Quantifying the Fate of Wastewater Nitrogen Discharged to a Canadian River

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Abstract:

Addition of nutrients, such as nitrogen, can degrade water quality in lakes, rivers, and estuaries. To predict the fate of nutrient inputs, an understanding of the biogeochemical cycling of nutrients is needed. We develop and employ a novel, parsimonious, process-based model of nitrogen concentrations and stable isotopes that quantifies the competing processes of volatilization, uptake, nitrification, and denitrification in nutrient-impacted rivers. Calibration of the model to nitrogen discharges from two wastewater treatment plants in the Grand River, Ontario, Canada show that ammonia volatilization was negligible relative to uptake, nitrification, and denitrification within 5 km of the discharge points.

Introduction:

Nitrogen (N) is essential for life but can be present in the environment in excess of growth requirements due to human activities. N is a common point-source pollutant to aquatic systems from waste-water treatment plants (WWTPs). Nitrate (NO_3^-) and total ammonia nitrogen (TAN; where TAN includes both ammonia (NH_3) and ammonium (NH_4^+)) are the two inorganic N forms that determine the critical loads beyond which aquatic ecosystems experience eutrophication or acidification (Posch *et al* 2001, Schindler *et al* 2006). The fate of these inorganic N species is a key determinant in the health of ecosystems and the services they provide to humans. TAN can be both a fertilizer of and detriment to aquatic life. At elevated concentrations, NH_3 is toxic to aquatic life.(Canadian Council of Ministers of the Environment 2010) Similarly, elevated concentrations of NO_3^- degrade water quality by harming aquatic life (Canadian Council of Ministers of the Environment 2012) and above drinking water limits can lead to adverse health effects in people (Iwanyshyn *et al* 2008). Consequently, understanding the environmental fate of TAN and NO_3^- discharged to surface waters is important for managing of human-disturbed aquatic ecosystems.

Many processes remove N from aquatic ecosystems. By understanding the relative contributions of each process and the factors that affect their rates, the environmental fate of N loading to aquatic ecosystems can be predicted (Iwanyshyn *et al* 2008). Successful nutrient mitigation strategies in larger aquatic ecosystems rely on using smaller, tractable ecosystems as realistic and replicatable systems (Schindler 1998, Sharpley *et al* 2009, Webster *et al* 2003, Dodds and Welch 2000, Withers and Lord 2002). The concept of nutrient spiralling in streams was developed to describe the cycling and transport of nutrients in small lotic ecosystem(Newbold *et al* 1981, 1982, 1983) and is based on downstream changes in nutrient concentrations. Isotope tracer experiments, where ^{15}N-enriched compounds are added and the tracer followed through different pools, have improved spiralling techniques.(e.g. Mulholland *et al* 2000, 2008, Tank *et al* 2000, Earl *et al* 2006, Hall *et al* 2009, Mulholland *et al* 2004). In a similar fashion, low nutrient streams can be spiked with nutrients and changes in the nutrient pulse can be used to understand ecosystem metabolism of nutrients (e.g. Davis and Minshall 1999, Hall and Tank 2003). These studies are often restricted to short lengths of streams where the hydrology can be well characterized and to smaller systems in general. The understanding of nutrient spiralling in large impacted rivers is often confounded by a heterogeneous river morphology, frequent run-of-the-river dams, groundwater and multiple nutrient inputs, and consequently relies on the intensive work conducted in these smaller systems supplemented by sampling campaigns of both concentration and stable isotopes of N species. Further, observed values are a cumulative result of a plethora of contemporaneous N cycling processes with rates that change in relative importance with distance from inputs and time of day. Disentangling the relative rates of these processes in large rivers is greatly aided by the additional information supplied by stable isotopes and the development of numerical model(Denk *et al* 2017).

Stable isotope studies in rivers have shown that (i) NH_4^+ is preferentially incorporated into the food web compared to NO_3^- and (ii) some TAN is lost to volatilization to the atmosphere while some is nitrified to NO_3^- (Loomer 2008, Murray 2008, Hood *et al* 2014). Denitrification results in N attenuation in rivers, but to a lesser extent in well oxygenated rivers(Rosamond *et al* 2011, 2012, Laursen and Seitzinger 2002, 2004). The rates of these processes change from day to night in response to the release of photosynthetic O_2 into the water(Venkiteswaran *et al* 2007, 2015, Wassenaar *et al* 2010). $\delta^{15}N$ values have been used to qualitatively identify anthropogenic N in coastal areas(Fourqurean *et al* 1997, Fry *et al* 2000, Savage and Elmgren 2004, Derse *et al* 2007). Few studies have attempted to quantify the importance of these competing processes and their role in attenuation of WWTP TAN and NO_3^- in lotic systems though these processes set the baseline $\delta^{15}N$ (isotopic ratios are hereafter reported as δ values) values used for benthic invertebrate and fish studies(e.g. Hood *et al* 2014, Loomer *et al* 2014).

Novel technical developments in the analysis of stable isotopes have allowed for improved assessment of nitrogen cycling in rivers including the use of the differences in $\delta^{15}N\text{-}N_2O$ and $\delta^{18}O\text{-}N_2O$ produced by nitrification versus denitrification (Thuss *et al* 2014). Similarly, ecosystem metabolism techniques (Venkiteswaran *et al* 2007, Tobias *et al* 2007, Holtgrieve *et al* 2010, Parker *et al* 2010) have recently been improved by the use of diel $\delta^{18}O\text{-}O_2$ and $\delta^{13}C$-DIC modelling (Murray 2008, Fourqurean *et al* 1997, Fry *et al* 2000, Savage and Elmgren 2004). The isotopic labelling of benthic biofilm by differing NH_4^+ and NO_3^- sources has recently been describe (Hood *et al* 2014, Loomer *et al* 2014, Peipoch *et al* 2014). Here, we build on these studies by developing and testing a model that uses changes in concentrations and natural abundance stable isotopic ratios to quantify the contributions of the various nitrogen-removal pathways in nutrient-impacted rivers. We applied this model to quantify the fate of N from the WWTP effluent discharges in a river highly impacted by both agricultural and

WWTP nutrients.

The objectives of this research are to (1) quantify changes in concentrations and $\delta^{15}N$ values of TAN and NO_3^- with distance downstream from WWTPs; (2) develop a parsimonious process-based model for N cycling and the fate of WWTP N in rivers, and assess model performance with field measurements; and (3) provide model-based estimates of the rates of nitrification, denitrification, NH_3 volatilization, and N assimilation in WWTP plumes in a river impacted by both WWTP and agricultural nutrient inputs.

Methods:

Field Site:

The Grand River is the largest river discharging into the Canadian side of Lake Erie (Figure 1). Almost 1 million people live in its watershed and more than half of those rely on the river for drinking water. There are 30 wastewater treatment plants of varying sizes in the watershed where agriculture is the dominant land use (80%). We have previously studied the N and O_2 cycling in the Grand River (Rosamond *et al* 2011, 2012, Jamieson *et al* 2013, Venkiteswaran *et al* 2014, 2015). Here, we focus on two large WWTPs in the central part of the watershed that serve a combined population of about 230,000.

