Biogeosciences Discussion Paper Response to Referee Comments

Michael J. Pennino et al. "Sources andTransformations of Anthropogenic Nitrogen along an Urban River-Estuarine Continuum" doi:10.5194/bg-2016-264

Note: We copied the referee comments below and responded directly after each comment or question. The referee comments have a hyphen at the beginning of each comment. Our responses follow directly after each referee comment or question.

Response to Jack Middelburg, Associate Editor

While preparing your revision, please change the use of ppt for salinity. Use either no unit (as oceanographers do), or use practical salinity unit (psu). ppt is part per trillion, part per thousands is permille.

We have updated Figures 3 and 7 so that the axis label only says salinity without units.

Response to Referee 1

This is an interesting paper that answers a number of important research questions, covering the attribution of sources, transformations of nitrogen, and the impact of the hydrological conditions over the Potomac river-estuary continuum of 150 km. Isotope and mass balance approaches are combined to track nitrogen sources and transformations along this distance. The results of this work can be very helpful in designing strategies to manage the water quality of this densely populated river basin.

The paper is well structured, and reads easily. I have a few concerns and a number of minor comments and suggestions.

-Lines 290-304: the reasoning why the 14 down-stream WWTPs have little effect is completely unclear to me. Particularly 301-304 is not clear.

We have modified this paragraph and no longer say that we assume the 14 WWTPs have little effect, but focus instead on how their effect is only to increase the loads along the estuary, and thus counteract the overall decline in loads that are observed along the estuary. And we also emphasize how there is likely little impact on the isotope levels due to the average isotope levels from primary and secondary WWTPs being much lower than what was measured at the Blue Plains WWTP (see further details in the response below).

-With all the uncertainties associated with the mixing model (line 204-206, line 214-216) and the caution (use for illustrative purposes only), I wonder if it makes sense to present it at all, since I do not know what the meaning is of "illustrative purposes" is if I do not know the uncertainty. The attribution of sources in the text looks pretty certain (no word about the illustrative purpose), and the uncertainty ranges are very small. That is surprising to me, and I wonder how these ranges are obtained? Is it the same error propagation method discussed in lines 267-274?

The uncertainties in the nitrate isotope sources came from the literature, except for wastewater nitrate which came from averaging about a year of monthly samples.

The method of error propagation described in lines 267-274 was only used for the box model mass balance estimates - not the isotope mixing model, which used a Bayesian approach (described in the methods).

We believe the results of the isotope mixing model are still useful (such as to show trends over distance) despite the potential variability. Also, the other reviewer liked the mixing model approach for illustrative purposes and thought it could be used to make stronger conclusions. For example, seasonal endmembers could provide more confidence in the results because we found that seasonality/temperature mattered in endmembers. Many isotopic studies do not always take this into account and sometimes they just use literature values – our work showed that there are important seasonal variations and thus seasonal changes in the other endmembers may need to be captured.

We have updated the discussion section to discuss how the longitudinal trends in nitrate sources along the Potomac Estuary correspond with the other results of this study and how future use of the isotope mixing model would benefit from conducting the model separately for each season to better constrain the differences between seasons.

-The range for the contribution of denitrification to the TN decline of 23-27% (Line 478; Line 543) suggests it is an uncertainty, but is simply is two different estimates, a direct and indirect one. It is possible to provide a real uncertainty here? The Burial rate presented by Boynton et al. is an average for upper and lower Potomac estuary, and it is not clear if this calculation was done by the original paper or in this study, but it is probably quite and uncertain number. Similar question about the average denitrification rate.

The estimates of % burial, % denitrification, and % assimilation have been modified. They use information from the Boynton et al. (1995) paper (Table 6) as well as new denitrification rate estimates from Cornwell et al. 2016 and burial rate from Harris (manuscript in prep). The Boynton et al. paper did not provide uncertainties in their estimates. But we now have error estimates for the burial and denitrification rates. Also, recent measurements of burial (manuscript in preparation) and denitrification (Cornwell et al. 2016) are in line with these estimates.

Citation:
Cornwell et al. 2016. Sediment-Water Nitrogen Exchange along the Potomac River Estuarine Salinity Gradient. JOURNAL OF COASTAL RESEARCH 32:776-787

-In various places the authors indicate that a statement or reason for a phenomenon is described “below”: e.g. line 292, 311, 318, 434, 514, 529, 547,597, 633. For readers this is awkward, because they start looking where this could be, because they want the explanation for something they read. Now either the word below can be avoided by

placing the discussion referred to directly after the statement, or the explanation comes first, and then the concluding statement.

We have removed most of the instances where we say “discussed below” because it was either unnecessary or sufficiently discussed in that section.

Minor comments

-I am not sure if present and past tense is consistently used correctly in the results and discussion sections. Please check.

We have checked this and fixed any incorrect use of past or present tense.

-I do not know how many times the words "additionally", "suggest" and "suggesting" are used, but it is a lot. Please try to vary.

We have removed or changed several of the instances where we previously used "additionally", "suggest" and "suggesting". Other words we used were “indicate” or “show” instead of “suggest,” for example.

-Line 126 and line 134: confusion between current concentration of 2.3 mg/L and 2001-2008 concentration of 4.1 mg/L. What is current? Has it gone down further, or what is the reason of the difference?

To clarify I added that the 2.3 mg/L value was from 2009 and the 4.1 mg/L was from directly after the year 2000.

-Line 187: atmospheric deposition.

We added the word deposition after atmospheric.

-It is not clear to me if the sampling locations in Figure 2 correspond to those in Figure 1. For example, the first point at about -17 km is not in Figure 1.

We have updated Figure 2 so that it lines up with Figure 1, such that zero is at the location of the WWTP. And the first sample point is located at -6 km (or 6 km up-estuary from the WWTP)

-Line 232: insert that after indicate.

We have added the word “that” here

-What is at distance zero in Figure 2? Is that the WWTP?

We have updated Figure 2 so that it lines up with Figure 1, such that zero is at the location of the WWTP.

-Lines 267-274: I assume that the errors are expressed as standard deviations? If so, please mention.

We clarified that error propagation was done using standard errors.

-Line 295-296: The isotope signal for the Blue Plains has been mentioned previously. These references provide numbers for the 14 down-stream WWTPs, I assume, but it suggests that they are for Blue Plains.

We've added that these values were for typical WWTP nitrate isotopes to make sure they were not confused with Blue Plains values.

-Line 362: what is directly down-estuary?

We added to this sentence that the Blue Plains WWTP is directly down-estuary.

-Line 385: up-estuary? Line 386: 2km down-estuary? Is it possible to attach a code to sampling locations, show that in Figure 1 (and 2) and refer to those codes instead of these up and down indicators of locations?

We believe that updating Figure 2 so that both show the Blue Plains WWTP at distance zero will help. We do not think attaching codes is necessary. For clarification we also added to the text and figure captions that the distance up-estuary or down-estuary was in references to distance from the Blue Plains WWTP.

-Line 506: it is not clear if there is a long-term warming or increasing warming; are temperatures warming?

We removed the word "warming" before "water temperatures," for clarification.

-Line 528: What is the unit "mgd".

This unit is defined earlier in the manuscript (line 128).

-Line 546: is it AND assimilation?

Yes we made the appropriate revision.

-Lines 565 and 671: shorter times instead of lower.

We changed lower to shorter here.

-Lines 547-551: remineralization leads to addition of TN, so I'd attribute a decrease in TN to uptake and subsequent deposition.

We changed this so that it just says “attributable to high rates of phytoplankton uptake and detrital deposition.”

-The header 4.2 and 4.3 read like a conclusion, not a section header. In addition, it looks like in 4.2 a few words are missing (indicate that) and dominate (two processes dominate); If this is actually the intention, then please be consistent, and change 4.1 in a similar way.

We have changed the headers of 4.2 and 4.3 to no longer read as conclusions. They now read as: “4.2 Spatial Trends in NO_3^- Sources and Role of Denitrification, Assimilation and Nitrification” and “4.3 Isotope and Salinity Mixing Models and Influence of Temperature and Residence Time.”

-Line 634: may suggest!suggest or indicate? Otherwise 2x suggest

We changed may suggests to may suggest.

-Line 638: caused.

We changed “cause” to “caused.”

-Line 644: is supported.

We added the word is.

-Line 674: nitrate produced by nitrification.

We changed “derived from” to “produced by.”

-Line 681: delete “the” and change the order of the sentence: there is more conservative behavior when flows are larger.

We have incorporated these changes.

-Line 695: dominant.

We have changed the word “dominate” to “dominant.”

Response to Referee 2

General comments:

-The authors have used mass balance models, constrained with stable isotopes to identify the sources and fate of nitrogen in the Potomac river-estuary continuum. A large outfall from a tertiary sewage treatment plant contributes 8-47% of the total upstream N loading depending upon the season. The goal of the study is to evaluate how well this high N load is attenuated before being transported downstream to Chesapeake Bay. They highlight the

importance of making these measurements under different flow regimes since many studies have shown that N assimilation can be very sensitive to discharge. The approach the authors have taken serves as an excellent model for other studies but also illustrates some of the difficulties in this approach. Overall it is a very useful contribution and the findings can also help inform management to help understand where source reduction might be the most effective.

-I do believe the authors could make better use of the data they have to constrain some of the possible findings.

We have incorporated the reviewer's suggestions to help make better use of the data.

Specific comments

-Line 141 and 381 – this spans the period of time both before and after the nitrate in the effluent decreased by nearly half from the treatment plant. Would Fig 4 be a better fit if the data was separated into pre and post periods?

The Figure 4 caption already said it was "for this study period," but I added "(2010-2012)" for clarification.

-173-179 – Given how well studied this system is I suspect there is nitrite data? Based upon that data is nitrite high enough to be of concern?

For Potomac Estuary stations TF2.1 through LE2.3 (stations from the top of the estuary to the bottom of the estuary) the mean nitrite concentration from 2010-2012 is 0.013 mg/L and the minimum = 0.0055 mg/L and maximum = 0.0183 mg/L. The mean nitrite is about 2.4% of the mean nitrate+nitrite concentration.

Fawcett et al. 2015 says "If nitrite is present in seawater, even at levels <= 0.5% of nitrate+nitrite, it can noticeably affect the measured δ18O of nitrate+nitrite (Casciotti and McIlvin, 2007; Granger and Sigman, 2009; Granger et al., 2006). This is because, during bacterial conversion to N2O, nitrite is subject to a smaller fractional loss of O atoms than nitrate (3/4 versus 5/6) such that O isotopic fractionation during nitrite reduction to N2O is lower (by ~25‰) than that for N2O generated from nitrate with the same initial δ18O(Casciotti et al., 2007)."

Consequently, even though the nitrite levels are a very small portion of the nitrate+nitrite, it is possible there is an impact. We added text to the methods to further acknowledge this and included the proportion of nitrite in the samples for the reader.

-192 – single values are given here but fig. 6 shows a range of values which makes more sense. These uncertainties could be incorporated into the estimates.

The nitrate source end-member values from the literature has standard deviations associated with them (provides in lines 194-198) and these uncertainties were already incorporated into the nitrate isotope mixing model estimates.

-195, 202 I was a bit confused by this, aren't manure based fertilizers also used in region as well? The discussion is section 4.2 suggests this is a major input. Was the nitrate fertilizer value chosen because the authors know that is what is used here and manure is only important upstream?

There are 171 confined animal feeding operations (CAFOs) in Upper Potomac, above DC and there are 25 CAFOs in the lower Potomac below DC. This information was added to this section of the manuscript with a citation where the CAFO data were obtained.

-204 – I thought putting this in the methods was an odd way to present this. In spite of the uncertainty the isotopes do put some constraints on the data. I think it makes more sense to present the data and then discuss the limitations and errors.

This is a good suggestion, but we respectfully disagree with moving this section to the discussion. We feel that it should be presented before the results so that the reader knows the potential limitations of the isotope mixing model prior to reading the results. We are appreciative of the experience this reviewer has with these data, but we have encountered concerns from other reviews regarding these results and are being responsive to those concerns in this organizational structure.

-218, and section starting on 422 – How are additional lateral inputs of freshwater being dealt with here? There has been a lot of modeling of this region so I'm sure they are known but it would be good to state the assumptions/data behind this. Lateral sources of freshwater might also have significant nitrate concentrations and different isotopic signatures. If the amounts are trivial this should be stated.

We assume that the lateral inputs will show up in the samples that are from the main stem of the Potomac River Estuary and invoke runoff as a potential explanatory factor in our discussion. The lateral inputs of different nitrate concentrations and isotopic signatures would be accounted for in those measurements. We used model output from the Chesapeake Bay program (using HSPF, a hydrological surface water runoff model that is used to compute TMDLs for this system) to constrain nutrient inputs from the watershed associated with each "box" of the mass balance box model. This is described in the paragraph on line 275-289 and we have further clarified that sentence to read "freshwater and N inputs from the land".

-290 – I did not like the assumption that these other treatment plants would have little impact. An additional 32% is significant and depending upon where it is added could be very important. The locations of the plants are not given but could this account for the lack of change further downsteam post treatment change (line 367)? The authors also don't mention what types of plant these other WTP are (secondary or tertiary). Some secondary plants get to very high values 15N values if there is extensive open aeration. I agree that the net impact of all of these plants will probably be to underestimate biological assimilation but it would seem to be beneficial to constrain the system to the extent possible. Instead, it is dismissed here and then brought up in the discussion (526)

where we find out that the total flows are nearly as large as the Blue Plains plant. It is then brought up again on line 674-5. I believe the authors will have a more robust story if these plants are incorporated into the model.

We modified the first part of this paragraph to say "In the box model we made two assumptions regarding the 14 other WWTPs that are dispersed along the estuary below Blue Plains. All, but one of these WWTPs has tertiary treatment (the other has secondary treatment) (www.epa.gov/npdes). These other WWTPs have a combined TN load that is 32% of the TN load from Blue Plains. While the loads from these WWTPs are indirectly accounted for in the box model due to their impact on the concentrations in the estuarine water, it was not feasible to directly incorporate the loads from each WWTP into the box model estimates and thus there may be some added uncertainties. However, we can first assume that the estimated decline in nitrogen loads from the Blue Plains wastewater treatment plant to the mouth of the Potomac River Estuary results in conservative estimates. The additional load from the other WWTPs only adds to the loads estimated further down estuary and consequently the measured loss in N load from the Blue Plains wastewater load down-estuary (the difference between the loads at the mouth and at the head of the estuary) is a conservative estimate because it is less then would be expected, underestimating biological assimilation and removal. Second, for modeling purposes, we can also assume here that the loads from the 14 other WWTPs have little effect on the nitrate isotope signal." And then the paragraph goes on to describe how the other WWTPs have little impact on the isotope signal. Overall, our point here is not that the other WWTPs are not significant, but that it was not feasible to include them in the box modeling effort.

It would have been ideal to include the WWTPs in the mixing model, but data for parameterizing their inputs was not available – especially in terms of isotopic signatures. It would be possible to do some calculations using the box model with hypothetical isotope signatures from the various WWTPs, but if this is required by the reviewer for acceptance of this manuscript we will need to request additional time for revisions.

