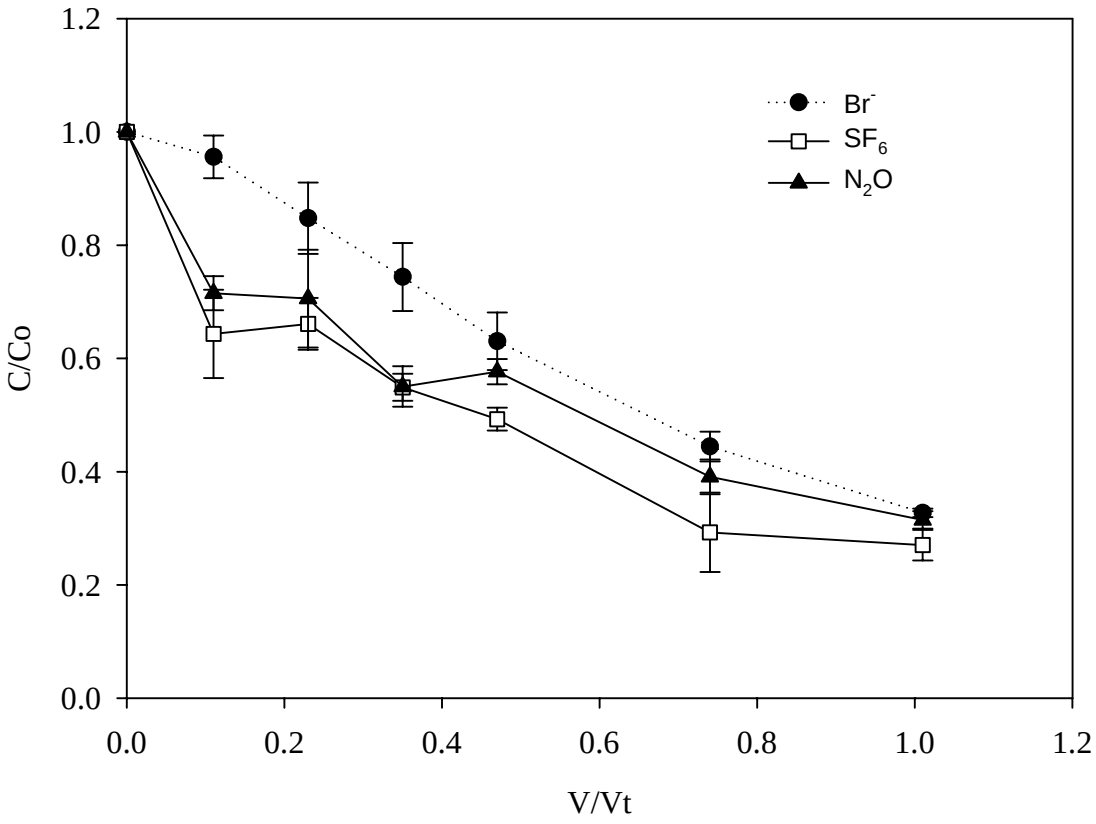

Dose-water in
inverted
volumetric flask
Water sampling port
He-filled
Tedlar bag
Peristaltic pump
peizometer
Top soil
Ground
Water-soil
matrix