Ecosystems the size of the Grand River are not amenable to experimental isotope tracer additions but nevertheless afford us the opportunity to assess many of the processes resultant from the discharge of nitrogen-rich WWTP effluent. These processes include assimilation of NH_4^+ by primary producers, nitrification of NH_4^+ to NO_3^-, loss of NH_3 to the atmosphere via volatilization, denitrification of NO_3^-, and dilution of both NH_4^+ and NO_3^-. Rather than simply a point-source addition of nutrients to a pristine ecosystem, WWTP effluent in the Grand River increases nutrients in an already nutrient-rich system (Venkiteswaran *et al* 2015).

The upstream Waterloo WWTP serves an urban population of approximately 120,000 and discharges a mix of NH_4^+ and NO_3^- via a pipe on the west side of the river. The plume hugs that bank of the river for several km downstream. At baseflow, WWTP discharge accounts for 10–25% of river flow along this reach. The downstream Kitchener WWTP serves about 205,000 and discharges mostly NH_4^+ via a diffuser in the middle of the river. The plume hugs the east bank of the river for several km downstream before several large river bends result in lateral mixing. The river is about 50m wide through the entire sampling area. Together, the WWTPs discharge about 900 tonnesN/yr (Table S1).

In the study reach, the Grand River flows over the stony and sandy Catfish Creek till (Karrow 1974). This forms a substrate for the patchy growth dominated by the macroalga *Cladophora* spp. and macrophytes *Myriophyllum spicatum* and *Stuckenia pectinatus*. Their biomass (about 1 kg/m^2) is greater below both WWTPs than above (Hood 2012).

Water in the Grand River is hard with dissolved inorganic carbon (DIC) concentrations around 50 mgC/L. Municipal drinking water, from both the Grand River and groundwater, is similarly hard, averaging 400 mg/L equivalents of $CaCO_3$. The use of residential water softeners produces WWTP effluent high in Cl^- (Region of Waterloo 2011). River dissolved organic carbon (DOC) concentrations are typically 6–8 mgC/L. The river is shallow through the sampled section, with mean depth at baseflow around 0.5 m. Typical river discharge during the sampling periods through these reaches was 2–13 m^3/s (Canadian Water Survey, https://wateroffice.ec.gc.ca/). Sampling occurred during stable base-flow conditions.

Agricultural activity and some small WWTPs in the watershed results in high nutrient concentrations in the river prior to the two main WWTPs in this study. Upstream NO_3^- concentrations are elevated (3–4 mgN/L) whereas NH_4^+ concentrations are low (<0.1 mgN/L) and both total phosphorus (TP) and soluble reactive phosphorus (SRP) are high (70 and 10 µg/L).

Below each of the two WWTPs, eight sampling points were established based on availability of access to the river (Figure 1). The first site was immediately downstream of the effluent discharge point, one was a few hundred kilometres downstream, and the others about every 800 m to 1000 m for about 5 km (Table S2). At each site, samples for NH_4^+, NO_3^-, Cl^-, DOC, $\delta^{15}N\text{-}NH_4^+$, and $\delta^{15}N\text{-}NO_3^-$ were collected from the centre of the plume as identified by *in situ* measurement of conductivity (YSI 556 MPS). Samples were collected in HDPE bottles and immediately chilled in a cooler for transport to the laboratory, filtered to 0.45 µm, and kept cold (4°C) until analyses. Samples for NH_4^+ and $\delta^{15}N\text{-}NH_4^+$ were immediately acidified to pH 4 with HCl and frozen until analyses. *In situ* measurements of temperature and pH were made (YSI 556 MPS) with reported accuracy on pH and temperature of ± 0.2 units and ± 0.15°C, respectively. To account for dilution of the effluent plume by river water, Cl^- at these elevated concentrations was assumed to be a conservative tracer and NH_4^+ and NO_3^-

concentrations were adjusted accordingly.

WWTP plumes were sampled downstream of both sites twice. The plume from the Waterloo WWTP was sampled on 2007-10-30 (typical discharge 2–7 m^3/s) and 2008-07-01 (typical discharge 2–4 m^3/s). The plume from the Kitchener WWTP was sampled on 2007-10-23 (typical discharge 11–17 m^3/s) and 2008-07-18 (typical discharge 8–11 m^3/s).

Analyses:

Anion concentrations were measured on a Dionex ICS-90 ion chromatograph. Precisions and detection limits of NO_3^- were 0.07 mgN/L (standard deviation of 15 replicates of a standard solution) and 0.05 mgN/L, respectively. NO_2^- concentrations were rarely detectable. Precision of Cl^- measurements was < 1 mg/L. NH_4^+ concentrations were measured colorimetrically by the phenate colourimetric method (EPA 350, (EPA 1993)) on a Beckman DU500 UV/VIS spectrophotometer with a precision and detection limit of 0.005 mgN/L and 0.015 mgN/L, respectively.

δ^{15}N-TAN was measured via the diffusion method on acidified discs(Zhang *et al* 2007). Briefly, NH_4^+ is converted to NH_3 by increasing the sample pH; NH_3 is trapped in a filter pack containing a 1 cm GF/D filter, acidified with H_2SO_4, trapped in a PTFE packet. The filter is dried and analysed for δ^{15}N on a Carlo Erba 1108 elemental analyzer (EA) coupled to a Micromass Isochrom isotope-ratio mass spectrometer (IRMS). Precision of δ^{15}N-NH_4^+ analysis was ± 0.3‰. δ^{15}N-NO_3^- was measured via the $AgNO_3$ method. Briefly, sample volumes were reduced by evaporation, SO_4^{2-} was removed by barium precipitation, and NO_3^- was collected on anion exchange resin in a column. After being eluted from the column, AgO was added to precipitate $AgNO_3$, which was analyzed on the same EA-IRMS as above. Precision of δ^{15}N-NO_3^- was ± 0.5‰. Methodological tests indicated that the $AgNO_3$ method can capture NO_2^- since NO_2^- oxidizes rapidly to NO_3^- even in filtered samples.(Spoelstra 2004) Since previous measurements showed there was little NO_2^- in this river (NO_2^- was < 5% of NO_3^-) the results presented here can be interpreted as δ^{15}N-NO_3^-.