The lack of change further down-estuary (noted on line 367) was actually attributed to the 14 other WWTPs on line 525, but the discussion is being revised to further clarify this and other sections of the discussion (as suggested in the comments below).

Because we don't accurately know the nitrate isotope signal from the 14 other WWTPs it is not feasible to incorporate their impact on the isotope mixing model directly. Also, based on the new discussion text referred to above for this same question, we assumed the other WWTPs had little impact on the isotope signal compared to the Blue Plains WWTP.

We also acknowledge the potential uncertainties in these assumptions within the discussion.

-414 – Fractionation will only be apparent if only part of the pool is used. While this would seem to be the case, because, nitrate does not completely disappear, there is data

from a variety of sources that indicates that sometimes denitrification occurs in hot spots (like hyporheic zones) where part of the pool is completely denitrified without any change in the isotopic composition. I think this at least deserves some mention.

I added this information to the discussion.

-458-475 - I think this could be made clearer. I was initially quite concerned about the very large error bars. The authors attribute some of this to the uncertainties in the last box but in looking at Table 1 things don't improve that much when box 6 is omitted. If I assume all of the seasons are of equal length (3 months) than the seasonal averages presented on 458-460 work out to a loss of 9.03x106 kg/year. With the propagated error this is nearly +/- 100%! But this can be compared to the independent estimates of burial and denitrification rates presented in Boynton et al. 1995 (lines 469-474) of 9.89 x106 kg/year. This agreement is quite good, and I wonder if these huge error bars are due to the method of error propagation. A monte carlo approach might result in smaller errors. I think I would point out the good agreement before going on to attribute the % loss to burial and denitrification.

When we propagate the error we find that on an annual average $9.1 \times 10^6 \pm 5.1 \times 10^6$ kg/yr of TN are exported to the Bay. We added this to the results and discussion and said that this is a close to Boynton et al. (1995) who estimated 14.1×10^6 kg/year are exported from the Potomac River (we did not see the 9.89 x106 kg/year value referred to in this comment).

Should the Monte Carlo error propagation be deemed critical for acceptance, we will need additional time. We have attempted to explore this, but with the multiple uncertainties in parameterization of the box model this has not been immediately obvious to constrain. Estimating errors in box models is a very new approach to take, in fact carrying out the error propagation efforts described here has been a point of discussion with many of our colleagues who regularly use box models (but rarely estimate uncertainty). We expect that a future manuscript will delve into these methods with greater attention.

-Section 4.2 and 4.3 This discussion is quite long and discusses many possible explanations for some of the data but seldom comes to strong conclusions. The authors have some great data here, I'm not sure they are making the most of it. These section contains a lot of statements such as those on line 607 "15N-NO3 values were likely higher in warmer months due to denitrification" since monthly measurements were made don't you know whether or not this is true? No mention is made on line 198 of seasonal changes so I had assumed this was not true. If it is true, the model should be run with different values for different seasons correct? The isotopes are not sufficient to tell when nitrate removal is due to assimilation or denitrification but doesn't the Boynton et al. 1995 data provide some insights that could be used? As mentioned above, on lines 674 the possible role of N from additional treatment plants is brought up when it had been dismissed earlier. So, overall, I think the authors may be able to constrain this system better and come up with more robust conclusions.

For line 607 we removed the word “likely” and instead said that the 15N values were higher “due likely to higher denitrification”, because we do know that 15N-NO3 values were higher in the warmer months (nitrate isotopes were measured monthly) but we are not certain that denitrification is the only cause.

A manuscript is in preparation that leverages this work and that of Cornwell et al. (2016) to establish nitrogen budgets for the Potomac estuary. That is the publication we are planning to use for exploration of the biogeochemistry of these rates in greater detail and where detailed flux measurements will be used to estimate assimilation, etc. That effort also included primary productivity estimates and N efficiency rates that we think will better inform the reviewer’s points here. We were able to use data from the manuscript in preparation to obtain an estimate for the burial rate in the Potomac

In the discussion we have acknowledge that it would be helpful to develop seasonal isotope mixing models due to our results showing that temperature and seasonality play a role. But due to lack of data on the seasonality of fertilizer and nitrification endmembers we do not think it is feasible for the scope of this paper.

We have changed the wording to the previous section about the 14 other WWTPs so that we are not dismissing the fact that they contribute a significant load or volume of water, but that their contribution does not adversely impact the trends.

-Conclusions – the importance of hydrology and temperature in N transformation is a critical issue for management and often discussed but removal is also a function of total load. I agree with all of the authors statements but differences in the N behavior in the manuscript is largely discussed by season and I think the conclusions could do a better job talking about all three factors.

We have added in further information for annual averages into the results and discussion section.

Sources and Transformations of Anthropogenic Nitrogen along an Urban River-Estuarine Continuum

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Abstract

Urbanization has altered the fate and transport of anthropogenic nitrogen (N) in rivers and estuaries globally. This study evaluates the capacity of an urbanizing river-estuarine continuum to transform N inputs from the world's largest advanced (e.g. phosphorus and biological N removal) wastewater treatment facility. Effluent samples and surface water were collected monthly along the Potomac River Estuary from Washington D.C. to the Chesapeake Bay over 150 km. In conjunction with box model mass balances, nitrate stable isotopes and mixing models were used to trace the fate of urban wastewater nitrate. Nitrate concentrations and $\delta^{15}N\text{-}NO_3^-$ values were higher down-estuary from the Blue Plains wastewater outfall in Washington D.C. (2.25±0.62 mg/l and 25.7±2.9‰, respectively) compared to upper-estuary concentrations (1.0±0.2 mg/l and 9.3±1.4‰, respectively). Nitrate concentration then decreased rapidly within 30 km down-estuary (to 0.8±0.2 mg/l) corresponding with an increase in organic nitrogen and dissolved organic carbon, suggesting biotic uptake and organic transformation. TN loads declined down-estuary (from an annual average of 48,000±5,000 kg/day at the sewage treatment plant outfall to 23,000±13,000 kg/day at the estuary mouth), with the greatest percentage decrease during summer and fall. Annually, there was a 36±19% loss in wastewater NO_3^- along the estuary, and 4–71% of urban wastewater TN inputs were exported to the Chesapeake Bay, with the greatest contribution of wastewater TN loads during the spring. Our results suggest that biological transformations along the urban river-estuary continuum can significantly transform wastewater N inputs from major cities globally,

and~~but~~ more work is necessary to evaluate the potential of organic nitrogen and carbon to contribute to eutrophication and hypoxia.

Key Words

Estuary, Mass Balance, Mixing Model, Nitrate Isotopes, Source Tracking, Wastewater

1 Introduction

Urbanization and agriculture have greatly increased the exports of nitrogen from coastal rivers and estuaries globally, contributing to eutrophication, hypoxia, harmful algal blooms, and fish kills (e.g. Aitkenhead-Peterson et al., 2009; Kaushal et al., 2014b; Nixon et al., 1996; Petrone, 2010; Vitousek et al., 1997). Despite billions of dollars spent on regulatory and technological improvements for wastewater treatment plants (WWTPs) and agricultural and urban stormwater runoff (e.g. US-EPA, 1972, 2009, 2011), many coastal waters are still impaired. Also, there are major questions regarding how far urban sources of N (wastewater and stormwater runoff) are transmitted along tidal river-estuarine networks to N-sensitive coastal receiving waters. This study evaluates the capacity of a major river-estuarine system to transform and attenuate N inputs from the world's largest advanced (e.g. phosphorus and biological nitrogen removal) wastewater treatment plant (Blue Plains) before being transported down-estuary to the Chesapeake Bay. We used a combination of stable isotope and box model mass balance approaches to track the fate and transport of anthropogenic nitrogen across space and time.

In addition to urban and agricultural inputs, altered river-estuarine hydrology can contribute to higher exports of N. Jordan et al. (2003) found that annual water discharge

522 increased as the proportion of developed land in a coastal watershed increased. Higher
523 flows, typically during winter and spring months, have also been associated with higher
524 N loads in coastal river-estuaries (Boynton et al., 2008). Furthermore, regional climate
525 variability amplifies pulses of nutrients and other contaminants in rivers (Easterling et al.,
526 2000; IPCC, 2007; Kaushal et al., 2010b; Saunders and Lea, 2008) and alters the biotic
527 transformation of N due to changes in hydrologic residence times (Hopkinson and
528 Vallino, 1995; Kaushal et al., 2014b; Wiegert and Penaslado, 1995). For example, high
529 flow periods related to storms can induce stratification and impact salinity regimes
530 (Boesch et al., 2001), which affects nutrient biogeochemistry like ammonium and
531 phosphate concentrations (Jordan et al., 2008). An improved understanding of the
532 longitudinal assimilatory capacity for nitrogen by large river-estuarine systems across
533 different flow regimes is needed for guiding effective coastal river and estuarine
534 management strategies.

535 One critical and innovative approach to effectively manage coastal nutrient
536 pollution is to 1) track the relative contributions of N export from different sources within
537 the watershed and 2) understand the potential for longitudinal transformation within
538 coastal rivers and estuaries. Recent studies using stable isotopes (Kaushal et al., 2011;
539 Kendall et al., 2007; Oczkowski et al., 2008; Wankel et al., 2006) have shown that these
540 methods can be helpful in elucidating sources and transformations of nitrogen. However,
541 these studies are typically conducted at relatively smaller spatial scales and without
542 coupling to mass balance approaches over both time and space.

543 Here, we combine isotope and mass balance approaches to track sources and
544 transformations of urban wastewater inputs to Chesapeake Bay over space and time

across an urban river-estuary continuum spanning over 150 km. The space-time continuum approach has previously been used in studying fate and transport of carbon and nitrogen in urban watersheds (Kaushal and Belt, 2012; Kaushal et al., 2014c), and here we explore extending it to river and estuarine ecosystems. Our overarching questions were: 1) how does the importance of point *vs.* non-point sources of N shift along a tidal and stratified urban river-estuary continuum across space and time? 2) What is the capacity of an urban river-estuary continuum to transform or assimilate anthropogenic N inputs? 3) How are transport and transformations of N affected by differences in season or hydrology? An improved understanding of how sources and transformations of N change along the urban river-estuarine continuum over space and time can inform management decisions regarding N source reductions along urbanizing coastal watersheds (e.g. Boesch et al., 2001; Kaushal and Belt, 2012; Paerl et al., 2006).

2 Methods

2.1 Site Description

This study is focused on the tidal Potomac River Estuary, which includes the section of the river from Washington D.C. to its confluence with the Chesapeake Bay (Fig. 1). The Potomac River Estuary begins as tidal freshwater, becoming oligohaline ~30-50 km below Washington D.C., and mesohaline at its mouth approximately 160 km below Washington D.C. (Jaworski et al., 1992). The Potomac River Estuary can be seasonally stratified (Hamdan and Jonas, 2006), especially in the southern portion of the system where intruding, saline bottom water from the main stem of the Chesapeake Bay leads to density driven estuarine circulation patterns (Elliott, 1976, 1978; Pritchard,

1956). Mixing is most evident at the estuarine turbidity maximum (Hamdan and Jonas, 2006), ~60-80 km below Washington D.C., and the water column is generally well mixed above the estuarine turbidity maximum zone in the tidal fresh and oligohaline regions of the estuary (Crump and Baross, 1996; Sanford et al., 2001).

The watershed draining to the Potomac River Estuary is classified as 58% forested, 23% agricultural, and 17% urban, based on Maryland Department of Planning data for 2002 (Karrh et al., 2007a). Based on the Chesapeake Bay Program (CBP) Model it was estimated that during 2005 total inputs of nitrogen were 33% from agriculture, 20% from urban (e.g. stormwater runoff and leaky sewers), 19% from point sources (wastewater treatment plants and industrial releases), 11% from forest, 10% from septic, 6 % from mixed open land, and 1 % from atmospheric deposition to water (Karrh et al., 2007b). The CBP model is developed using long-term monitoring data and the non-point loads are estimated from a variety of sources including land cover and agriculture records (Karrh et al., 2007b).

The Potomac River Estuary also receives N inputs from the Blue Plains wastewater treatment plant, located in Washington, D.C. In 2009 Blue Plains ~~currently~~ discharge~~d~~s 2.3 mg/L of NO_3^- and 3.7 mg/L of TN, on average, and export~~ed~~s~~d~~ loads of approximately 2,300 kg/day of NO_3^- and 3,900 kg of TN. Overall, Blue Plains treats and discharges approximately 280 million gallons per day (mgd), almost 5% of Potomac River's annual discharge. In the past several decades, Blue Plains has undergone several technological improvements with phosphorus removal in the 1980s and enhanced N removal beginning in the year 2000. Since the implementation of advanced wastewater treatment technologies at Blue Plains, there has been a significant decrease ($p < 0.01$) in the

concentration of nitrate in effluent discharge, from an average of 7.2 ± 0.3 mg/L before the year 2000 (years 1998 and 1999) to an average of 4.1 ± 0.4 mg/L directly after 2000 (years 2001 through~~to~~ 2008).

2.2 Analysis of long-term spatial and temporal water chemistry data

Surface and bottom water N and carbon data collected by the Maryland Department of Natural Resources (DNR) and accessed through the Chesapeake Bay Program's data hub website (Chesapeake Bay Program, 2013) was used to look at historical (1984 to 2012) monthly nutrient concentrations from stations located longitudinally along the Potomac River Estuary (Fig. 1). These data were used to look at the spatial and temporal trends for dissolved and particulate forms of N and dissolved organic carbon (DOC) in the Potomac River Estuary prior to and during this study.

2.3 Water Chemistry Sampling

Water chemistry samples along the Potomac River estuary were collected monthly for one year from April 2010 to May 2011; from 12 km to 160 km below the Blue Plains wastewater treatment plant (See Fig. 1). Water was collected from the surface (top 0.5 m) and bottom water depths. ~~Additionally, s~~Surface water samplings from 6 km above to 12 km below the Blue Plains wastewater treatment plant effluent outfall were collected seasonally during this time (Fig. 1). Water temperature and salinity was also measured during each water chemistry sampling.

2.4 Nitrate $\delta^{15}N$ and $\delta^{18}O$ Isotope Analysis

Surface samples for $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ isotopes of dissolved nitrate were filtered (0.45 μm), frozen, and shipped to the UC Davis Stable Isotope Facility (SIF) for analysis. The isotope composition of nitrate was measured following the denitrifier method (Casciotti et al., 2002; Sigman et al., 2001). In brief, denitrifying bacteria are used to convert nitrate in samples to N_2O gas, which is collected and sent through a mass spectrometer for determination of the stable isotopic ratios for N and O of nitrate ($^{15}N/^{14}N$ and $^{18}O/^{16}O$). Values for $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ are reported as per mil (‰) relative to atmospheric N_2 ($\delta^{15}N$) or Vienna Standard Mean Ocean Water (VSMOW) ($\delta^{18}O$), according to $\delta^{15}N$ or $\delta^{18}O$ (‰) = [(R)sample / (R)standard - 1] × 1000, where R denotes the ratio of the heavy to light isotope ($^{15}N/^{14}N$ or $^{18}O/^{16}O$). For data correction and calibration UC Davis SIF uses calibration nitrate standards (USGS 32, USGS 34, and USGS 35) supplied by NIST (National Institute of Standards and Technology, Gaithersburg, MD). The long-term standard deviation for nitrate isotope samples at UC Davis SIF is 0.4 ‰ for $\delta^{15}N\text{-}NO_3^-$ and 0.5 ‰ for $\delta^{18}O\text{-}NO_3^-$. Previous studies (Kaushal et al., 2011; Kendall et al., 2007) indicate that the relative amounts of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ can be used to determine specific sources of nitrate (i.e. fertilizer, nitrification, atmospheric, or sewage derived nitrate).

