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, Sharples 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 $\delta$ 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-N$_2$O and $\delta^{18}$O-N$_2$O
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-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
The objectives of this research are to (1) quantify changes in concentrations and δ¹⁵N values of TAN and NO₃⁻ 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, NH₃ 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 O₂ 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 NH₄⁺ by primary producers, nitrification of NH₄⁺ to NO₃⁻, loss of NH₃ to the atmosphere via volatilization, denitrification of NO₃⁻, and dilution of both NH₄⁺ and NO₃⁻. 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 NH₄⁺ and NO₃⁻ 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 NH₄⁺ 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/m²) 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 CaCO₃. 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 m³/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 NO₃⁻ concentrations are elevated (3–4 mgN/L) whereas NH₄⁺ 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 NH₄⁺, NO₃⁻, Cl⁻, DOC, δ¹⁵N-NH₄⁺, and δ¹⁵N-NO₃⁻ 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 NH₄⁺ and δ¹⁵N-NH₄⁺ 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 NH₄⁺ and NO₃⁻...
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 m³/s) and 2008-07-01 (typical discharge 2–4 m³/s). The plume from the Kitchener WWTP was sampled on 2007-10-23 (typical discharge 11–17 m³/s) and 2008-07-18 (typical discharge 8–11 m³/s).

**Analyses:**

Anion concentrations were measured on a Dionex ICS-90 ion chromatograph. Precisions and detection limits of NO₃⁻ were 0.07 mgN/L (standard deviation of 15 replicates of a standard solution) and 0.05 mgN/L, respectively. NO₂⁻ concentrations were rarely detectable. Precision of Cl⁻ measurements was < 1 mg/L. NH₄⁺ 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.

δ¹⁵N-TAN was measured via the diffusion method on acidified discs(Zhang et al 2007). Briefly, NH₄⁺ is converted to NH₃ by increasing the sample pH; NH₃ is trapped in a filter pack containing a 1 cm GF/D filter, acidified with H₂SO₄, trapped in a PTFE packet. The filter is dried and analysed for δ¹⁵N on a Carlo Erba 1108 elemental analyzer (EA) coupled to a Micromass Isochrom isotope-ratio mass spectrometer (IRMS). Precision of δ¹⁵N-NH₄⁺ analysis was ± 0.3‰. δ¹⁵N-NO₃⁻ was measured via the AgNO₃ method. Briefly, sample volumes were reduced by evaporation, SO₄²⁻ was removed by barium precipitation, and NO₃⁻ was collected on anion exchange resin in a column. After being eluted from the column, AgO was added to precipitate AgNO₃, which was analyzed on the same EA-IRMS as above. Precision of δ¹⁵N-NO₃⁻ was ± 0.5‰. Methodological tests indicated that the AgNO₃ method can capture NO₂⁻ since NO₂⁻ oxidizes rapidly to NO₃⁻ even in filtered samples.(Spoelstra 2004) Since previous measurements showed there was little NO₂⁻ in this river (NO₂⁻ was < 5% of NO₃⁻) the results presented here can be interpreted as δ¹⁵N-NO₃⁻.

**Model Setup**

To interpret patterns in the data, a dynamic model (hereafter NANNNO: nitrate, ammonia, nitrite, nitrous oxide) was developed to describe the dynamics of TAN, NO₂⁻, NO₃⁻, N₂O and their δ¹⁵N 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$_4^+$ → NO$_2^-$ and NO$_2^-$ → NO$_3^-$), though see complete ammonia oxidation by a single organism (comammox (van Kessel et al 2015)), denitrification (NO$_3^-$ → N$_2$O), and biological assimilation of NH$_4^+$ (Figure 2). In the model, N$_2$O produced by denitrification is allowed to accumulate rather than being further reduced to N$_2$; this choice was made because the N$_2$O: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 ε = α−1 and α = 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, Dincier and Kargi 2000), NH$_4^+$ uptake (MacIsaac and Dugdale 1969, D’Elia and DeBoer 1978), and denitrification (Dincier and Kargi 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, Meszéros 1992)). Ranges of potential gas exchange coefficients can be estimated from river channel geometry (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 $\alpha$ 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, $\delta^{15}$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 $\delta^{15}$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.

$\delta^{15}$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 $\delta^{15}$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
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$_2$O 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$_3^-$ (<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 ($\alpha_{nit1}$ and $\alpha_{nit2}$) 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$_2$O and a transient dip in $\delta^{15}$N-N$_2$O values that depends on initial conditions and the strength of $\alpha_{denit}$.

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 ($\alpha_{ge}$ (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 $k_{\text{nit2}}$ and $\alpha_{\text{nit2}}$ 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 ($k_{\text{nit1}}$ and $\alpha_{\text{nit1}}$) 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$_2$O, $\delta^{15}$N-TAN, $\delta^{15}$N-NO$_3^-$, and $\delta^{15}$N-N$_2$O. 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 $\alpha$ estimates are less confident. Two of the best-fit $\alpha$ value estimates, $\alpha_{\text{dnit}}$ and $\alpha_{\text{amup}}$, are at the bounds of the range of $\alpha$ 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 $\delta^{15}$N values is small despite the change in concentration being large.