Model Setup

To interpret patterns in the data, a dynamic model (hereafter NANNO: nitrate, ammonia, nitrite, nitrous oxide) was developed to describe the dynamics of TAN, NO_2^-, NO_3^-, N_2O and their δ^{15}N values (eight states, in total). The model was implemented in R(R Core Team 2016) using the simecol

(Petzoldt and Rinke 2007) package. Five processes were modelled: volatilization of NH_3, two-step nitrification ($NH_4^+ \rightarrow NO_2^-$ and $NO_2^- \rightarrow NO_3^-$), though see complete ammonia oxidation by a single organism (comammox (van Kessel *et al* 2015)), denitrification ($NO_3^- \rightarrow N_2O$), and biological assimilation of NH_4^+ (Figure 2). In the model, N_2O produced by denitrification is allowed to accumulate rather than being further reduced to N_2; this choice was made because the N_2O:N_2 ratio produced during denitrification varies widely and once nitrogen is removed from the TAN and NO_3^- pools, it is very unlikely to return to those pools especially in a system where N is in excess. Similarly, the biological assimilation of NO_3^- was not included given that NH_4^+ is in excess. Metabolic costs suggest NH_4^+ is the preferred source of nitrogen over NO_3^- for phytoplankton and aquatic plant(Mariotti *et al* 1982, Yoneyama *et al* 1991, Collier *et al* 2012) and that cycling of NH_4^+ uptake is rapid (Mulholland *et al* 2000). Isotopic evidence suggests this is also true for macrophytes in the Grand River, Ontario(Hood 2012, Hood *et al* 2014). Each process is associated with isotopic fractionation (ε, where $\varepsilon = \alpha - 1$ and $\alpha = R_{product}/R_{reactant}$). Fractionation factors for physical processes are typically known with greater precision than biological ones. Since the Grand River is very well buffered, average measured pH values were used for TAN speciation calculations. Model parameters and commonly reported values are summarized in Table 1.

To simplify modelling efforts, the common time-for-distance substitution was made. Additionally, to avoid requiring river surface area for gas exchange with the atmosphere, all rate constants, including the gas exchange coefficient, were modelled with units of per time. First-order rate kinetics were used for nitrification (Chen *et al* 2006, Dinçer and Kargı 2000), NH_4^+ uptake (MacIsaac and Dugdale 1969, D'Elia and DeBoer 1978), and denitrification (Dinçer and Kargı 2000). NH_3 gas exchange was modelled with the thin-boundary layer equation(Denmead and Freney 1992) assuming the atmospheric NH_3 concentration was negligible (ppb-range range concentration (Finlayson-Pitts and Pitts 1986, Mészáros 1992)). Ranges of potential gas exchange coefficients can be estimated from river channel geometr(Raymond *et al* 2012) and converted for use with NH_3 via Schmidt number scaling (Jähne *et al* 1987) (for example, at 20°C the Schmidt number for NH_3 in freshwater is 585 (Kreith 2000).)

For the Grand River, the gas exchange coefficient for O_2 has been estimated for its length with focus on the areas including the WWTPs (Jamieson *et al* 2013, Venkiteswaran *et al* 2015). There k_{ge} values for O_2 were 0.1–0.5 m/h. Measured river depths at all sampling sites were 0.5–1.0 m. Modelled river velocities were 0.2–0.4 m/s for typical flow conditions (MJ Anderson, pers. comm.). This produces a range of k_{ge} values for NH_3 of 0.0001–0.0006 m^{-1} downstream of the WWTPs.

An initial best-fit solution for each set of field data was found by allowing the model to find a combination of rate constants (greater than or equal to 0), isotopic fractionation factors (between the lowest literature α values, i.e. the strongest values, and 1), and initial values that minimized the sum of squared errors between field data and model output.

Results:

Field measures of N concentrations and isotopes in WWTP plumes

Waterloo

On both dates, TAN concentrations declined from 5–7 mgN/L to $\ll$ 1 mgN/L by the 5 km sampling point, although the rate of decline was much faster on 2008-07-01 than on 2007-10-30. Nitrate response in the plumes was different on both dates: on 2007-10-30, there was a gradual decline in NO_3^- but on 2008-07-01 there was an increase of > 1 mgN/L. Together, these results suggests different fates for N in the Waterloo plume on each date.

On 2007-10-30, δ^{15}N-TAN values increase rapidly from 12‰ to 30‰ in the first 500m before concentrations became too low for analyses. On 2008-07-01, there were no data below the initial sample but TAN persists at a greater distance downstream that on 2007-10-30. On both dates, the δ^{15}N-NO_3^- values declined from 16‰ to 11‰ within the first 1km, and then rose gradually.

Kitchener

TAN concentrations downstream of the Kitchener WWTP declined to < 1 mgN/L over the 5.5 km sampling transect from initial values of 14 mgN/L and 4 mgN/L on 2007-10-23 and 2008-07-18. Nitrate concentrations increased substantially on 2007-10-23, from 0.8 mgN/L to 5.9 mgN/L, and to a lesser degree on 2008-07-18 from 1.3 mgN/L to 3.1 mgN/L.

δ^{15}N-TAN values increased on both days, by 22‰ on 2007-10-23 and by 12‰ on 2008-07-18 over the 5.5 km. Unlike at Waterloo, the δ^{15}N-NO_3^- values changed only by around 3‰; decreasing on 2007-10-23 and increasing on 2008-07-18.

Model development: Effect of N cycling processes on coupled N concentrations

and isotopes

The coupling of concentrations and isotopes in a simple process-based model shows that the various N cycling processes result in different patterns at the river scale. These results suggest the model may reproduce the variety of expected patterns from each process in the model. Additionally, as we describe next, the dynamic features of each process are sufficiently distinct that we would expect the model to be identifiable. That is, we would expect to arrive at a tight estimate of the kinetic parameters given a sufficiently rich field data set. If this were not the case, then there would be less likelihood that a unique model solution describing TAN, NO_2^-, NO_3^-, N_2O and their $\delta^{15}N$ values could be found.

Both NH_3 degassing and NH_4^+ uptake result in expected and rapid increases in $\delta^{15}N$ values but increases in $\delta^{15}N$-TAN have different values with similar decreases in TAN due to the difference in isotopic fractionation between the two processes. Nitrification alone produces a transient but small increase in NO_2^- (<20% of DIN at its greatest) as NH_4^+ is oxidized to NO_3^-. There is a characteristic initial dip in $\delta^{15}N$-NO_3^- that must be a function of the initial $\delta^{15}N$ values and isotopic fractionation (α_{nit1} and α_{nit2}) and may be exploitable as an identifier of significant nitrification. Denitrification alone, unlikely in a WWTP plume in a shallow oxygenated river during the day, results in a clear decline in NO_3^- associated with an increase in $\delta^{15}N$-NO_3^- and no changes in TAN or $\delta^{15}N$-TAN. There is a corresponding increase in N_2O and a transient dip in $\delta^{15}N$-N_2O values that depends on initial conditions and the strength of α_{denit}.

An initial structural identifiability analysis (Bellman and Åström 1970) (see Supporting Information) of the model dynamics and outputs confirmed that with the available data, it would not be possible to separately estimate the rates at which the equilibrated TAN pool (NH_3 and NH_4^+) is lost through gas exchange and NH_4^+ uptake processes. This conclusion is valid regardless of the quality of the concentration data and $\delta^{15}N$ values (see also Supporting Information.)

Consequently, we simplified the model by fixing from independent sources the gas exchange coefficient (k_{ge} (Venkiteswaran *et al* 2015, Jamieson *et al* 2013) and its isotope fractionation factor (α_{ge} (Kirshenbaum *et al* 1947, Thode *et al* 1945, Norlin *et al* 2002).