It should be noted that while the denitrifier method converts sample NO_3^- and NO_2^- to N_2O gas, in marine systems, NO_2^- has been shown to complicate interpretations of the N and O isotopes of NO_3^- if it remains unaccounted for (e.g. Fawcett et al., 2015; Marconi et al., 2015; Rafter et al., 2013; Smart et al., 2015). This is partially because during the reduction of NO_3^- and NO_2^- to N_2O by the denitrifiers, the O isotope effects

are different (and thus need to be corrected for). In addition, the $\delta^{15}N$ of NO_2^- can be extremely different from that of NO_3^-, potentially further complicating interpretation of the data. We found that in the Potomac Estuary stations TF2.1 through LE2.3 (stations from the top of the estuary to the bottom of the estuary) the mean nitrite concentration from 2010-2012 is 0.013 mg/L and the minimum = 0.0055 mg/L and maximum = 0.0183 mg/L. The mean nitrite is about 2.4% of the mean nitrate+nitrite concentration. Based on the literature (Fawcett et al., 2015), this level of nitrite is still high enough to have some impacts on the nitrate isotope values, with differences up to 5‰ for both N and O isotopes of nitrate when using the denitrified method with and without nitrite mixed with nitrate in the samples (Casciotti & McIlvin 2007).

2.5 Nitrate Isotope Mixing Model

To distinguish between the different potential nitrate sources we used a Bayesian isotope mixing model (Parnell et al., 2010; Parnell et al., 2013; Xue et al., 2012; Yang and Toor, 2016). For the Bayesian isotope mixing model, the Stable Isotope Analysis in R (SIAR) package was used to determine the fraction of nitrate in each sample from four different sources: wastewater, atmospheric deposition, nitrification, and nitrate fertilizer (Parnell et al., 2010; Parnell et al., 2013; Xue et al., 2012; Yang and Toor, 2016). The SIAR mixing model is able to incorporate uncertainty in nitrate source estimates based on the uncertainty in the nitrate source endmembers ~~(see below)~~ (Parnell et al., 2010; Parnell et al., 2013; Xue et al., 2012; Yang and Toor, 2016).

Nitrate source end-member values, for $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ were obtained from the literature, except wastewater nitrate, which was obtained from this study. The

end-member values for $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ were -10.3±1.7 and 10.1±1.5, respectively for nitrate from nitrification (Mayer et al., 2001), 0±3 and 22±3, respectively for NO_3^- fertilizer (Mayer et al., 2002), and 3±3 and 69±5, respectively for atmospheric nitrate (Burns and Kendall, 2002; Divers et al., 2014). The wastewater $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ end-member values (31.5±7.8 and 11±4.5, respectively) were based on averaging the effluent nitrate isotope values measured monthly from Blue Plains during the study period. The nitrification source represents NO_3^- from nitrification in the water as well as nitrification of ammonia fertilizer in the watershed. The fertilizer source represents synthetically produced NO_3^- fertilizer, not the more common ammonia fertilizer. Animal manure was not used as one of the end-members because this source is more significant in the upper Potomac River, above Washington, D.C. compared to the Lower Potomac River watershed. For example, there are 171 concentrated animal feeding operation (CAFOs) in Upper Potomac compared to 25 CAFOs in the lower Potomac below DC (U.S. EPA, 2016).

Due to the variability in nitrate source endmembers, the mixing model was used primarily for illustrative purposes and should be viewed with caution (particularly with regard to identifying other sources besides wastewater). For example, there ~~is high~~ can be high variability in the nitrification source endmembers because nitrate from nitrification can come from ammonia fertilizer, manure fertilizer, particulate organic matter within the water column, etc. The nitrate from nitrification will therefor carry a range of nitrate isotope values reflecting its original source (Kendall et al., 2007). ~~Additionally~~Also, because denitrification is known to cause the increase in $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values through isotopic fractionation in approximately a 2:1 relationship

(Divers et al., 2014; Kendall et al., 2007), this isotopic enrichment can complicate the identification of wastewater nitrate. As a result, water samples with increased wastewater nitrate, based on the mixing model, may also ~~suggest~~ indicate denitrification has played a role in the isotopic levels of the sample nitrate.

2.6 Salinity *vs*. Nitrate Concentrations ~~-~~and Isotope Mixing Plots

An additional method using plots of salinity *vs*. NO_3^- concentration or NO_3^- isotopes was used to assess whether there is conservative mixing (dilution), or mixing with additional NO_3^- sources down-estuary, or losses of NO_3^- through biotic uptake or denitrification (Middelburg and Nieuwenhuize, 2001; Wankel et al., 2006). Mixing line equations for NO_3^- concentrations were based on equations 1-3 from Middelburg and Nieuwenhuize (2001) and isotopes mixing lines were based on equation 4 from Middelburg and Nieuwenhuize (2001). The mixing line equations and endmember values used for salinity and nitrate isotopes are provided in supporting information (Table S2). Based on those equations, the salinity *vs*. NO_3^- concentration mixing lines are linear, while the mixing lines for NO_3^- isotopes are non-linear (Middelburg and Nieuwenhuize, 2001). Wankel et al. (2006) suggests that when nutrient concentrations fall above the mixing line this indicates an additional source to raise the concentrations, while concentrations that fall below the mixing line indicate that there is a nutrient sink (e.g., denitrification, assimilation, etc.). For nitrate isotopes, when the $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values fall above this mixing line, this could indicate an additional source or the fractionation of nitrate from assimilation or denitrification that would increase the heavy isotope levels, while isotope values below the mixing line could indicate an additional

source of nitrate with lighter isotope values, such as from nitrification or fertilizer sources (Wankel et al., 2006).

2.7 Estuarine ~~Nitrogen~~ Net Fluxes of Nitrogen

A box model was used to estimate net fluxes of TN, NO_3^-, and nitrate isotope loads along the Potomac River Estuary using methods modified from Officer (1980), Boynton et al. (1995), Hagy et al. (2000), and Testa et al. (2008), which are widely used methods for tracking nutrient fluxes in estuaries between different salinity zones. First, the Potomac Estuary was divided into 6 boxes in order to accommodate adequate sampling stations per box, and to evaluate net fluxes at key locations along the estuarine gradient (Fig. 2). Next, due to the Potomac Estuary having a semi-diurnal tidal cycle, where there is movement back and forth across boundaries of the box model, mean monthly freshwater discharge inputs to the first box (USGS, 2014) and interpolated salinity values (measured monthly from surface and bottom waters throughout the system) were used to calculate advective and diffusive exchanges of water and salt between adjacent boxes. Salt balances were then used to compute net exchanges at the boundaries of the six model boxes, similar to previous estuarine box model studies (e.g. Boynton et al., 1995; Hagy et al., 2000). Average monthly TN, NO_3^- and NO_3^- isotope concentrations (collected from the surface and bottom water at each station, except for NO_3^- isotopes, which were collected from the surface only) were multiplied by net estimated exchange values at the box boundaries and summed to calculate the N load leaving or entering each box. In order to calculate the loads for NO_3^- isotopes, the δ^{15}N-NO_3^- and δ^{18}O-NO_3^- values in per mil (‰) were converted to concentrations (µg/L) by

multiplying the NO_3^- concentration of the sample by R, the ratio of the heavy to light isotope ($^{15}N/^{14}N$ or $^{18}O/^{16}O$). Fluxes were estimated for each month during the sampling period and then averaged to find seasonal estimates of N fluxes for the Potomac. The box model results were used to compute: (1) the total inputs of N, (2) the % inputs of loads from Blue Plains, (3) the net export of N to the Chesapeake Bay, (4) the % of Blue Plains inputs that are exported, (5) the net loss in loads along the estuary, and (6) the contribution of N loads from the Chesapeake Bay through tidal inflow.

To account for uncertainty in monthly load estimates, error propagation (using standard errors) was used for each of the hydrologic and nutrient inputs to the model. For example, the error in discharge data came from averaging the mean daily discharge for each month, the error in water concentrations came from averaging the surface and bottom water concentrations, and the error in N from atmospheric deposition came from averaging the weakly deposition data for each month. These uncertainties in the inputs to the box model were then propagated for each of the box model calculations, similar to Filoso and Palmer (2011).

Inputs to the box model include, total monthly precipitation data based on averaging data from three stations along the Potomac Estuary (Precipitation data is from the NOAA National Centers for Environmental Information, Climate Data Online), monthly estimates of atmospheric deposition for NH_4^+, NO_3^-, and DIN (obtained from the National Atmospheric Deposition Program / National Trends Network), NO_3^- concentrations and isotope levels in atmospheric deposition (from Buda and DeWalle, 2009, for the nearby central Pennsylvania region for the year 2005, which was a similar year hydrologically (as described below)), freshwater and N inputs from the land (from

Chesapeake Bay model output from 2005), surface and bottom water nutrient and salinity concentrations (from MD DNR), and inputs from the Blue Plains wastewater treatment plant. Also, while there are no USGS gages located along the Potomac Estuary, there is one USGS gage (USGS 01646580) located directly above the Estuary, above the fall line (the location where the hydryodynamics of the river cease being tidally influenced) and this gage was used to account for freshwater inputs into the first box. The model also takes into account water temperature and evaporation.

In the box model we made two assumptions regarding the 14 other WWTPs that are dispersed along the estuary below Blue Plains. All, but one of these WWTPs has tertiary treatment (the other has secondary treatment) (www.epa.gov/npdes). These other WWTPs have a combined TN load that is 32% of the TN load from Blue Plains. While the loads from these WWTPs are indirectly accounted for in the box model due to their impact on the concentrations in the estuarine water, it was not feasible to directly incorporate the loads from each WWTP into the box model estimates and thus there may be some added uncertainties. However, we can first assume that the estimated decline in nitrogen loads from the Blue Plains wastewater treatment plant to the mouth of the Potomac River Estuary ~~are~~results~~s~~ in conservative estimates. The additional load from the other WWTPs only adds to the loads estimated further down estuary and consequently the measured loss in N load from the Blue Plains wastewater load down-estuary (the difference between the loads at the mouth and at the head of the estuary) is a conservative estimate because it is less then would be expected, underestimating biological assimilation and removal. ~~and~~Second, ~~F~~for modeling purposes, we ~~can assume~~also assume here that the loads from the 14 other WWTPs have little effect on the nitrate

isotope signal. ~~w~~While $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ isotope values were not measured directly for the 14 other down-estuary wastewater treatment plants, based on the literature, the values for average WWTP nitrate isotopes are typically lower (~10‰ for $\delta^{15}N\text{-}NO_3^-$ and ~0 for $\delta^{18}O\text{-}NO_3^-$) compared to 31.5‰ for $\delta^{15}N\text{-}NO_3^-$ and 11‰ $\delta^{18}O\text{-}NO_3^-$ for Blue Plains (Kendall et al., 2007; Wang et al., 2013; Wankel et al., 2006). As a result, we expected the other WWTPs to have a similar or an even less pronounced wastewater isotope signal compared to Blue Plains, which has biological nitrogen removal (i.e. denitrification is promoted within the Blue Plains WWTP), elevating the $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ isotope values at Blue Plains more (Kendall et al., 2007). Consequently, the estimated nitrate loads down-estuary incorporate Blue Plains and nitrate inputs from the other WWTPs. They~~, and~~ are considered conservative estimates because the additional WWTPs only add to the TN loads and wastewater NO_3^- isotope signal~~s~~, so any decline in an isotope signal that we attribute to Blue Plains would likely be greater if data availability permitted us to specifically parameterize the isotope values for additional WWTP inputs~~, and thus lessening the potential decline in loads or isotope values down estuary~~.

A~~nother second~~ assumption was made for the box model related to estuarine mixing. Although portions of the lower estuary can be seasonally stratified, we assumed each box to be well mixed vertically as no bottom water isotope values were available to constrain a 2-layer box model. This assumption is supported by other bottom water data that is available and by samples taken along the width of the estuary. For example, we have conducted the box model and other analyses with and without bottom water isotope data and found minimal change in results (Fig. S1~~, see below~~). Our measurements of

various biogeochemical signatures at the station close to the estuarine turbidity maximum suggests that there is intense mixing at this site, and prior studies have documented extensive mixing in the freshwater tidal portion of the system (Elliott, 1976, 1978; Pritchard, 1956). Also, it can be assumed that because wastewater effluent inputs are freshwater, much of the effluent plume would likely not sink in the more dense estuarine waters moving up from the bay. Additionally, our box model estimates of net fluxes was compared to a complex, 3 dimensional hydrodynamic model (described below) that incorporates stratification, and this comparison provided support for the low impact of assuming mixing in our approach.

Only surface water samples were analyzed for $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ isotopes, and as a result our box model was not able to directly incorporate the potential impacts of stratification on the estimated flux of NO_3^- isotopes. However, ~~W~~while seasonal stratification has been found close to the mouth of the of the Potomac estuary (Hamdan and Jonas, 2006), using documented nitrate bottom water isotope values from near the mouth of the estuary (Horrigan et al., 1990) we calculate that incorporating bottom water isotope values would have a minimal impact on the flux estimates of our box model, particularly when not including spring 2011 (Fig. S1). But when including spring 2011, and using the reported values of 10‰ for bottom water $\delta^{15}N\text{-}NO_3^-$, based on Horrigan et al. (1990), in Boxes 5 and 6 where stratification is most likely, our estimates for the flux of $\delta^{15}N\text{-}NO_3^-$ from these boxes increases by 20% on average, and the net loss in load from box 1 to box 6 increases by 12% on average. This indicates that our estimates are conservative because by not using bottom water we estimate a smaller net loss in $\delta^{15}N\text{-}NO_3^-$ (Fig. S1).

For the box model we also assumed the estuary to be well mixed laterally. In terms of potential variability for samples taken at different locations along the width of the estuary, there was found for surface water samples, on average, a 6±3% difference in $\delta^{15}N\text{-}NO_3^-$, a 7±3% difference in $\delta^{18}O\text{-}NO_3^-$, a 24±8% difference in NO_3^-, and a 15±3% difference in TN (based on samplings that were done at two or more locations along the same longitudinal transect at approximately the same distance down-estuary, but at different locations horizontally at that location). Based on this, the nitrate isotopes values and NO_3^- and TN concentrations appear to show that the estuary is fairly well mixed laterally.