Figure 2



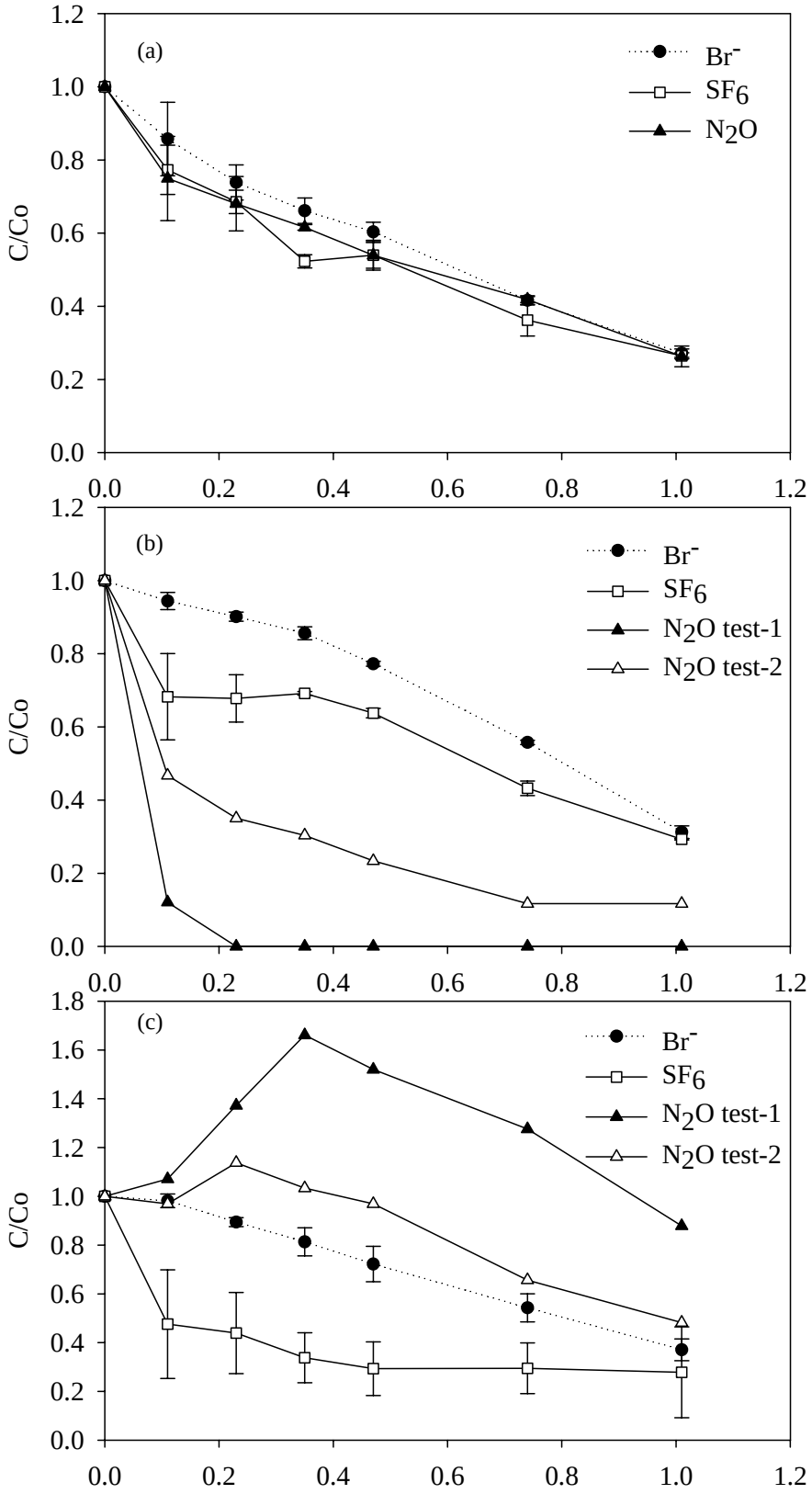

(a)
Br-
SF6
N2O
C/Co
(b)
Br-
SF6
N2O test-1
N2O test-2
C/Co
(c)
Br-
SF6
N2O test-1
N2O test-2
C/Co
V/Vt