We fit the resulting model separately to the four field data sets and then, in each case, applied uncertainty analysis as described in Methods. The results varied, but from this preliminary analysis (results not shown) we discovered that in every case the available data was not sufficient to provide accurate estimates of the 8 free parameters. In particular, the k_{nit2} and α_{nit2} parameters could not be well-estimated from any of the datasets. Consequently, we reduced the model further, by removing NO_2^- and instead describe a single-step nitrification process (k_{nit1} and α_{nit1}) where NH_4^+ is oxidized to NO_3^-; justified given that NO_2^- concentrations are low compared to NO_3^- and TAN and not accumulating. The resulting system has 6 states: TAN, NO_3^-, N_2O, δ^{15}N-TAN, δ^{15}N-NO_3^-, and δ^{15}N-N_2O. Results of fitting and uncertainty analysis, as described in Methods, are shown in the Supplementary Information Tables 3–6.

In the case of the Kitchener 2007 dataset (Supporting Information Table 6), the k parameters for nitrification and denitrification all appear to be reasonably well constrained. The α estimates are less confident. Two of the best-fit α value estimates, α_{dnit} and α_{amup}, are at the bounds of the range of α allowed (Table 1; [0.975,1]), suggesting the data provide minimal useful information about their values. Moreover, while two of the sensitivities are not unreasonably low, the confidence intervals are considerably larger than the search space, which has a width of only 2.5% (25‰).

For the Kitchener 2008 best fit, the trend in certainty is similar but the data constrain the parameter estimates to a lower degree. Data from Waterloo (2007 and 2008, Tables S4 and S5) provide even less ability to constrain the rates likely because the system behaviour is not as dynamic, i.e. the range in δ^{15}N values is small despite the change in concentration being large.

Discussion:

The process-based NANNO model was able to reproduce the observed dynamics in concentrations and the δ^{15}N values of TAN and NO_3^- (Supporting Information Tables 3–6). Results from two seasons, with different proportional fates of N processing, at two different WWTPs with different TAN:NO_3^- ratios in their effluent indicate a good degree of coherence between model results and field data (Figures 3–6 and Supplementary Tables 3–6.) Additionally, the shapes of the curves (increases, decreases, and pleateaux) were all generally reproducible by the model. The model was least successful in reproducing behaviour when there were increases in NO_3^- concentration without a change in δ^{15}N-NO_3^-. This scenario suggests nitrification where the new NO_3^- has the same δ^{15}N-NO_3^- as the extant NO_3^-.

In all four cases, N is lost from the river downstream of the WWTPs. Rates for each N process can be summarized by their rate constants (Tables S4–S7) but are better compared as the mass of N transformed by each process (Table 2). In three of four cases, NH_3 loss via volatilization was much lower than NH_4^+ loss via update or nitrification (Table 2). With river pH values of 7.6–8.4, well below the pK_a value of 9.4, and high rates of community metabolism (Venkiteswaran *et al* 2015, Jamieson *et al* 2013) the N from WWTP effluent largely remained in and was transformed in the Grand River. Previous work has shown that photosynthesis in the Grand River can be inhibited by high light in summer (Chen *et al* 2014, Chen 2013) and autumn storms that serve to remove biomass may have increased the availability of substrate for growth.

In both Waterloo cases, denitrification played a modest role in reducing N concentrations (Table 2). N_2O concentrations in and fluxes from the Grand River are high downstream of these WWTPs (Rosamond *et al* 2011, 2012, Venkiteswaran *et al* 2014). More detailed sampling of N_2O and its $\delta^{15}N$ values may provide additional constraints to improve the model fit.

Nitrification played a moderate role in N cycling in all four cases. There were no clear correlations between nitrification rates and rates of other N processes suggesting that predictions about the fate of N in the Grand River cannot be simply derived from other components of ecosystem metabolism. Where measurable, NO_2^- concentrations and $\delta^{15}N$ values may provide additional information to the model by constraining nitrification.

The $\delta^{15}N$ of benthic periphyton and invertebrate (Loomer 2008, Loomer *et al* 2014) as well as macrophte (Hood 2012, Hood *et al* 2014) are often used as indicators of different N sources and N pollution because they form the base of the food web. Interpreting these data requires an ability to understand and predict the fate of large isotopically distinct N sources like WWTP effluent since the $\delta^{15}N$ values measured in biota ultimately depend on the source of N and isotopic fractionation during uptake. Moreover, macrophytes integrate N over a much longer time scale than the effluent-plume travel time or diel variability (Loomer *et al* 2014, Hood *et al* 2014).

There are several key model parameters that are insufficiently characterized, such as isotopic fractionation during TAN and NO_3^- uptake, preferential uptake of different N species, and release of TAN and NO_3^-. The variability in isotopic fractionation during NH_4^+ uptake is large and varies non-linearly with concentration (Hoch *et al* 1992, Pennock *et al* 1996, Yoneyama *et al* 2001). This poses a vexing problem at the ecosystem scale since the isotopic enrichment – concentration relationship varies between species and both concentrations and species vary within ecosystems.

The mass and $\delta^{15}N$ of river biomass are difficult to capture in the parsimonious NANNO model structure; model fitting may be improved if the release of TAN and NO_3^- by biomass contributes significantly to river N relative to WWTP effluent (Loomer *et al* 2014). Nitrogen uptake and release rates can be estimated with nutrient spiralling techniques but this analysis often conflates TAN and NO_3^-. It is therefore difficult to discern which N form is used, which is released, and how these results apply to a river with more than 100 km of upstream nutrient inputs. The degree of importance, if any, to dissolved organic N mineralization or N release from microbes and macrophytes in the nutrient-replete WWTP plumes is unknown.

Understanding the ecosystem effects of changes in nitrogen sources, such as altering WWTPs to produce only NO_3^- instead of NH_4^+ in order to improve river O_2 concentrations, requires knowledge about which N enters the base of the foodweb via primary producers and consumers. In cases where $\delta^{15}N$-NO_3^- and $\delta^{15}N$-TAN values are far enough apart, or one is changing while the other is constant, the use of each by primary producers and consumers may be teased apart. NO_3^- uptake is associated with little to no isotopic fractionation (Mariotti *et al* 1981, Yoneyama *et al* 1998, 2001) and in the WWTPs' effluent plumes in the Grand River $\delta^{15}N$-NO_3^- values do not vary as much as $\delta^{15}N$-TAN values. In such scenarios, response to increasing $\delta^{15}N$-TAN may be observable as a concomitant increase in the $\delta^{15}N$ of primary producers and consumers (Loomer *et al* 2014, Hood *et al* 2014).

Since O_2, N, and phosphorus cycles are strongly linked, improving the understanding of nitrogen processes allows previous work on O_2 and phosphorus cycling in the Grand Rive(Barlow-Busch *et al* 2006, Venkiteswaran *et al* 2014, 2015) to be extended to process-based biogeochemical models that incorporate multiple elements and their isotopes. Components that may be added to NANNO to improve constraints on nitrogen processes include $\delta^{18}O$-NO_3^- values. However, recent work has demonstrated that predicting the $\delta^{18}O$ values of nitrogenous species is more complicated than originally thought because there are poorly understood abiotic factors that alter the $\delta^{18}O$ value of NO_2^- and NO_3^- as well as multiple pathways to produce N_2O (Snider *et al* 2010, 2012, 2013, 2015, Casciotti *et al* 2010, Buchwald and Casciotti 2010, Buchwald *et al* 2012). Nevertheless, there are opportunities to produce a more constrainable model.