To assess the accuracy of the box model assumptions and results, estimated net fluxes of total N were compared to simulation output from the Chesapeake Bay Water Quality Model. This model was developed by the U.S. EPA to aid in efforts to set TMDLs for the Chesapeake Bay (Cerco et al., 2010), and combines a 3-D hydrodynamic model (CH3D) with a water quality model (CE-QUAL-ICM). Simulation output data were available for 1996, 2002, and 2005. We selected a simulation year (2005) because it had similar river discharge conditions to 2010, and compared modeled net fluxes of TN at three boundary locations to estimates at the same (or nearby) box model boundaries.

2.8 Statistical Analyses

Statistical analyses were performed using the statistical package R (R Development Core Team, 2013). Linear regression was used to test for significant changes in stream chemistry and nitrate isotope data with distance down estuary.

Repeated measures analysis of variance (ANOVA) was used to test for seasonal differences in nitrate isotopes trends with distance.

3 Results

3.1 Spatial and Temporal Trends in N Concentrations

Longitudinal patterns of dissolved inorganic nitrogen (DIN) in the lower Potomac River showed an increase in concentrations near and directly below the Blue Plains wastewater treatment plant and then a steady decline in concentrations down to the Chesapeake Bay (Fig. 3a). The implementation of tertiary treatment in 2000 coincided with a significant drop in annual average DIN concentration directly down-estuary of the Blue Plains WWTP (from 1.7 ± 0.02 to 1.3 ± 0.01 mg/l, $p < 0.05$) (Fig. 3a), when comparing years directly prior (1997-1999) and the years directly after 2000 (2001-2005). However, the impact of the wastewater treatment plant improvements on reducing longitudinal patterns of DIN was only apparent for the first 30 km down-estuary. After this, both the pre- and post-2000 DIN concentrations overlapped (Fig. 3a). As DIN decreased longitudinally down-estuary of the wastewater treatment plant, there was also a small, but significant increase in total organic nitrogen (TON) after the year 2000 ($p < 0.01$, Fig. 3a), not including the last sample near the mouth of the estuary, which is likely influenced by tidal inflow.

There were seasonal variations in DIN concentrations along the Potomac River Estuary with the greatest concentrations in the winter and spring (Fig. 3b). There is also a steeper decline in DIN with distance during fall, winter, and summer compared to the spring ($p < 0.05$, Fig. 3b). The average molar ratio of DIN to PO_4^{-3} (N:P ratio) showed

an initial increase, then a decrease as estuarine salinity started to increase (Fig. 3c). During the summer and fall, the N:P ratio fell below the Redfield ratio (16:1, the atomic ratio of nitrogen and phosphorus found in oceans and phytoplankton), around 40 km down-estuary and stayed below 16, which indicated a shift from P to N limitation. During the winter and spring, the N:P ratio never fell below 16 and increased steadily after 50 km down-estuary (Fig. 3c). There was also a significant negative relationship between NO_3^- and DOC concentration during the study period ($p < 0.01$, Fig. 4).

3.2 Spatial and Seasonal Trends in NO_3^- Isotopes and Sources

During each season, except spring, $\delta^{15}N\text{-}NO_3^-$ values increased sharply at the Blue Plains outfall, from 9.3 ± 1.4 ‰ up-estuary to 25.7 ± 2.9 ‰ at the outfall ($p < 0.05$), and then rapidly decreased within 2 km down-estuary of the Blue Plains WWTP to 15.7 ± 2.2 ‰ ($p < 0.05$, Fig. 5a). During the summer and fall, the $\delta^{15}N\text{-}NO_3^-$ values showed the largest increase near the effluent outfall (except for one very high winter value) and then a significant decrease ($p < 0.05$) with distance down-estuary. There was also a slight increase in $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values from 1 to 6 km down-estuary (Fig. 5a,b). During the winter and spring, the $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values remained relatively constant throughout the estuary, even near Blue Plains (Fig. 5a,b), while during the summer and fall the $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values steadily declined after 6-10 km down-estuary (Fig. 5a,b). At the mouth of the estuary, the $\delta^{15}N\text{-}NO_3^-$ values for all seasons were roughly equivalent (Fig. 5a). During the summer and fall, the $\delta^{18}O\text{-}NO_3^-$ values showed a steady decrease after 12 km down-estuary, while they increased during spring and winter (Fig. 5b).

Based on the nitrate isotope mixing model, nitrate contributions from wastewater ranged from 80 ± 13% at the wastewater outfall to 57 ± 11% within the first 1 km down-estuary. Wastewater nitrate contributions then decreased to 44 ± 14% at the confluence of the Potomac River Estuary with Chesapeake Bay (Fig. 5c), suggesting that there was a 36 ± 19% loss in wastewater NO_3^- along the estuary annually. Nitrate from nitrification (of N from upriver manure or ammonia fertilizer and also Blue Plains wastewater N) increased from 13 ± 12% at the wastewater outfall to 29 ± 22% at the confluence of the Potomac River Estuary with Chesapeake Bay (Fig. 5c). Nitrate from fertilizer increased from 6 ± 6% at the wastewater outfall to 22 ± 22% at the confluence of the Potomac River Estuary with Chesapeake Bay (Fig. 5c). Nitrate from atmospheric deposition changed little along the Potomac Estuary from 1 ± 1 at the wastewater outfall to 5 ± 5 at the confluence with the Chesapeake Bay (Fig. 5c). At the last two sampling stations near the mouth of the Potomac River Estuary, NO_3^- from fertilizer showed an increase, while NO_3^- from nitrification showed a corresponding decline (Fig. 5c).

3.3 $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$, NO_3^- Concentration, and Salinity Relationships

The Blue Plains effluent and Potomac River samples within 20 km downriver of the wastewater treatment plant showed a significant positive relationship between $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ ($p < 0.05$) (Fig. 6a). When denitrification and biotic uptake occurs, plotting $\delta^{15}N\text{-}NO_3^-$ *vs.* $\delta^{18}O\text{-}NO_3^-$ shows a 2:1 relationship (Kendall et al. 2007). The Blue Plains effluent samples showed approximately a 2.4 to 1 relationship. The samples within 20 km downriver showed a 3:1 ratio (Fig. 6a). The nitrate samples within the first 6 km showed a 2.4 to 1 relationship (Fig. 6a). There were also seasonal differences in the

relationship between $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ (Fig. 6b); spring, summer, and fall were characterized by close to a 2:1 relationship between $\delta^{15}N\text{-}NO_3^-$ *vs.* $\delta^{18}O\text{-}NO_3^-$, while winter showed a ~8:1 relationship.

Because salinity is a conservative tracer, plots of salinity *vs.* NO_3^-, $\delta^{15}N\text{-}NO_3^-$, and $\delta^{18}O\text{-}NO_3^-$ can indicate effects of mixing between water at the tidal freshwater section with water from the mesohaline section of the Potomac River Estuary. Deviations from the mixing lines can indicate additional sources or biological transformations (Middelburg and Nieuwenhuize, 2000; Wankel et al., 2006). Surface water NO_3^- concentrations and nitrate isotopes fell on (for $\delta^{18}O\text{-}NO_3^-$) or slightly below mixing line (for $\delta^{15}N\text{-}NO_3^-$) during the spring (Fig. 7a,b,c), which indicated mostly conservative mixing (dilution or inputs from low $\delta^{15}N\text{-}NO_3^-$ like nitrification~~, see discussion below~~). But during the summer and fall, the NO_3^- concentration and isotope values fell well below the mixing lines. During the winter, the values fell both above and below the mixing line (Fig. 7a,b,c), which indicated non-conservative mixing ~~(please see discussion below)~~.

3.4 Spatial and Seasonal Trends in N Loads

Our comparisons of box model net exchange estimates with simulation output provided by the Chesapeake Bay Program Eutrophication Model ("Bay Model") revealed similar TN loads between our results and the Bay Model in the winter, spring, and fall, with the largest differences in the models evident in the summer months at the boundary location where tidal fresh transitions to oligohaline conditions and at the mouth of the estuary (Table S3 and Figures 8 and 9). Even so, these differences are smaller than a

factor of 2 for winter and spring and for most of the summer and fall.~~,~~ D~~d~~espite the assumption of complete mixing in our box model, this is a good agreement considering the simplification of hydrodynamics inherent to a box modeling approach when compared to the highly constrained CH3D hydrodynamic modeling platform (Cerco et al., 2010). The Potomac estuary is well mixed along two thirds of its length, and this likely contributes to our success in applying a single layer box model to this system. The box model also permitted estimates of TN loads at smaller spatial scales than the three boundaries available from the Chesapeake Bay Program, which could enable a better interpretation of where Blue Plains effluent was subject to transformations in the oligohaline portion of the estuary (Fig. 8). The caveat here is that box-modeled summer loads should be interpreted with caution because they show the greatest differences from the CH3D model.

Results of the box model indicate~~d~~ that ~~on~~ an annual average of $8.4 \times 10^6 \pm 4.8 \times 10^6$ kg/yr of TN are exported to the Bay and the net loss in load for TN along the estuary (from Blue Plains to the mouth of the estuary), attributed to assimilation, burial and denitrification, was $9.1 \times 10^6 \pm 5.1 \times 10^6$ kg/yr of TN. Using an ~~estimated~~ N burial ~~-~~rate of 2.49~~9.32~~ $\times 10^6 \pm 3.1 \times 10^5$ kg/yr (Harris, unpublished data), a denitrification rate of 6~~3~~.17~~98~~ $\times 10^6 \pm 8.3 \times 10^4$ kg/yr (Cornwell et al., 2016) and a fisheries yield rate of 0.82×10^6 kg/yr (Boynton et al., 1995) ~~for the lower Potomac Estuary from Boynton et al. (1995)~~, we see that our box model estimate is nearly balanced by independently estimated values for these loss terms. ~~it was calculated that,~~ O~~o~~n a~~n~~ mean annual basis, ~~burial~~denitrification accounts for about 68 ± 1%~~5~~6 ~~($6.0 \times 10^6 \pm 3.4 \times 10^6$ kg/year)~~ of the loss in TN, ~~denitrification~~burial is estimated to account for 27 ± 3%~~6~~.2~~8.2~~ ~~($2.6 \times 10^6 \pm$~~

~~1.5 × 10^6 kg/year)~~ of the loss in TN, and assimilation into fisheries accounts for approximately 9% of loss in TN load along the Potomac Estuary.

T~~t~~he net load (kg/day) of TN, NO_3^-, and $\delta^{15}N\text{-}NO_3^-$ decreased down-estuary during each season (Fig. 10a-c, $p < 0.05$ for winter and spring and $p < 0.1$ for summer and fall). N loads were highest along the estuary during spring and winter (Fig. 10), and there was a greater decline in TN loads on average from box 1 to box 6 during winter and spring (a loss of ~27,000 ± 15,000 and 50,000 ± 52,000 kg/day, respectively) (Table 1) compared to summer and fall (a loss of ~7,000 ± 8,000 and 15,000 ± 13,000 kg/day, respectively). However, the summer and fall months showed a greater percent decline in TN (75 ± 75% and 112 ± 95%, respectively) compared to winter and spring (54 ± 40 and 36 ± 43%, respectively). The relatively high errors are primarily from the larger uncertainty found in the last box, at the mouth of the estuary, due to the larger size of this box and greater uncertainty in fluxes at the mouth of the estuary; the uncertainties are much smaller further up-estuary (See Fig. 10a). NO_3^- and $\delta^{15}N\text{-}NO_3^-$ follow the same seasonal patterns as TN. Also, winter, along with summer and fall, showed a greater percent decline in NO_3^- and NO_3^- isotope loads compared to spring (Table 1).

~~Using an estimated N burial rate of 7.09 × 10^6 kg/yr(which is an average of burial rate estimates for the upper and lower Potomac Estuary) from Boynton et al. (1995), it was calculated that, on an mean annual basis, burial accounts for about 77% of the loss in TN. Denitrification was then calculated, by difference, to account for the remaining 23% loss in TN load. Using a different independent method, based on the average annual estimated denitrification rate (2.8 ×10^6 kg/yr) from the upper and lower Potomac (Boynton et al., 1995), and the box model results, it is estimated that denitrification~~

~~accounts for about 27% of the TN removal. Consequently denitrification is estimated to account for 23 to 27% of the loss in TN load along the Potomac Estuary.~~

The percent contribution of TN inputs from the Blue Plains wastewater treatment to the main stem of the Chesapeake Bay ranged from 8 to 47 % (Table 1). The contribution was significantly lower during the winter and spring (10 ± 13 and 8 ± 1%, respectively) compared to summer and fall (38 ± 3 and 47 ± 13%, respectively, Table 1), when TN fluxes from all sources are relatively low. The percent ~~contribution~~ of Blue Plains wastewater TN inputs~~, which~~ that are exported to the Chesapeake Bay ranged from <4 to 71%, and they were highest in the spring (71 ± 20%, Table 1). There were also N inputs to the Potomac river-estuarine continuum from the Chesapeake Bay during each season, except spring, due to higher flows (Table 1 & 2) because flow in spring was too high to allow the inputs from the Bay that occurred in the other seasons. NO_3^- and $\delta^{15}N$-NO_3^- follow the same seasonal patterns as TN, showing the greatest percentage of inputs from Blue Plains exported during the spring.

4 Discussion

While coastal urbanization can have a major impact on water quality in receiving waters, the results of this study suggest that rivers and estuaries also show a large capacity to transform and~~or~~ bury anthropogenic N. In particular, our results ~~suggest~~ indicate that ~~30-96~~up to 95% of inputs of N from the Washington D.C. Blue Plains wastewater treatment plant were removed *via* burial or denitrification along the Potomac river-estuarine continuum, depending on the season (Table 1). Recent work shows that

urban watersheds and river networks can also be "transformers" of nitrogen across similar broad spatial scales, which impacts downstream coastal water quality (Kaushal et al., 2014a). Here, we show that the urban river-estuarine continuum also acts as a transformer and can have large impacts on the sources, amounts, and forms of nitrogen transported to the Chesapeake Bay. Our results showed that N transformation varied across seasons and hydrologic conditions with important implications for anticipating changes in sources and transport of coastal nitrogen pollution in response to future climate change. This is particularly significant, given long-term increases in ~~warming~~ water temperatures of major rivers and increased frequency and magnitude of droughts and floods in this region and elsewhere (e.g. Kaushal et al., 2010a; Kaushal et al., 2014b).