Figure 3

Figure 4

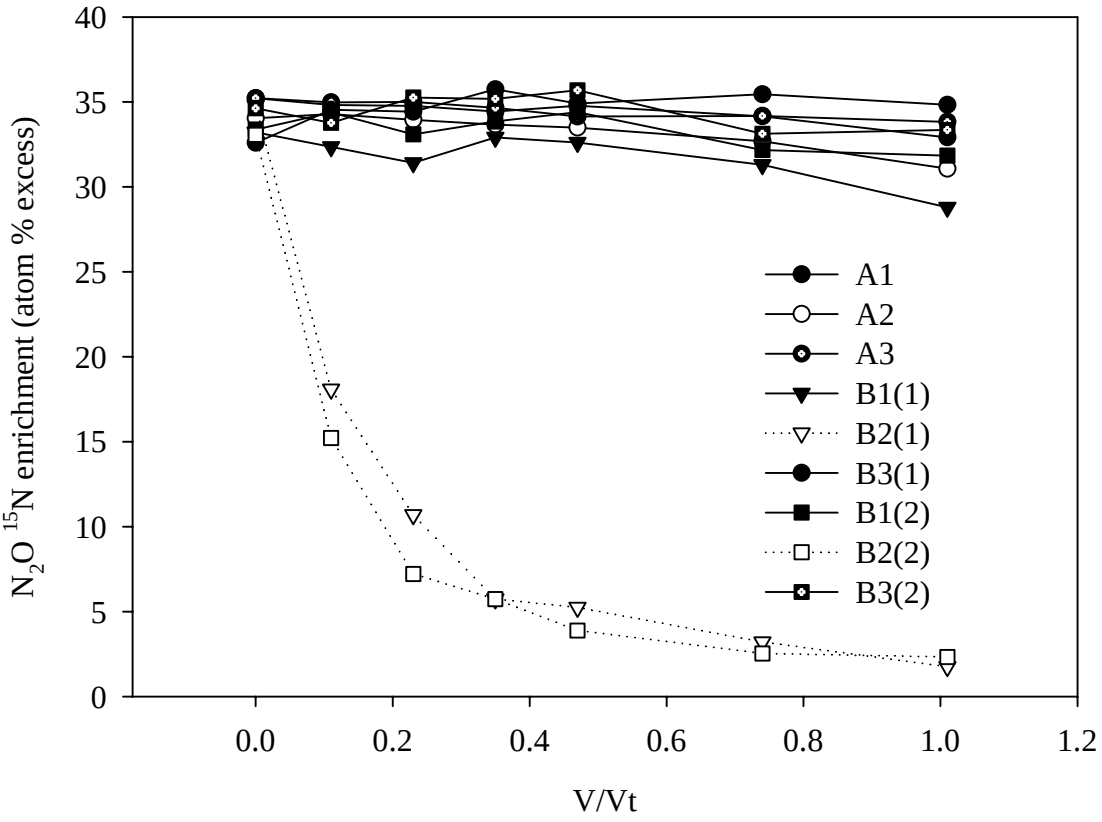
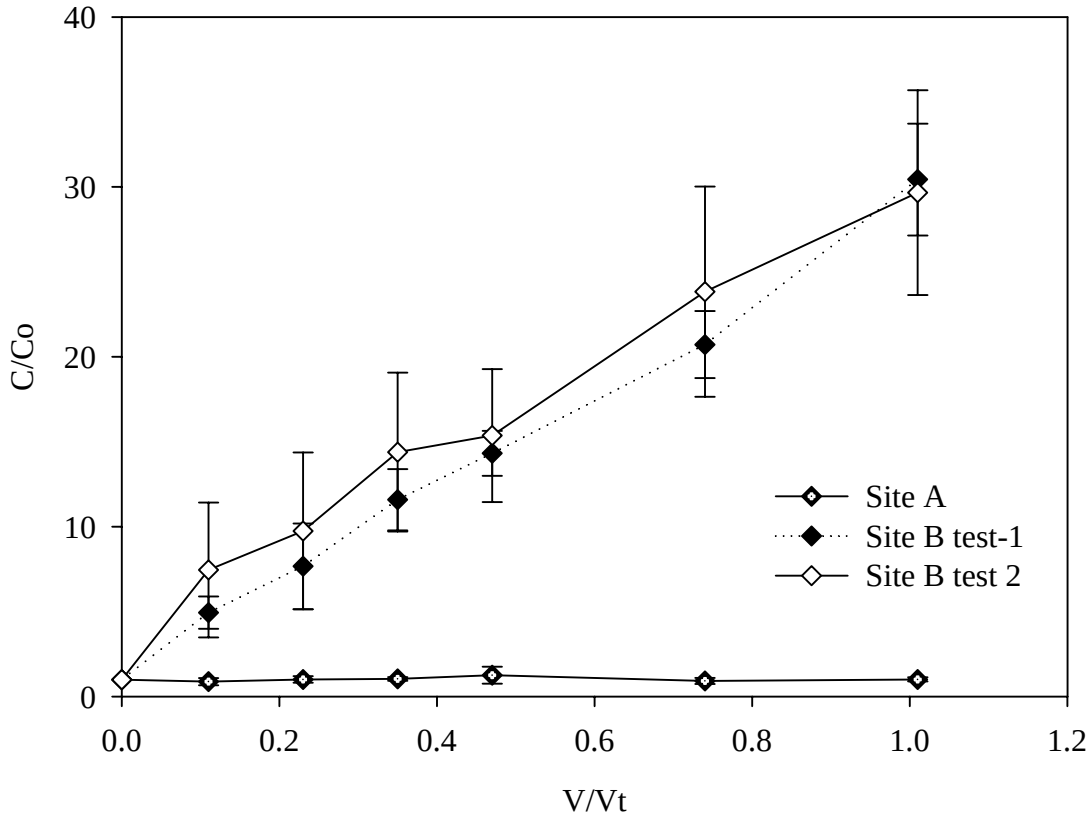

40
35
30
25
20
15
10
5
0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
V/Vt
N_2O ^{15}N enrichment (atom % excess)
A1
A2
A3
B1(1)
B2(1)
B3(1)
B1(2)
B2(2)
B3(2)

Figure 5


40
30
20
10
0
C/Co
0.0
0.2
0.4
0.6
0.8
1.0
1.2
V/Vt
Site A
Site B test-1
Site B test 2