**Discussion:**

The process-based NANN0 model was able to reproduce the observed dynamics in concentrations and the $\delta^{15}$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 $\delta^{15}$N-NO$_3^-$. This scenario suggests nitrification where the new NO$_3^-$ has the same $\delta^{15}$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, NH₄⁺ loss via volatilization was much lower than NH₃ loss via update or nitrification (Table 2). With river pH values of 7.6–8.4, well below the pKₐ 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). N₂O 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 N₂O and its δ¹⁵N 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, NO₂⁻ concentrations and δ¹⁵N values may provide additional information to the model by constraining nitrification.

The δ¹⁵N of benthic periphyton and invertebrate (Loomer 2008, Loomer et al 2014) as well as macrophyte (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 δ¹⁵N 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 NO₃⁻ uptake, preferential uptake of different N species, and release of TAN and NO₃⁻. The variability in isotopic fractionation during NH₄⁺ uptake is large and varies nonlinearly 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 δ¹⁵N of river biomass are difficult to capture in the parsimonious NANNO model structure; model fitting may be improved if the release of TAN and NO₃⁻ 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 NO₃⁻. 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 NO₃⁻ instead of NH₄⁺ in order to improve river O₂ concentrations, requires knowledge about which N enters the base of the foodweb via primary producers and consumers. In cases where δ¹⁵N-NO₃⁻ and δ¹⁵N-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. NO₃⁻ 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 δ¹⁵N-NO₃⁻ values do not vary as much as δ¹⁵N-TAN values. In such scenarios, response to increasing δ¹⁵N-TAN may be observable as a concomitant increase in the δ¹⁵N of primary producers and consumers (Loomer et al 2014, Hood et al 2014).

Since O₂, N, and phosphorus cycles are strongly linked, improving the understanding of nitrogen processes allows previous work on O₂ and phosphorus cycling in the Grand River (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 δ¹⁸O-NO₃⁻ values. However, recent work has demonstrated that predicting the δ¹⁸O values of nitrogenous species is more complicated than originally thought because there are poorly understood abiotic factors that alter the δ¹⁸O value of NO₂⁻ and NO₃⁻ as well as multiple pathways to produce N₂O (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 \( \text{NO}_3^- \) concentrations and their \( \delta^{15}\text{N} \) 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.

**Acknowledgements:**

Canada’s Natural Science and Engineering Research Council funded the field research under STPGP 336807-06 and STPGP 381058-09. Grand River Conservation Authority provided assistance with field data. A portion of this was work performed while under the sponsorship of the International Atomic Energy Agency Collaborative Research Program F32007. Field and laboratory assistance was provided by Richard Elgood, Neus Otero, Marilla Murray, Sarah Sine, David Snider, Madeline Rosamond. We thank Thomas Petzoldt for the ease with which the simecol package can be used and Hadley Wickham and Winston Chang for their work on and documentation of ggplot2.

**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](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 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 ($\text{NH}_3$) and ammonium ($\text{NH}_4^+$). The ratio is set by measured temperature and pH, and calculated pK 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 $\text{NH}_4^+$. Two-step nitrification (nit1, nit2) is modelled as $\text{NH}_4^+$ to $\text{NO}_2^-$ to $\text{NO}_3^-$. Denitrification (denit) is modelled as a loss of $\text{NO}_3^-$ to $\text{N}_2\text{O}$. Each stock is modelled independently for each isotope and flows are adjusted by isotope fractionation factors ($\alpha$).
3. Waterloo wastewater treatment plants plumes on 2007-10-30. Measured field data (adjusted for WWTP plume dilution using Cl\textsuperscript{−} 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\textsubscript{3}\textsuperscript{−} and mass-weighted $\delta^{15}$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 $\delta^{15}$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 $\delta^{15}$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 $\delta^{15}$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                 | \(t^{-1}, m^{-1}\) |                                                                            |
| \(\alpha_{NH3NH4}\) | equilibrium isotope fractionation factor between \(NH_3\) and \(NH_4^+\) | 1.047                  | unitless | (Li *et al* 2012, Hermes *et al* 1985)                                     |
| \(\alpha_{ge}\) | 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)      |
| \(\alpha_{nit1}\) | isotope fractionation factor for nitrification: \(NH_4^+\) to \(NO_2^-\) | 0.990–1.000            | unitless | (Gammons *et al* 2010)                                                   |
| \(\alpha_{nit2}\) | isotope fractionation factor for nitrification: \(NO_2^-\) to \(NO_3^-\) | 0.990–1.000            | unitless | (Gammons *et al* 2010)                                                   |
| \(\alpha_{denit}\) | isotope fractionation factor for denitrification: \(NO_3^-\) to \(N_2O\)  | 0.985–1.000            | unitless | (Sebilo *et al* 2003)                                                    |
| \(\alpha_{amup}\) | isotope fractionation factor 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 |
| pKₐ      | acid dissociation constant for NH₄⁺ | 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.

| Process          | 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                      |