4.1 Spatial and Temporal Trends in N Concentrations and Loads

The decrease in DIN concentrations with distance down-estuary is largely from denitrification, assimilation, and burial, as indicated by the inverse relationship between NO_3^- concentrations and DOC and TON concentrations, the NO_3^- isotope data, and N mass balance data ~~discussed below~~. Dilution from tidal marine waters plays a minor role in the decrease in DIN and the incoming tidal waters may even contribute to DIN as suggested by the decrease in DIN slope after 130 km down estuary (Boynton et al., 1995), depending on the season. The installation of tertiary wastewater treatment technology at Blue Plains in the year 2000 showed a significant drop in DIN concentrations within 20-30 km of Blue Plains. However, the DIN concentrations below 30 km down-estuary were approximately the same based on an annual average, before and after the year 2000. One explanation is that the dissolved wastewater N is

completely assimilated into particulate organic matter (supported by the inverse NO_3^- *vs.* TON or DOC relationships (Fig.s 3a and 4) or removed by denitrification (as suggested by the isotope data ~~discussed below~~) within the first 10 km down-estuary, and thus the majority of DIN below 30 km is from other inputs than the Blue Plains wastewater treatment plant. For example, there are 14 other smaller wastewater treatment plants along the Potomac River Estuary, which contribute a total of about 270 mgd (almost as much as the amount Blue Plains contributes) and they could offset further decreases in NO_3^- concentrations down-estuary. Also, our isotope mixing model data ~~(discussed more below)~~ ~~suggests~~ shows that nitrification (likely of upriver manure or ammonia fertilizer inputs) and fertilizer are important sources further down-estuary; and 42% of the land-use along the Potomac Estuary is agriculture (Karrh et al., 2007b). A second explanation could be related to a change in N:P ratio with distance down-estuary. Specifically, there was a rise in estuarine salinity around 30 to 50 km down-estuary and a coinciding increase in dissolved PO_4^{-3} concentration (typical of the estuarine salinity gradient) (Jordan et al., 2008). When the N:P ratio fell below the Redfield Ratio of 16:1, the estuary could shift from P limitation to N limitation (Fisher et al., 1999). The potential shift from P to N limitation occurred 40-50 km down-estuary, around the estuarine turbidity maximum, which is associated with higher estuarine bacterial productivity (Crump and Baross, 1996), and may be driving DIN removal further down-estuary.

Mass balance indicates that TN and NO_3^- loads decreased down-estuary each season (despite inputs from the 14 other wastewater treatment plants down-estuary). The $8.4 \times 10^6 \pm 4.8 \times 10^6$ kg/year of TN exported to the Bay annually is close to the 14.1×10^6 kg/yr estimated by Boynton et al. (1995). The net loss in load for TN along the

estuary ($9.1 \times 10^6 \pm 5.1 \times 10^6$ kg/yr), attributed to burial and denitrification was also similar to the sum of the burial and denitrification rates estimated by Boynton et al. (1995) for the lower Potomac (13.3×10^6 kg/year of TN). Also, our comparison of net losses in TN along the estuary with independent estimates of burial (Harris, unpublished data), denitrification rate (Cornwell et al., 2016), and assimilation (Boynton et al., 1995) also closely align with our estimate for the net loss in load for TN along the estuary. ~~On an annual average, it was estimated that approximately 23-27% of the loss in TN could be attributed to denitrification, while 73-77% was lost through burial into the estuarine sediment~~. The large loss in TN load attributed to denitrification (68 ± 1%) ~~This~~ is supported by the NO_3^- isotope data indicating that there was likely denitrification (and assimilation) of NO_3^-, particularly within 6 km down-estuary from the Blue Plains wastewater treatment plant ~~(discussed further below)~~. Over seasonal time scales, there was a greater percent decline in TN loading during summer and fall, likely due to warmer temperatures and increased biological transformation (attributable to high rates of phytoplankton uptake and ~~,~~ detrital deposition~~, and remineralization for subsequent recycling~~) (Eyre and Ferguson, 2005; Gillooly et al., 2001; Harris and Brush, 2012; Nowicki, 1994), which suggested that the urban river-estuarine continuum may be more efficient at removing TN during the summer and fall. Compared to summer and fall, winter also had a relatively high percent decline in NO_3^- loads possibly driven by the higher concentrations typically found in winter months, which could result in quicker assimilation through first order reaction rate kinetics (Betlach and Tiedje, 1981). Since there was no evidence for denitrification during the winter, burial could also be a mechanism for the relative high decline in winter months, which is typical of higher

flows (Boynton et al., 1995; Milliman et al., 1985; Sanford et al., 2001). However, more work is necessary to evaluate the fate of nitrate using ecosystem process level measurements.

The higher total exports of TN and NO_3^- to Chesapeake Bay during the winter and spring are due to greater N inputs from the upper and lower watershed and/or greater flow rates. The proportion of N exports attributed to Blue Plains wastewater treatment plant were the highest in the spring, likely due to ~~lower~~ shorter water residence times (Table 2), resulting in less time for biological uptake, removal, or burial of N. The greater decline in N loads during the spring, however, may be attributed to multiple factors, such as greater N loads being imported from the upper estuary and higher concentrations, compared to summer and fall (Table 1) and thus driving greater losses (from burial and denitrification) due to first order reaction rate kinetics (Betlach and Tiedje, 1981) similar to winter (described above), stratification that is characteristic of higher flows (Boesch et al., 2001), and increased burial rates due to greater sediment loads during higher flows (Milliman et al., 1985; Sanford et al., 2001). As mentioned previously, more work is necessary regarding linking ecosystem processes and microbial dynamics with the fate of nitrate in the estuary. Nonetheless, the decline in TN and NO_3^- loads down-estuary each season provide strong evidence for the transformation and retention of N along estuaries.

4.2 Spatial Trends in NO_3^- Sources ~~Indicate~~ and Role of Denitrification~~, and Assimilation of NO_3^- initially Dominates~~ and ~~then~~ Nitrification ~~Dominates Further Down Estuary~~

The Potomac River estuary was a transformer of wastewater N inputs from the Washington D.C. metropolitan area to its confluence with Chesapeake Bay. The values for $\delta^{15}N\text{-}NO_3^-$ above the wastewater treatment plant were relatively high, suggesting upriver sources may primarily be from animal waste (Burns et al., 2009; Kaushal et al., 2011; Kendall et al., 2007). This is consistent with a previous study, which found that 43% of N inputs to the upper Potomac River are from manure (Jaworski et al., 1992), while the lower Potomac River has more fertilizer and combined animal feeding operations (CAFOs) (U.S. EPA, 2016). Effluent inputs from the Blue Plains wastewater treatment plant significantly increased the $\delta^{15}N\text{-}NO_3^-$ values even further, yet this NO_3^- signal from wastewater disappeared after 20-30 km down-estuary. The increase in $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values within the first 1 to 6 km down-estuary suggest denitrification or assimilation of nitrate, due to the lighter $\delta^{14}N\text{-}NO_3^-$ and $\delta^{16}O\text{-}NO_3^-$ isotopes being preferentially denitrified or assimilated and leaving behind the heavier nitrate isotopes (Granger et al., 2008; Granger et al., 2004; Kendall et al., 2007) ~~(see further discussion below)~~. But the gradual decline in both $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values from 6 km to 160 km down-estuary ~~suggests~~ indicates nitrification dominates this portion of the estuary (supported by the nitrate isotope mixing model results) because the process of nitrification, which converts ammonia to nitrate results in lighter nitrate isotopes being generated through fractionation (Kendall et al., 2007; Vavilin, 2014) ~~(see further discussion below)~~. However, the decline in $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ loads

corresponding with the decline in overall NO_3^- loads down-estuary also suggests that the heavy nitrate isotopes are being removed as well as the light isotopes. The disappearance of $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ down-estuary where NO_3^- concentrations are very low (~0.01 mg/l) may indicate that assimilation or even denitrification is occurring on the remaining heavy $\delta^{15}N\text{-}NO_3^-$ or $\delta^{18}O\text{-}NO_3^-$ after the lighter $\delta^{14}N\text{-}NO_3^-$ or $\delta^{16}O\text{-}NO_3^-$ is all used up (Fogel and Cifuentes, 1993; Vavilin et al., 2014; Waser et al., 1998a; Waser et al., 1998b).

Seasonal differences in the longitudinal trends for $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ suggest differences in biological transformations of nitrate due to differences in water temperature, hydrology, and/or N inputs. The $\delta^{15}N\text{-}NO_3^-$ values from effluent inputs were ~~likely~~ higher in warmer months due likely to higher denitrification rates in the wastewater treatment plant associated with warmer water temperatures (Dawson and Murphy, 1972; Pfenning and McMahon, 1997), resulting in elevated $\delta^{15}N\text{-}NO_3^-$ values produced by isotopic fractionation (Kendall et al., 2007; Mariotti et al., 1981). An increase in $\delta^{15}N\text{-}NO_3^-$ between 2 and 6 km down-estuary during summer and fall (Fig. 5b) further ~~suggested~~ shows increased denitrification or biological uptake due to warmer water temperatures and fractionation (Eyre and Ferguson, 2005; Gillooly et al., 2001; Harris and Brush, 2012; Nowicki, 1994). The significant drop in $\delta^{15}N\text{-}NO_3^-$ beyond 10 km down-estuary during summer and fall may have been due to mixing with other N sources and increased nitrification (Wankel et al., 2006), indicated by the salinity mixing line results ~~(see further discussion below)~~. During the spring, there was also a significant decline in $\delta^{15}N\text{-}NO_3^-$ between 10 and 160 km down-estuary, but this was likely attributed to dilution and nitrification, based on the conservative mixing results ~~discussed below~~.

The lack of a significant change during the winter, may be due to shorter residence times (Table 2) and cooler temperatures, contributing to lower biological transformation rates. Further down-estuary, near the mouth of the estuary, the increase in $\delta^{18}O\text{-}NO_3^-$ in winter and spring might indicate denitrification in the estuary but in spring nitrate seems conservative based on the salinity mixing plots. The decline in $\delta^{18}O\text{-}NO_3^-$ down-estuary in summer and fall suggest that processes other than denitrification in the estuary are controlling the $\delta^{18}O\text{-}NO_3^-$, such as nitrification.

4.3 Isotope and Salinity Mixing Models ~~Suggest Seasonal Patterns in N Transformation~~and Influence~~d~~ of ~~by~~ Temperature and Residence Time

Seasonally, the ~2:1 relationship between $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ during spring, summer and fall, may indicate denitrification or assimilation, but the salinity mixing plots ~~discussed below~~ suggests no denitrification in the spring. The fact that the $\delta^{15}N{:}\delta^{18}O$ ratio is between 1 and 2 for summer and fall may ~~suggests~~mean assimilation plays a role, which is supported by previous studies ~~which~~ that found a 1:1 relationship for assimilation in the marine environment (Granger et al., 2004; Karsh et al., 2012; Karsh et al., 2014). However, other previous studies suggest that a $\delta^{15}N{:}\delta^{18}O$ ratio between 1 and 2 can also be caused by denitrifying bacteria (Granger et al., 2008; Lehmann et al., 2003). The divergence from 2:1 ratio may also be attributed to hotspots of denitrification, such as in hyporheic zones where nitrate is completely consumed by denitrification, resulting in no fractionation (Fogel and Cifuentes, 1993; Vavilin et al., 2014; Waser et al., 1998a; Waser et al., 1998b). Additionally, the divergence from the 2:1 ratio in samples further down-estuary ~~samples~~ may indicate mixing between two or

more NO_3^- sources, such as between atmospheric, marine, or nitrification (Kaushal et al., 2011; Wankel et al., 2006). Due to water column dissolved oxygen levels averaging over 4 mg/L (data from Chesapeake Bay program, not shown), assimilation likely dominates NO_3^- removal in the water column, while denitrification likely dominates nitrate removal from the sediment, which is supported by previous work (Cornwell et al., 2014; Kemp et al., 1990).

Based on the nitrate isotope mixing model, the longitudinal trends in nitrate sources along the Potomac Estuary correspond with the other results of this study. The decline in wastewater nitrate matched the decline in nitrate concentrations and loads, while the slight increases in nitrification and fertilizer both correspond with decline N and O isotopes values down-estuary and the increase agricultural land use in the lower Potomac watershed. Future research would benefit from doing the mixing model separately using different endmembers for the different seasons in order to better constrain the differences between seasons. But due to lack of data on the seasonality of fertilizer and nitrification endmembers it was not feasible for the scope of this paper. Seasonal endmembers could provide more confidence because we found that seasonality and temperature mattered in the N sources and loads. Many isotopic studies do not always take this into account and typically just use literature values; our work showed that there are important seasonal variations and in order to improve the isotope mixing model to capture difference between seasons, the seasonal changes in the endmembers may need to be captured.

Denitrification is likely a sink for NO_3^- during the summer and fall based on the increases in $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ within 6 km down-estuary and due to warmer

water temperatures, while there is no evidence for denitrification in the winter due to reduced biological activities typical in cooler winter temperatures (Eyre and Ferguson, 2005; Gillooly et al., 2001; Harris and Brush, 2012; Nowicki, 1994). Nevertheless, nitrate removal was significant in all seasons, including winter ~~suggesting~~ proposing other mechanisms, as indicated by the salinity based mixing lines.

Plots of salinity *vs.* NO_3^-, $\delta^{15}N\text{-}NO_3^-$, and $\delta^{18}O\text{-}NO_3^-$ were used to provide evidence for conservative mixing, uptake, production, or contributions from other NO_3^- sources. NO_3^- concentrations fell below the mixing lines during the summer, fall, and winter, suggesting non-conservative mixing behavior due to the presence of a NO_3^- sink, such as assimilation or denitrification (Wankel et al., 2006). During the spring NO_3^- concentrations fell on the mixing line, however, ~~suggesting~~ indicating that there were no important sources or sinks. This may be due to higher flows and shorter residence times in the spring (Table 2), which can result in less biological transformations of NO_3^-. In the salinity *vs*. $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ plots, when the isotope values fell below the mixing lines, this suggested the contribution of NO_3^- from sources with lower $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$, such as fertilizer inputs or nitrification, which produces nitrate with lower $\delta^{15}N\text{-}NO_3^-$ and $\delta^{18}O\text{-}NO_3^-$ values through fractionation (Kaushal et al., 2011; Kendall et al., 2007). An increase in nitrification down-estuary is likely attributed to the conversion of remineralized N to nitrate or from down-estuary inputs of wastewater ammonia that is converted to nitrate (Middelburg and Nieuwenhuize, 2001). During the spring, $\delta^{18}O\text{-}NO_3^-$, isotope values again fell mostly on the mixing line, which may indicate the Potomac River Estuary is acting more like a transporter instead of a transformer (e.g. Kaushal and Belt, 2012), transporting NO_3^- without there being any significant sinks of

NO_3^- or mixing with additional sources, likely due to lower residence times (Table 2) in the spring. However, the fact that during the spring the $\delta^{15}N\text{-}NO_3^-$ values were slightly below the mixing line indicates there may have been an increased amount of nitrate inputs from the watershed through runoff carrying nitrate produced ~~derived from~~ by nitrification. During the winter, $\delta^{15}N\text{-}NO_3^-$ values also fell above the mixing line for some samples, which suggested the contribution of heavy $\delta^{15}N\text{-}NO_3^-$ from an additional down-estuary source (~~there are~~potentially from one of the 14 other wastewater treatment plants in the lower Potomac watershed). This was likely not the case during the summer and fall when other sources and sinks may dominate due to greater biological activities (Eyre and Ferguson, 2005; Gillooly et al., 2001; Harris and Brush, 2012; Nowicki, 1994) or during the spring when there is more conservative behavior due to higher flows. ~~when flows are higher the there is more conservative behavior~~. Even though only surface water salinity, nutrient, and isotope values were used in these mixing line plots, when bottom water nutrient and isotope data was averaged with the surface water values, the mixing lines plots and results did not change (data not shown).