We have presented a process based-isotopic model of key nitrogen species for use in nutrient plumes in rivers. The NANNO model successfully reproduced observed dynamics in TAN and NO_3^- concentrations and their $\delta^{15}N$ values including seasonal differences in the way N species were processed. The ability to model these processes is a key step to making predictions about how improvements in WWTP effluent will affect receiving waters.

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Author Contributions:

Conceived and designed the experiments: JJV, SLS. Performed the experiments: JJV, SLS. Analyzed the results: JJV, BPI, SLS. Contributed reagents/materials/analysis tools: JJV, SLS, BPI. Wrote and reviewed the paper: JJV, SLS, BPI.

Data Deposition:

Data and code are available as part of the NANNO package https://github.com/jjvenky/NANNO (reviewers can anonymously review the code at this URL; upon the paper's acceptance, a DOI will be obtained and used).

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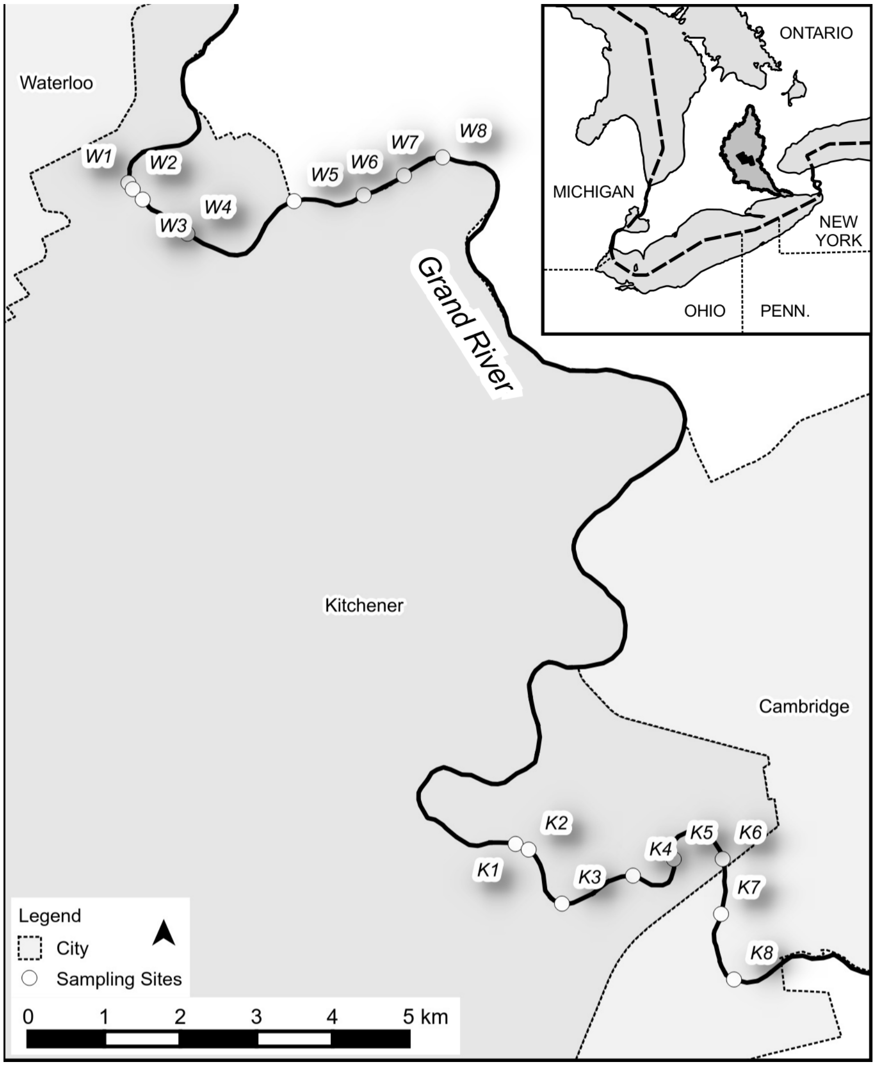
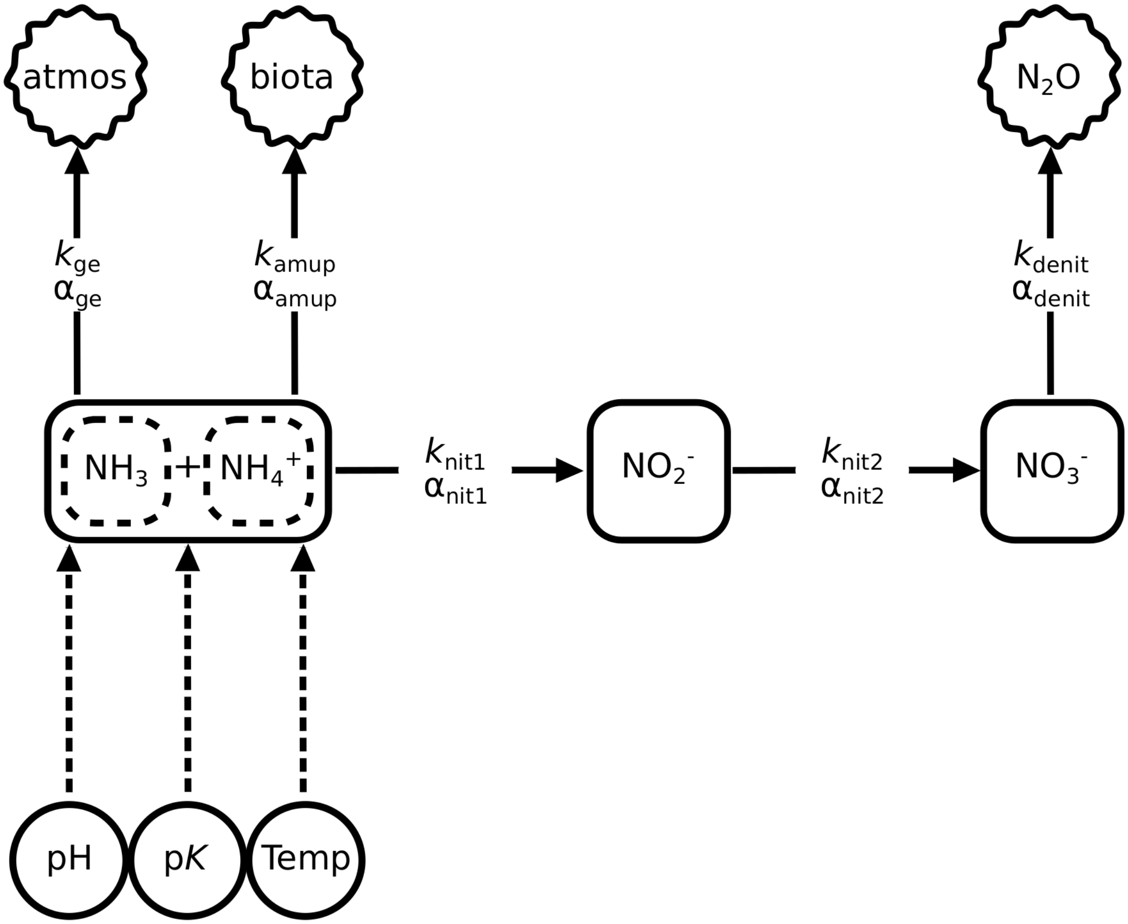
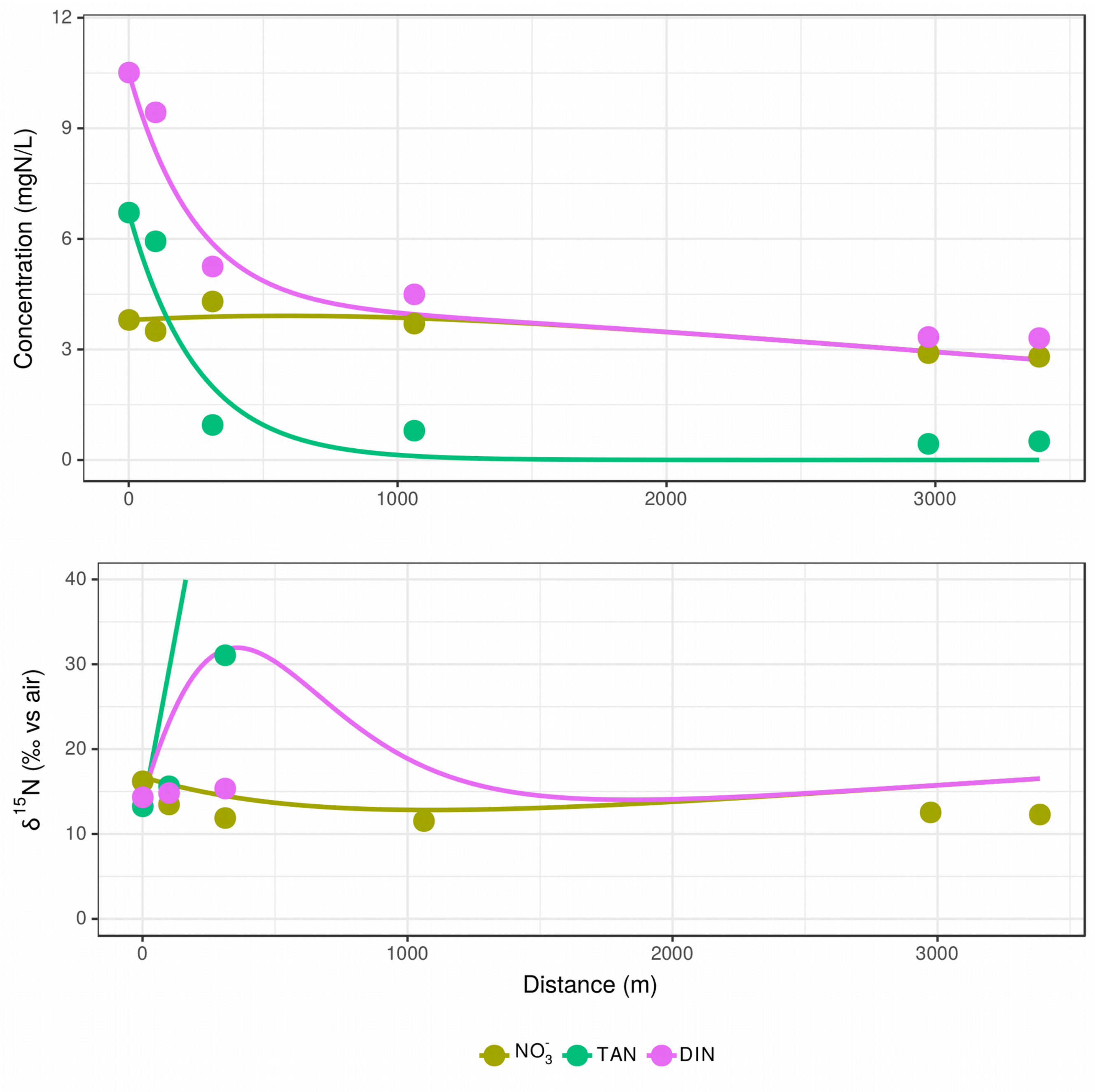
Figures:



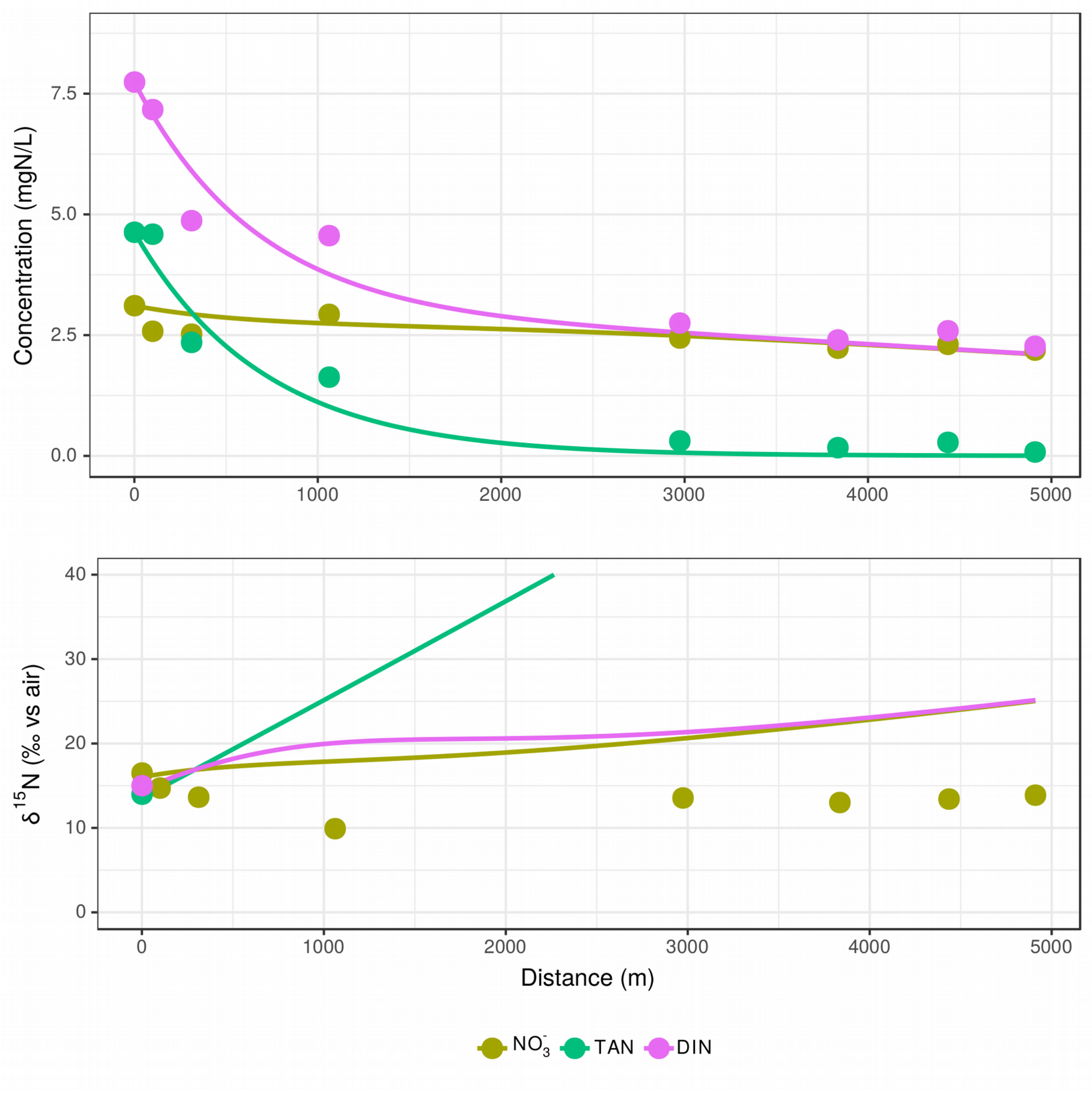
1. Central portion of the Grand River watershed in south-western Ontario, Canada. River flow is from north to south. Sampling sites below the Waterloo (W) and Kitchener (K) waste-water treatment plants are numbered 1–8. The cities of Cambridge, Kitchener, and Waterloo are highlighted as black blocks in the middle of the Grand River watershed in the inset map. Made with Natural Earth data (naturalearthdata.com) and information under licence with the Grand River Conservation Authority.