5 Conclusion

By coupling isotope tracking techniques and a mass balance over broader spatial and temporal scales, we found that an urban river-estuarine continuum in the Chesapeake Bay, and likely similar estuaries globally can transform anthropogenic inputs of N over relatively short spatial scales. Only a small fraction of N inputs from a major wastewater treatment plant were exported out of the estuary. However, processing of N by estuaries can vary considerably across seasons and hydrologic extremes, with greater exports

during periods of higher flows and cooler temperatures, and greater transformations and retention during longer hydrologic residence times and warmer temperatures. In particular, this study supports previous work, showing that non-point sources of N were more dominant ~~dominate~~ during winter and spring when runoff from the watershed and estuarine flows were higher compared to summer and fall when the point-sources were more dominant, due to lower flows. These differences suggest N processing in urban rivers and estuaries would differ from those in non-urban estuaries. Also, the potential for long-term and widespread increase in water temperatures and frequency and magnitude of droughts and floods through climate change (Kaushal et al., 2010a; Kaushal et al., 2014b; Kaushal et al., 2010b), will likely influence the sources and transformation of nitrogen to the Chesapeake Bay and estuaries globally. Consequently, future efforts to manage nutrient exports along rivers and estuaries would benefit from better understanding the interactive effects of land use and climate variability on the sources, amounts, and transformations of N exported to coastal waters and targeting critical times for more intensive wastewater treatment.

Details on Supporting Information

- Additional site information and details on methods
- Table with site coordinates
- Table with mixing model
- Table comparing between box model (this study) and Chesapeake Bay Model.
- A figure comparing box model results with and without bottom water isotope data

Data Availability

Data used for the research in this paper is available through 4TU.centre at the following DOI and URL: doi:10.4121/uuid:e68c6141-f83e-4375-ac3b-088ddf4eff51 http://doi.org/10.4121/uuid:e68c6141-f83e-4375-ac3b-088ddf4eff51

Author contribution

This paper is based on work from Michael Pennino's PhD dissertation. Dr. Michael Pennino collected water samples, conducted data analysis, and wrote the manuscript. Dr. Sujay Kaushal contributed to the study design, and provided helpful feedback on data analysis and manuscript writing. Dr. Sudhir Murthy contributed to study design, provided data, and contributed to manuscript revisions. Joel Blomquist contributed to study design, sample collection, and manuscript revisions. Dr. Jeff Cornwell contributed to manuscript revisions and provided feedback on data analysis. Dr. Lora Harris contributed to study design, ~~and~~ helped with manuscript writing, and provided significant contributions to data analysis (particularly for the box model mass balance)~~, and manuscript writing~~.

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Table 1. Seasonal comparison of N and C inputs, exports, and losses along the Potomac River Estuary (mean ± standard error).

	Nutrient	Total Inputs (kg/day)	% of Inputs from Blue Plains*	Net Export (kg/day)	% of Blue Plains Inputs Exported	Net Loss in Load along Estuary, Box 1 to 6 (kg/day)	% Net Loss in Load along Estuary, Box 1 to 6	Net Loss in Load along Estuary, Box 1 to 5 (kg/day)	% Net Loss in Load along Estuary, Box 1 to 5	Net Loads from Bay to Estuary (kg/day)
Winter	TN	49150 ± 30323	10 ± 13	19844 ± 13728	3.7 ± NA	27369 ± 14597	54 ± 40	16426 ± 9509	28 ± 25	473 ± 414
Spring	TN	135317 ± 14614	8 ± 0.8	68431 ± 48060	71 ± 20	49672 ± 52116	36 ± 43	29515 ± 32908	26 ± 21	-127 ± 480
Summer	TN	13888 ± 596	38 ± 3	4853 ± 8326	19 ± 11	7155 ± 8370	75 ± 75	5739 ± 1832	44 ± 21	380 ± 164
Fall	TN	15334 ± 3700	47 ± 13	-1613 ± 12124	18 ± 10	15364 ± 12548	112 ± 95	4140 ± 6607	30 ± 43	264 ± 290
Winter	NO_3^-	37749 ± 23574	5.7 ± 4.6	2080 ± 6235	3 ± NA	31791 ± 7417	93 ± 29	26299 ± 10069	74 ± 33	32 ± 58
Spring	NO_3^-	95395 ± 10416	7.4 ± 0.6	30039 ± 161747	52 ± 70	40206 ± 161977	60 ± 187	30998 ± 26791	46 ± 34	8 ± 109
Summer	NO_3^-	7066 ± 364	49 ± 6.3	105 ± 4130	17 ± 2	5166 ± 4143	96 ± 141	4223 ± 763	77 ± 19	11 ± 10
Fall	NO_3^-	10526 ± 3006	53 ± 18.2	-204 ± 6278	13 ± 35	7291 ± 6812	108 ± 181	5637 ± 6817	85 ± 122	13 ± 35
Winter	$\delta^{15}N\text{-}NO_3^-$	130 ± 10	4 ± 0.4	4 ± NA	2.7 ± NA	130 ± NA	97 ± NA	77 ± NA	68 ± NA	86 ± NA
Spring	$\delta^{15}N\text{-}NO_3^-$	374 ± 3	7 ± 0.1	170 ± 547	52 ± 136	88 ± 547	48 ± 136	42 ± 71	26 ± 31	-412 ± 1471
Summer	$\delta^{15}N\text{-}NO_3^-$	30 ± 1	53 ± 1.6	5 ± 1	17 ± 3	27 ± 1	83 ± 3	18 ± 1	83 ± 3	NA
Fall	$\delta^{15}N\text{-}NO_3^-$	40 ± 5	55 ± 5.8	7 ± 8	13 ± 68	26 ± 8	87 ± 105	26 ± 13	87 ± 105	NA

TN = Total Nitrogen. NA – indicates there was only one month with data for that season and thus no S.E. value.

*Blue Plains is a wastewater treatment plant.

Table 2. Comparison of mean (± standard error) seasonal discharge and residence time within the Potomac River Estuary

	Mean Discharge (m^3/s)	Mean Residence time (days)
Winter	187 ± 60	26 ± 18
Spring	545 ± 214	57 ± 36
Summer	81 ± 29	129 ± 85
Fall	81 ± 27	196 ± 102

Data is based on discharge and box model results for the period from April 2010 to March 2011.

Figures

Figure 1. Map showing the Potomac River sampling stations (black diamond) and the location of the Blue Plains Wastewater Treatment plant (WWTP, black X) just south of Washington D.C., within the Chesapeake Bay watershed. The larger figure shows the location of monthly extensive synoptic surveys sites and the smaller figure on upper left shows the locations of the shorter intensive synoptic surveys. The larger figure also shows the location for the historical Maryland DNR surface water sampling sites.

Figure 2. Plot of the Potomac Estuary depth with distance down-estuary, with the Blue Plains wastewater treatment plant at distance zero, showing the location of the 6 boxes used in the box model calculations.

Figure 3. Longitudinal patterns in Potomac River Estuary: (a) mean annual dissolved inorganic nitrogen (DIN) and total organic nitrogen (TON) spanning 1997 to 2005, (b) mean seasonal DIN before year 2000 (1994 to 1999), and post 2000 (2001 to 2012), and (c) mean (1994 to 2012) seasonal molar N:P ratio (DIN/PO_4^{-3}), with salinity averaged from all seasons (1984 to 2008). Note: errors bars are provided, but S.E. is relatively small compared to concentrations. This data was obtained from the Maryland DNR and the Chesapeake Bay Program Data Hub.

Figure 4. Comparison of NO_3^- *vs.* dissolved organic carbon (DOC). N and C data was obtained from the Maryland DNR and the Chesapeake Bay Program Data Hub for this study period (2010-2012).

Figure 5. Trends in (a) $\delta^{15}N\text{-}NO_3^-$, (b) $\delta^{18}O\text{-}NO_3^-$, and (c) percent contribution of nitrate from wastewater, the atmospheric, and nitrification, based on isotope mixing model, with distance down-estuary from wastewater treatment plant input. Error bars are standard errors of the mean. N = 1 for winter, N = 3 for spring and fall, and N = 2 for summer.

Figure 6. (a) Plot of $\delta^{15}N\text{-}NO_3^-$ *vs.* $\delta^{18}O\text{-}NO_3^-$ of nitrate from effluent water samples and Potomac River Estuary samples, showing samples from different locations along the estuary; the grey arrow indicates the 2:1 relationship characteristic for denitrification; and (b) Same plot as (a), but seasonally and without the effluent or wastewater outfall values. Not included in these plots is the box indicating the region where atmospheric nitrate samples generally lie, from -10 to +15 for $\delta^{15}N\text{-}NO_3^-$ and from 60 to 100 for $\delta^{18}O\text{-}NO_3^-$.

Figure 7. Comparison of salinity *vs.* (a) NO_3^-, (b) $\delta^{15}N\text{-}NO_3^-$ and (c) $\delta^{18}O\text{-}NO_3^-$. Mixing lines connect the mean NO_3^- concentration or isotope values at the lowest and highest salinity values. Error bars are standard errors of the mean. For panel (a), N = 3 for all seasons, for panels (b) and (c), N = 1 for winter, N = 3 for spring and fall, and N = 2 for summer. Mixing line equations for NO_3^- concentrations and isotopes were obtained from Middelburg and Nieuwenhuize (2001). NO_3^- data was obtained from the Maryland DNR and the Chesapeake Bay Program Data Hub, covering spring 2010 to spring 2011, the same dates as the NO_3^- isotope data.

Figure 8. Comparing the TN fluxes along the Potomac River Estuary estimated from the Box Model used in this study and from the results from the Chesapeake Bay nutrient model.

Figure 9. Correlation between the fluxes estimated from the Box Model used in this study and the Chesapeake Bay nutrient model.

Figure 10. Seasonal Box Model results showing how (a) TN, (b) NO_3^-, and (c) $\delta^{15}N\text{-}NO_3^-$ loads vary down-estuary. Error bars are standard errors of the mean. For panels (a) and (b), N = 3 for all seasons. For panel (c), N = 1 for winter, N = 3 for spring and fall, and N = 2 for summer. TN and NO_3^- data was obtained from the Maryland DNR and the Chesapeake Bay Program Data Hub.

1640 Figure 1.

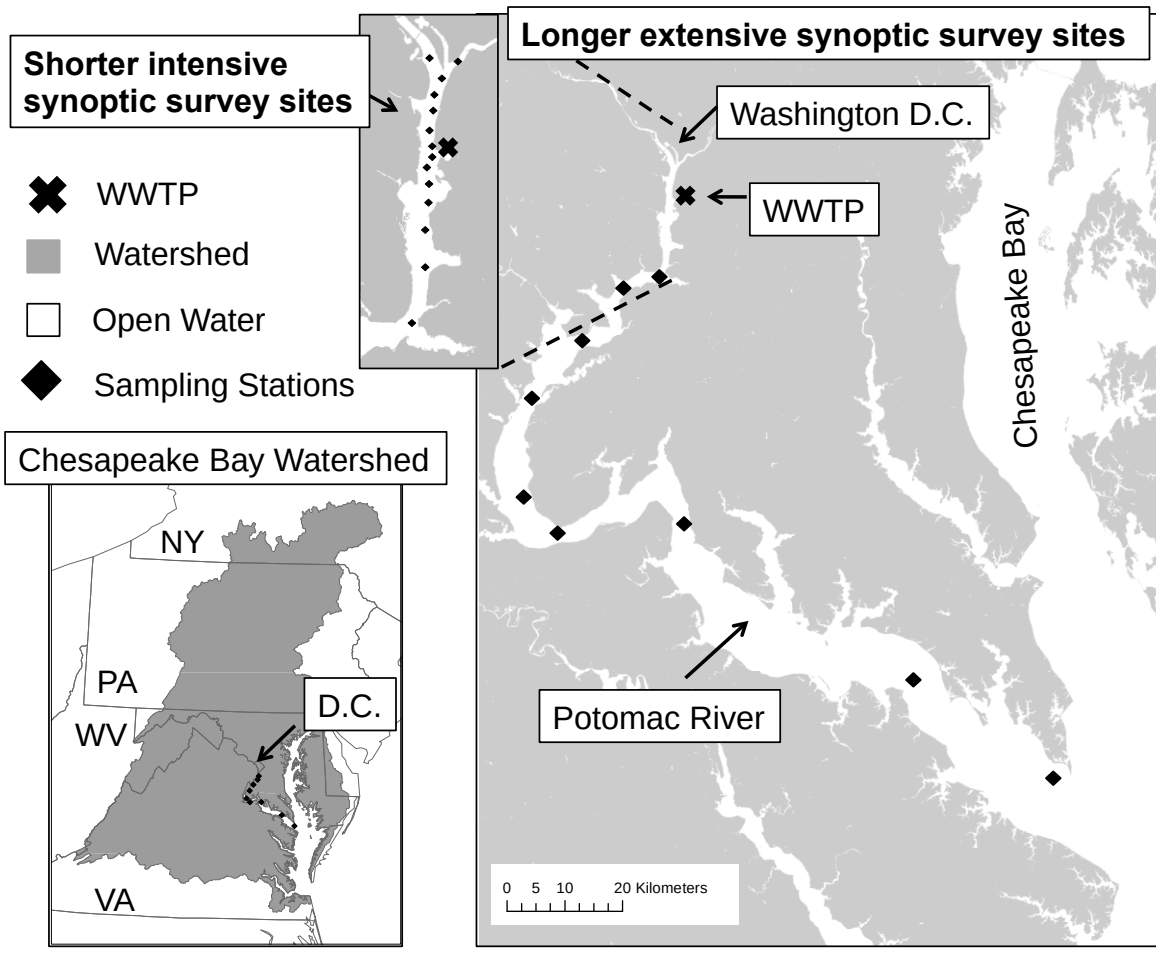

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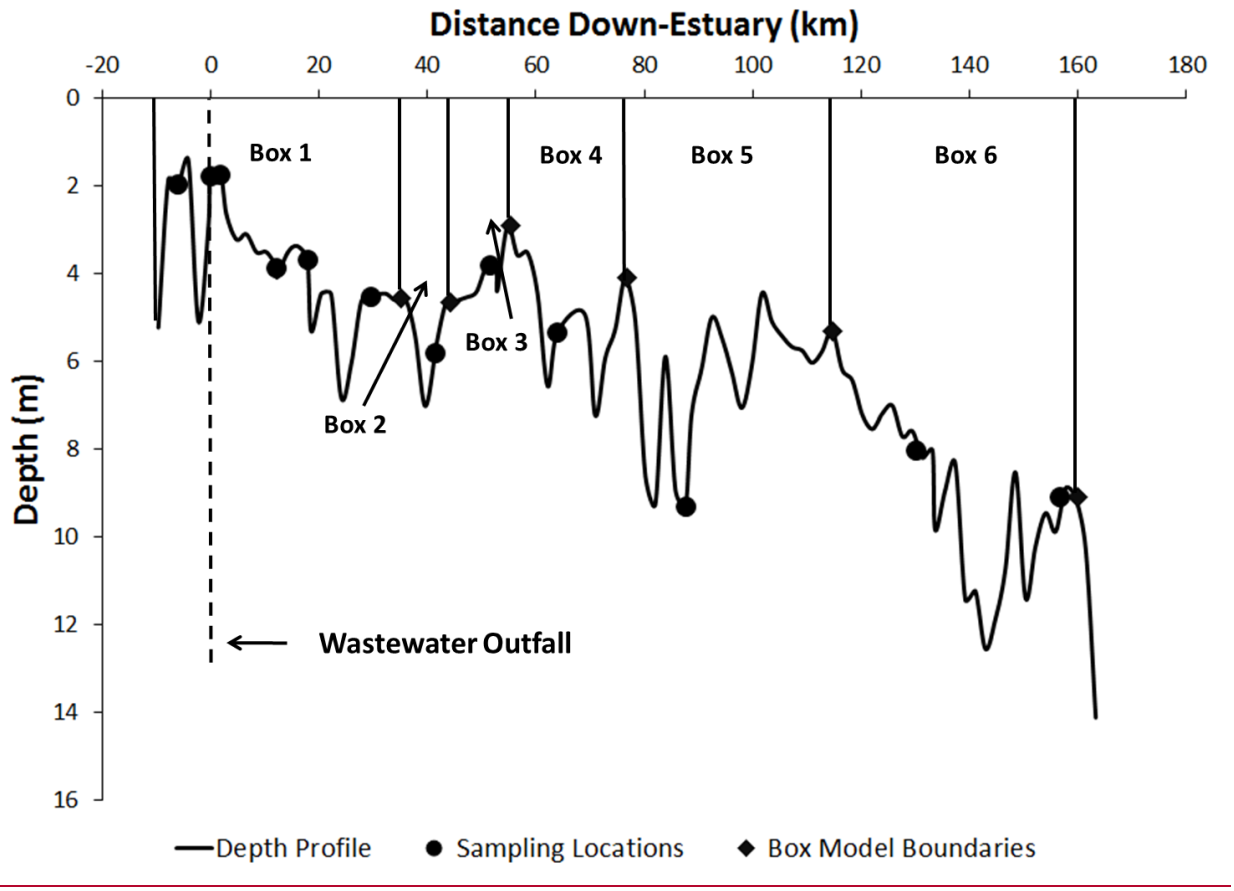
Figure 2.


Distance Down-Estuary (km)
-20
0
20
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60
80
100
120
140
160
180
Depth (m)
0
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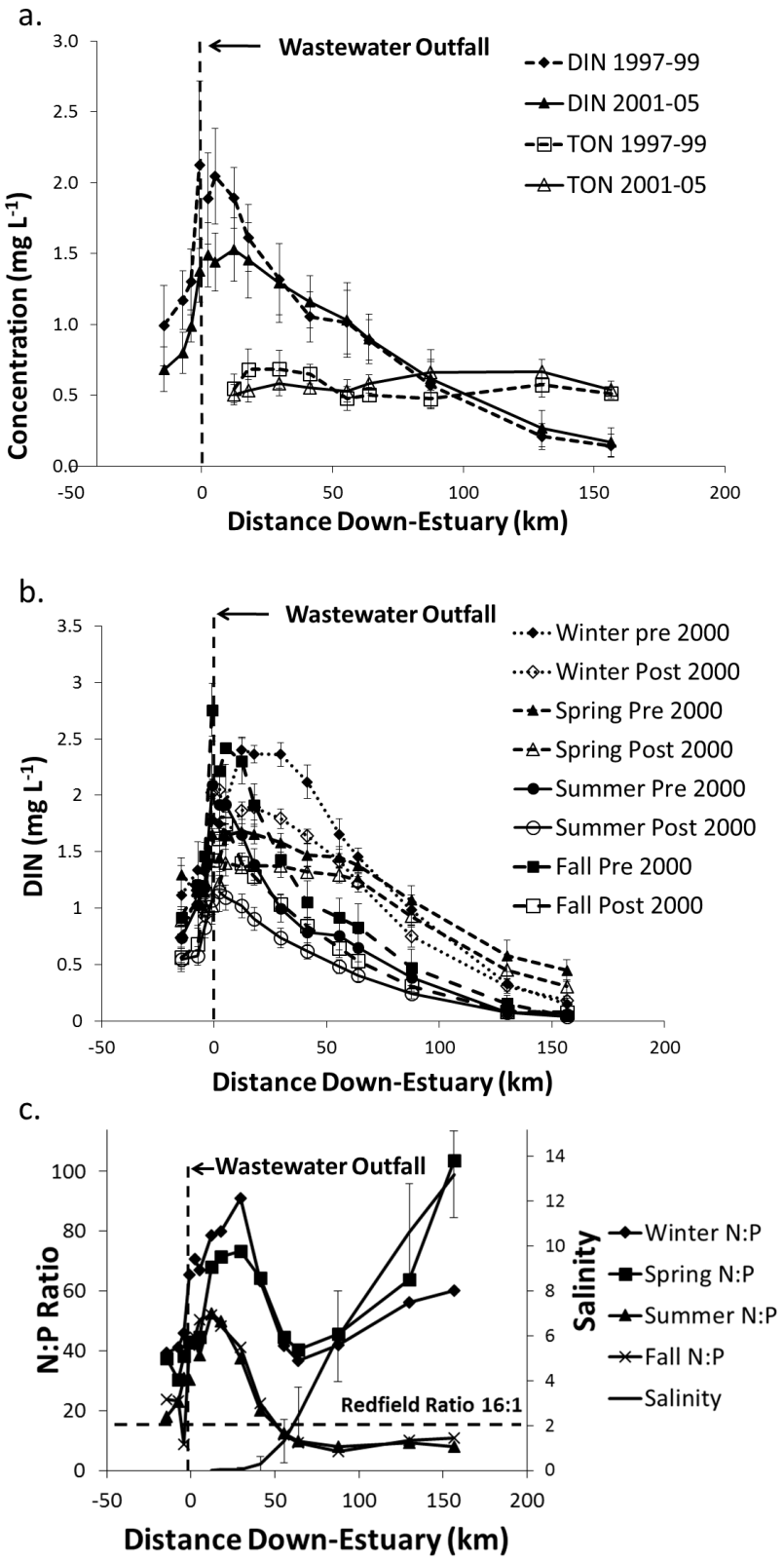
4
6
8
10
12
14
16
Box 1
Box 2
Box 3
Box 4
Box 5
Box 6
Wastewater Outfall
Depth Profile
Sampling Locations
Box Model Boundaries

1671 Figure 3.



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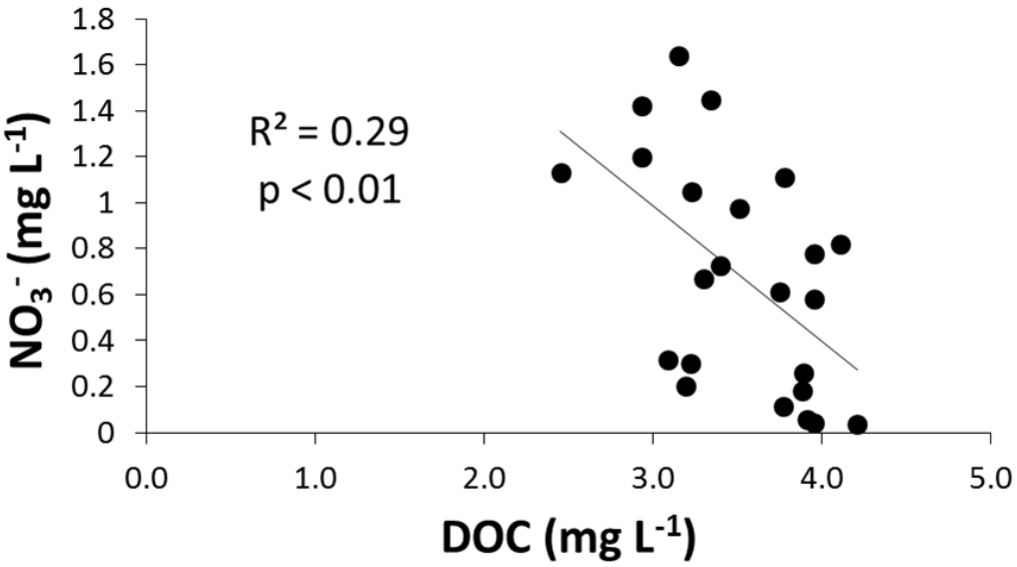

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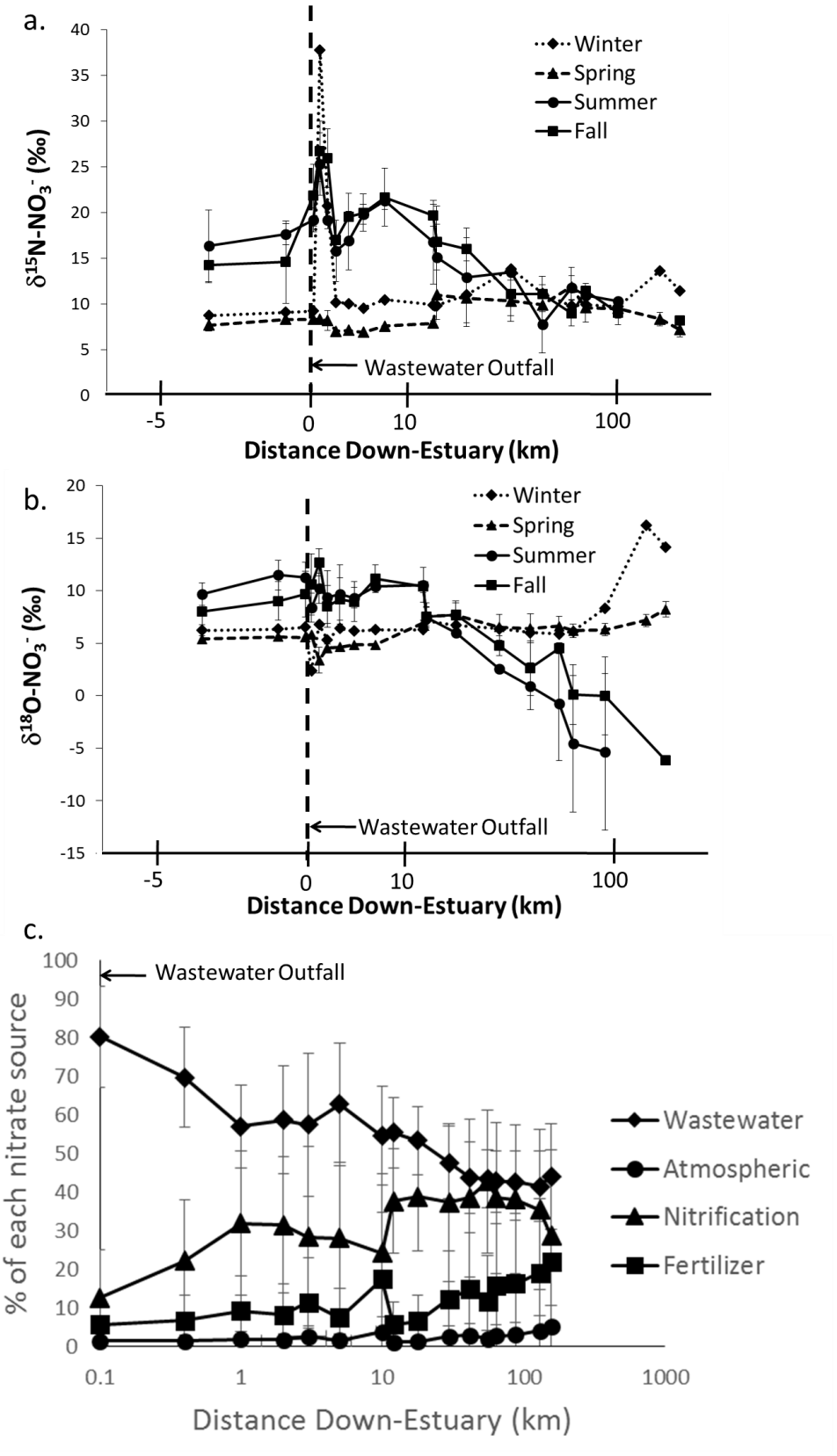
1704 Figure 5.



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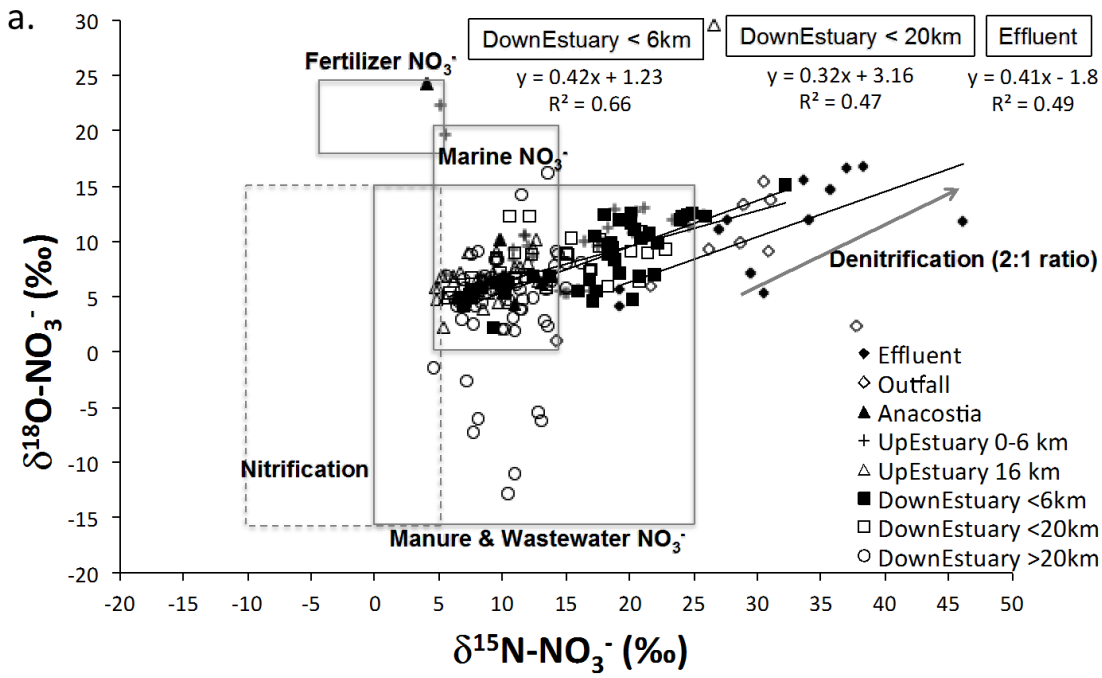