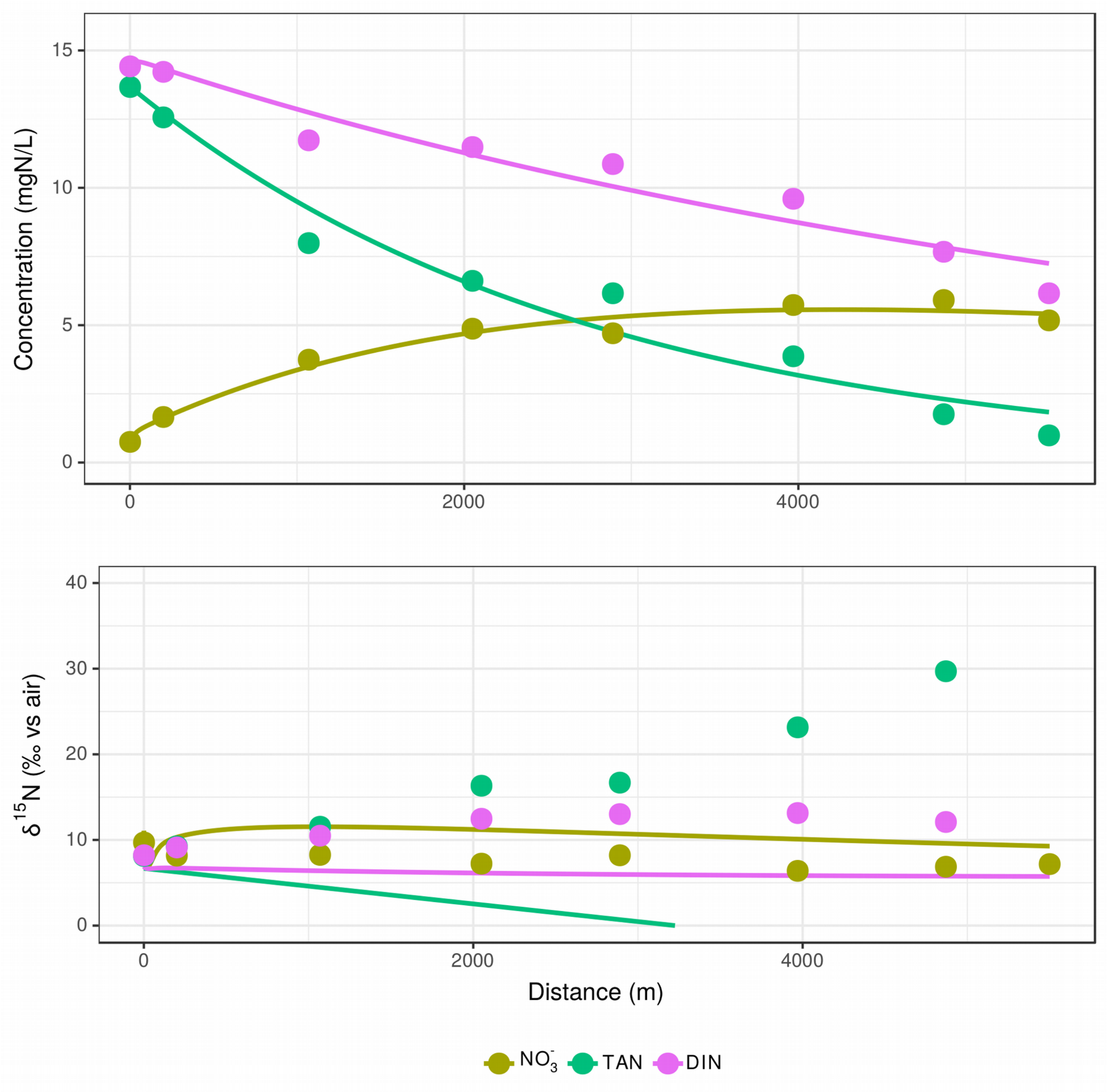
2. Nitrogen pathways in the eight-state NANNO model. Each box represents represents a stock or pool. Circles represent data inputs to the model. Flows of nitrogen between stocks are identified with arrows, accompanied by first-order rate constants, *k*. Clouds indicate a loss of nitrogen from the system. Total ammonia nitrogen is modelled as ammonia (NH_3) and ammonium (NH_4^+). The ratio is set by measured temperature and pH, and calculated p*K* values. Ammonia gas exchange (ge) is modelled via the thin boundary layer model (Denmead and Freney 1992). Ammonium assimilation (amup) by biota is modelled as a loss of NH_4^+. Two-step nitrification (nit1, nit2) is modelled as NH_4^+ to NO_2^- to NO_3^-. Denitrification (denit) is modelled as a loss of NO_3^- to N_2O. Each stock is modelled independently for each isotope and flows are adjusted by isotope fractionation factors (α).