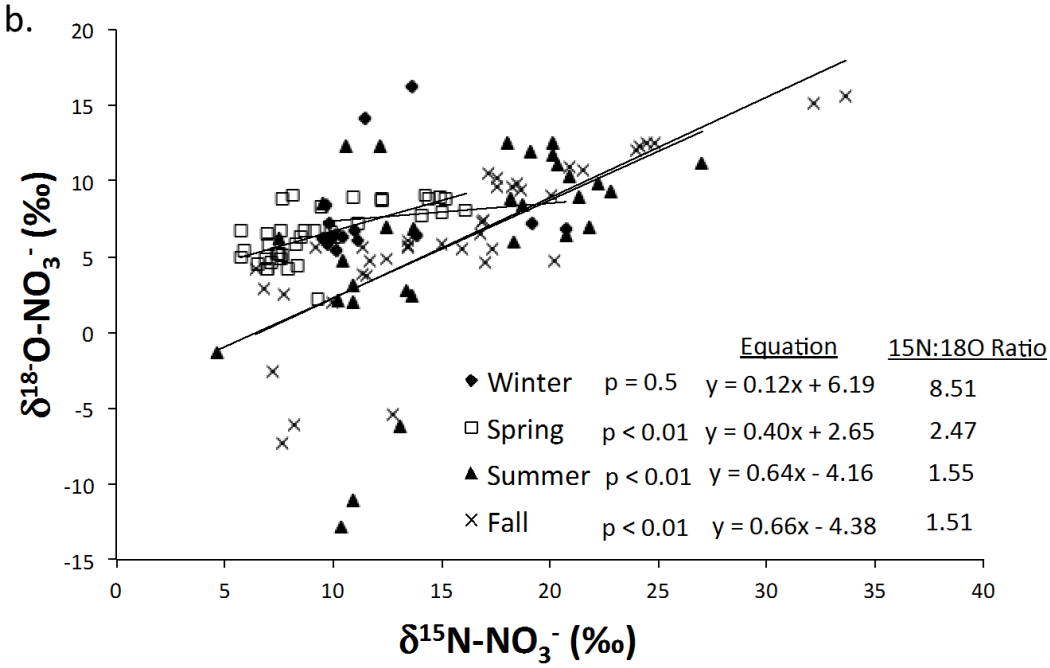

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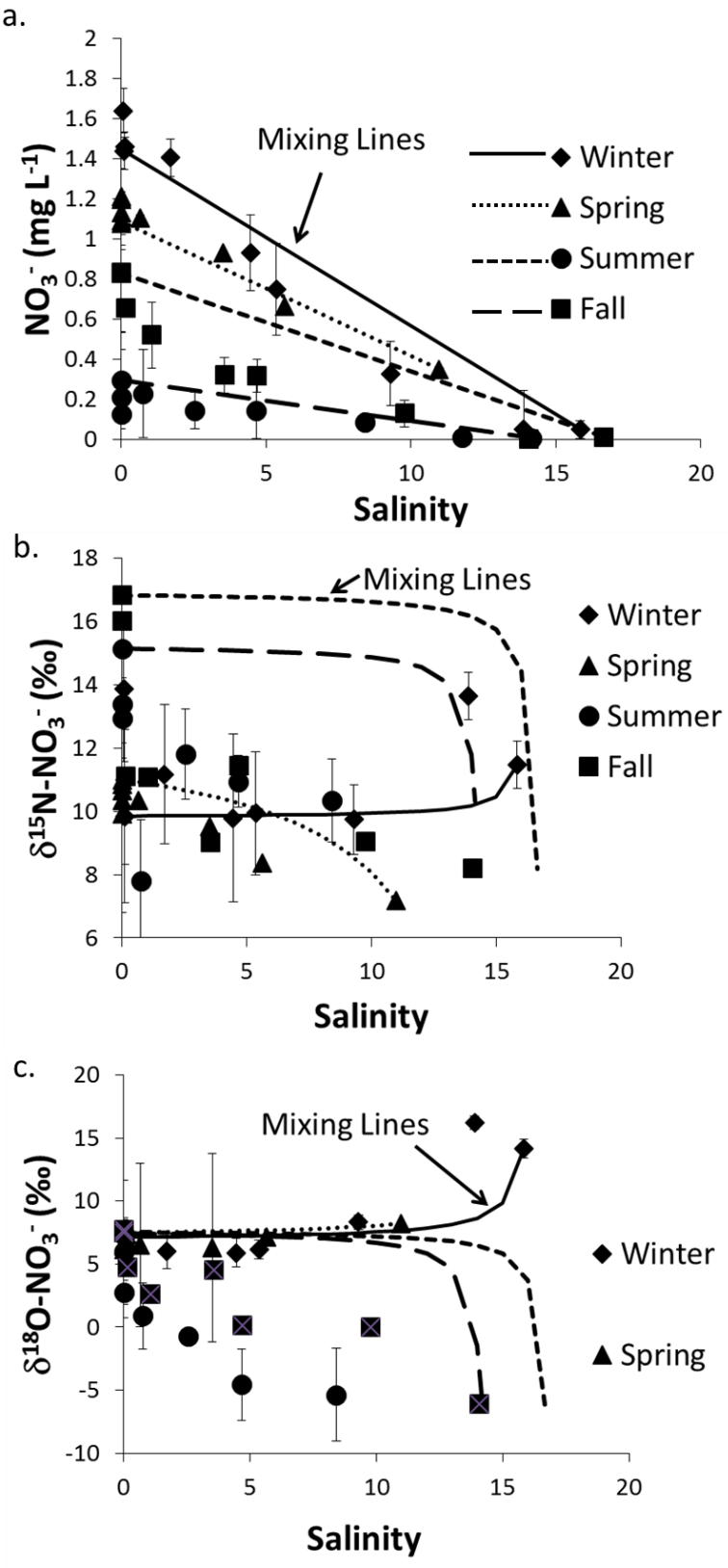

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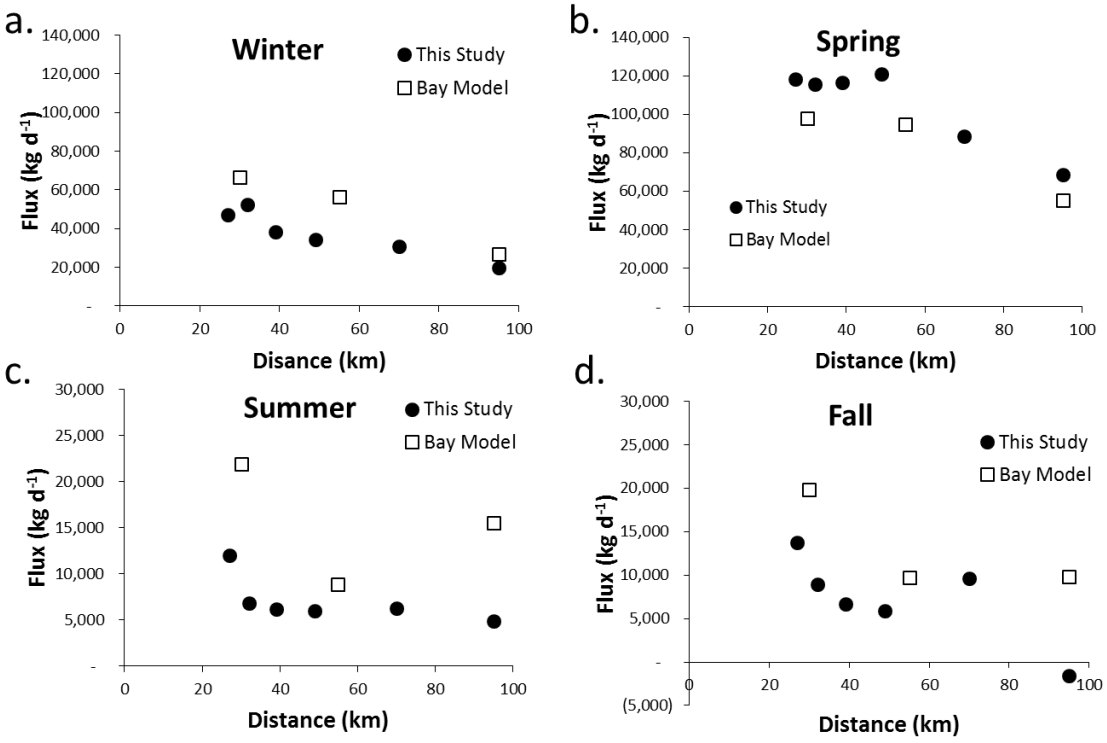
Figure 8.


a.
Winter
This Study
Bay Model
Flux (kg d-1)
Disance (km)
b.
Spring
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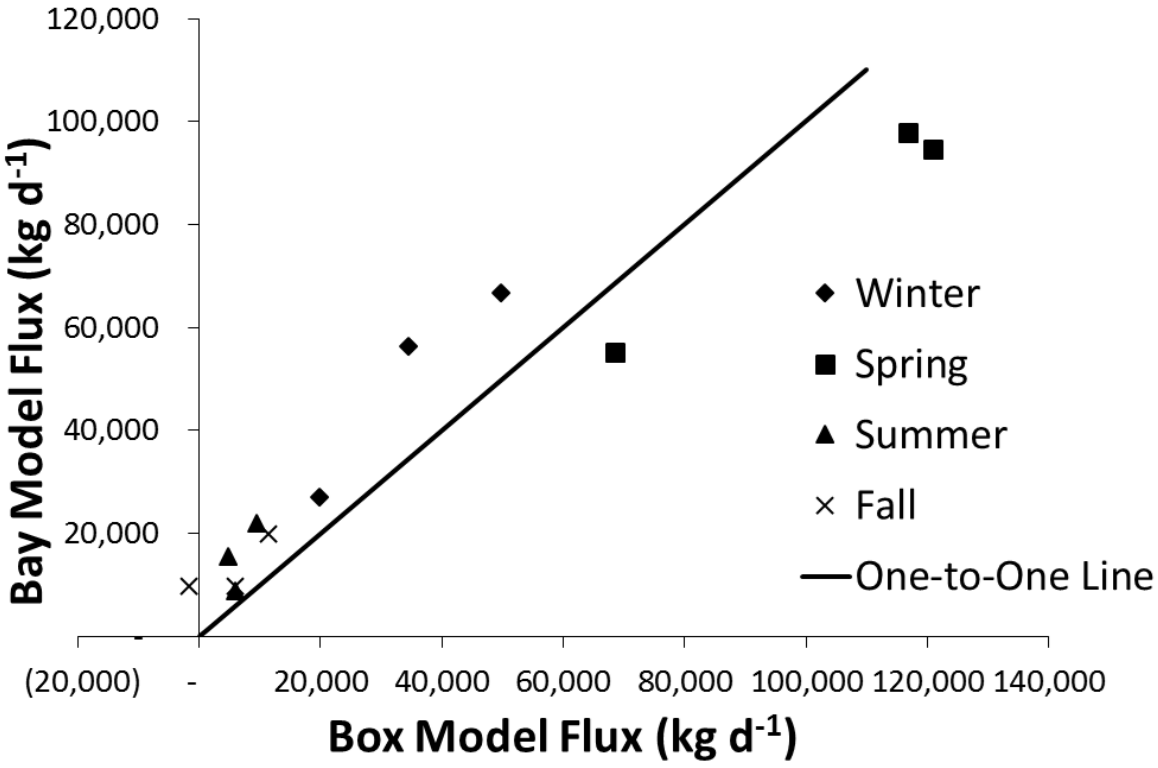
Bay Model
Flux (kg d-1)
Distance (km)
c.
Summer
This Study
Bay Model
Flux (kg d-1)
Distance (km)
d.
Fall
This Study
Bay Model
Flux (kg d-1)
Distance (km)

1746 Figure 9.



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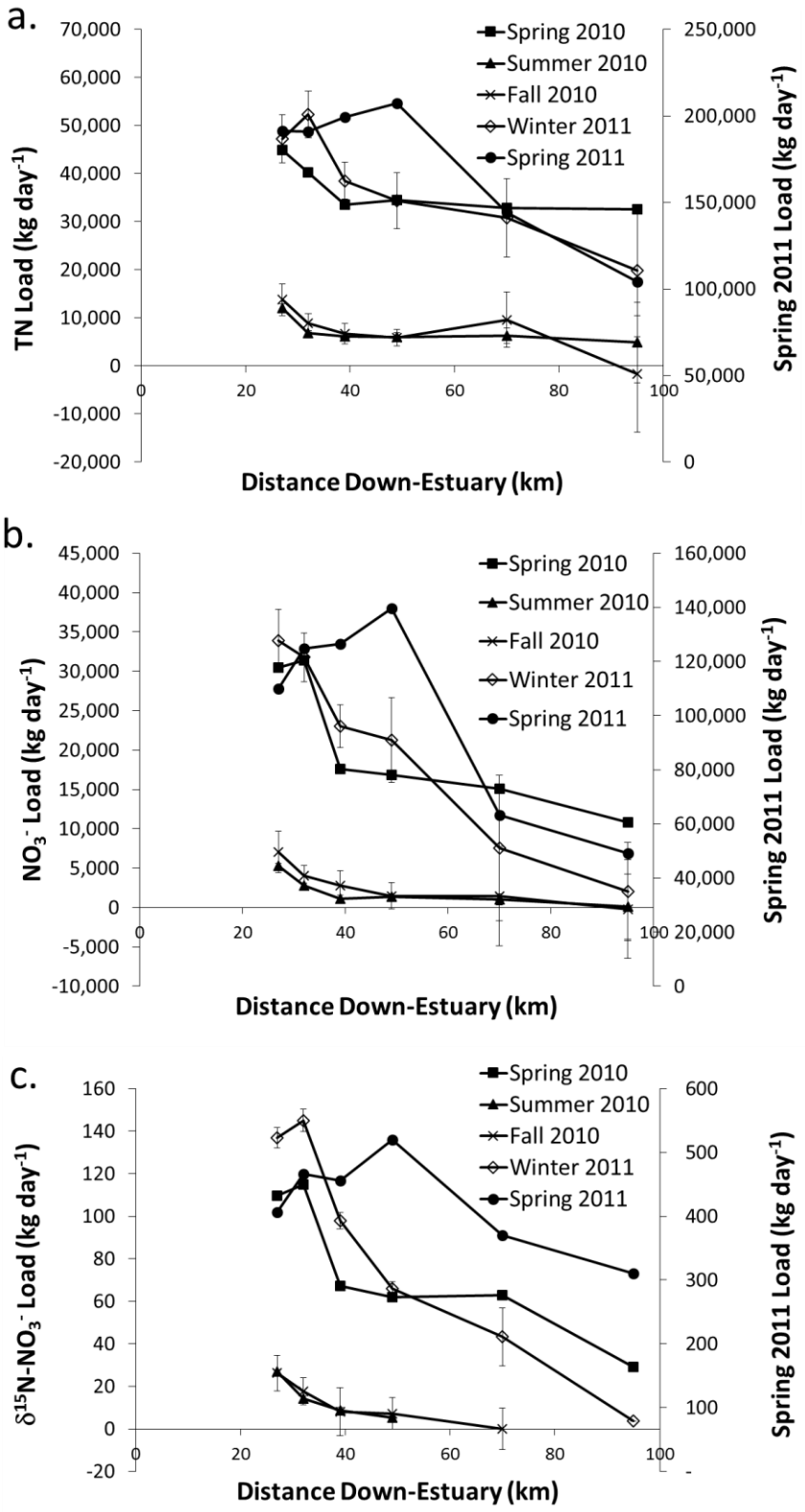
1772 Figure 10.



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