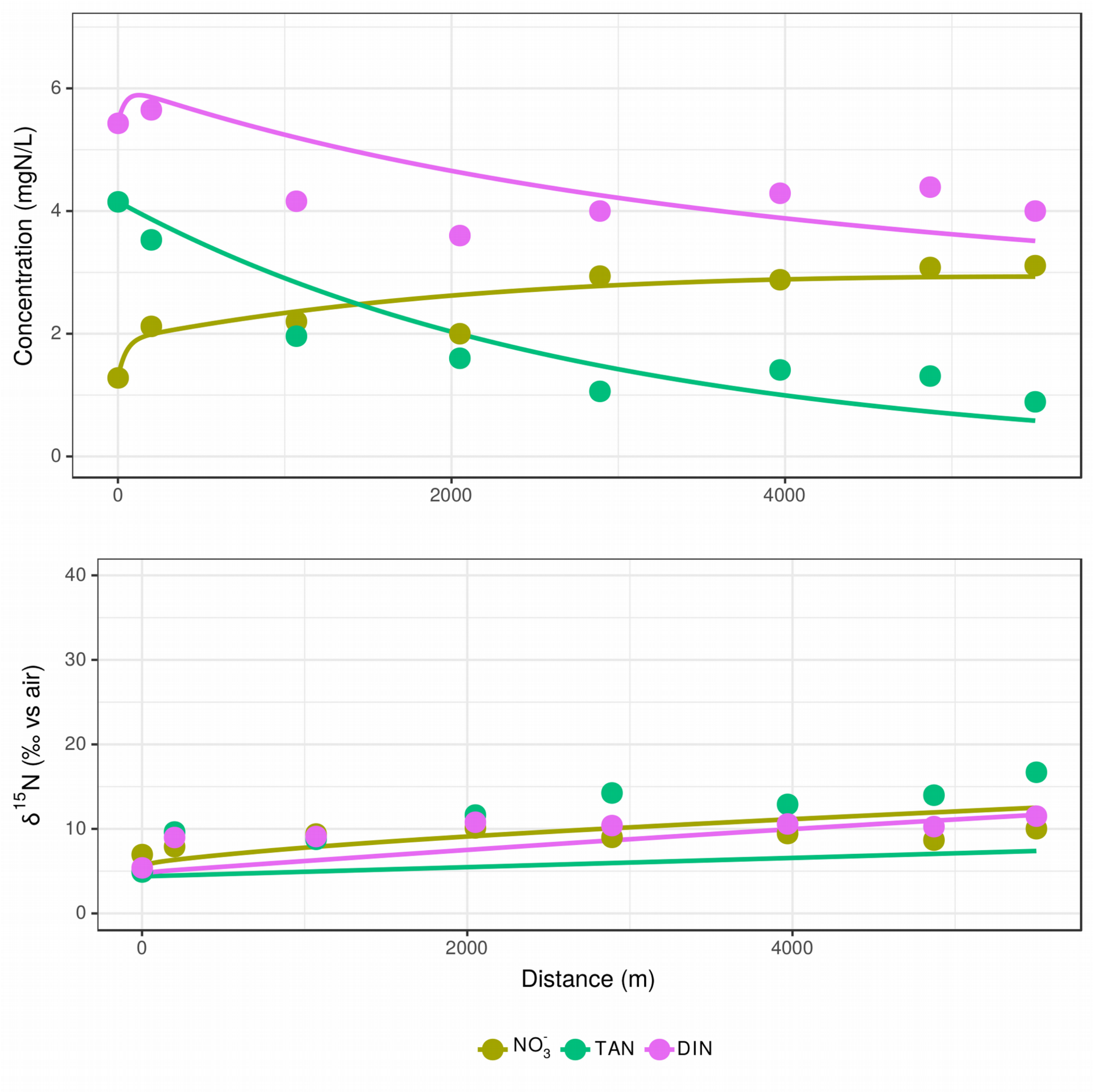
3. Waterloo wastewater treatment plants plumes on 2007-10-30. Measured field data (adjusted for WWTP plume dilution using Cl^- data) are shown as points. Best-fit model results are shown as curves. Parameters used in the model are given in Table 1. Dissolved inorganic nitrogen (DIN = TAN + NO_3^- and mass-weighted δ^{15}N-DIN) is also plotted to show where there is N loss from the system either through degassing, assimilation or denitrification.



4. Waterloo wastewater treatment plants plumes on 2008-07-01. Measured field data are shown as points. Best-fit model results are shown as curves. Parameters used in the model are given in Table. Dissolved inorganic nitrogen (DIN = TAN + NO_3^- and mass-weighted δ^{15}N-DIN) is plotted to show where there is nitrogen loss from the system either through degassing, assimilation or denitrification.



5. Kitchener wastewater treatment plants plumes in 2007-10-23. Measured field data are shown as points. Best-fit model results are shown as curves. Parameters used in the model are given in Table. Dissolved inorganic nitrogen (DIN = TAN + NO_3^- and mass-weighted δ^{15}N-DIN) is plotted to show where there is nitrogen loss from the system either through degassing, assimilation or denitrification.



6. Kitchener wastewater treatment plants plumes in 2008-07-18. Measured field data are shown as points. Best-fit model results are shown as curves. Parameters used in the model are given in Table. Dissolved inorganic nitrogen (DIN = TAN + NO_3^- and mass-weighted δ^{15}N-DIN) is plotted to show where there is nitrogen loss from the system either through degassing, assimilation or denitrification.

Tables:

Table 1. Parameters and typical values used in modelling wastewater treatment plant plumes in the Grand River, Ontario via NANNO.

Parameter	Description	Typical value or range	Units	Reference
k_{ge}	gas exchange coefficient	0.0001–0.001	t^{-1}, m^{-1}	(Jamieson *et al* 2013, Venkiteswaran *et al* 2015)
k_{nit1}	nitrification rate constant: NH_4^+ to NO_2^-	0–0.01	t^{-1}, m^{-1}	
k_{nit2}	nitrification rate constant: NO_2^- to NO_3^-	0–0.01	t^{-1}, m^{-1}	
k_{denit}	denitrification rate constant	0–0.001	t^{-1}, m^{-1}	
k_{amup}	ammonium uptake rate constant	0–0.01	t^{-1}, m^{-1}	
α_{NH3NH4}	equilibrium isotope fractionation factor between NH_3 and NH_4^+	1.047	unitless	(Li *et al* 2012, Hermes *et al* 1985)
α_{ge}	kinetic isotope fractionation factor for gas exchange of NH_3	0.995–1.000	unitless	(Kirshenbaum *et al* 1947, Thode *et al* 1945, Norlin *et al* 2002)
α_{nit1}	isotope fractionation factor for nitrification: NH_4^+ to NO_2^-	0.990–1.000	unitless	(Gammons *et al* 2010)
α_{nit2}	isotope fractionation factor for nitrification: NO_2^- to NO_3^-	0.990–1.000	unitless	(Gammons *et al* 2010)
α_{denit}	isotope fractionation factor for denitrification: NO_3^- to N_2O	0.985–1.000	unitless	(Sebilo *et al* 2003)
α_{amup}	isotope fractionation for NH_4^+ uptake	0.973–1.000	unitless	(Delwiche and Steyn 1970,

				Mariotti *et al* 1981, Fogel and Cifuentes 1993, Hoch *et al* 1992)
pH	pH	7.5–8.5	unitless	Measured in situ
Temp	temperature	10–20	°C	Measured in situ
pK_a	acid dissociation constant for NH_4^+	9.4	unitless	Calculate(Olofsson 1975)

Table 2. Summary of all rates from best-fits. Reported as X mgN/L or mass of N transformed by each process in the river reaches under study..

	Waterloo WWTP 2007-10-30	Waterloo WWTP 2008-07-01	Kitchener WWTP 2007-10-23	Kitchener WWTP 2008-07-18
NH_3 Volatilization	2.3	0.11	0.02	0.12
NH_4^+ Uptake	0.16	0.37	5.0	1.9
Nitrification	2.1	7.3	8.4	3.0
Denitrification	3.0	4.1	0.30	0.41