# Dissolved Organic Nitrogen Variability at a Permaculture Livestock Farm in Central Virginia

Adam Chaffin
Charlottesville, VA

B.S. Environmental Chemistry, University of Virginia

**Abstract**

In analyzing water quality dynamics in inland aquatic ecosystems, dissolved organic nitrogen (or DON), including proteins, DNA, and other types of organic N-bearing compounds, is one of the important factors to consider as it is present in all life forms. This study focused on the seasonal variability in surface water DON in the pond and surrounding streams of Timbercreek Farm (TCF), a semi-permaculture livestock farm in central (Charlottesville) Virginia. Water samples were chemically analyzed using the Lachat Automated Ion Analyzer. This study found that DON concentrations in the outflow of the pond increased from 10% to 52% of the total dissolved nitrogen from April to July of 2015, but then decreased from 52% to 20% from July of 2015 to April of 2016, thus creating a seasonal pattern. This was mainly accredited to biological activity within the pond – more specifically, the accumulation of biomass associated with increased epilimnetic primary productivity in the summer relative to the spring and the winter. Additionally, the data gathered from 9 other ponds in Albemarle County (along with those from Timbercreek) strongly suggest that higher degrees of agricultural activity in a watershed directly contribute to elevated DIN concentrations (relative to DON). Thus, having agricultural and non-agricultural reference points, DIN and DON data from Timbercreek were found to be characteristic of heavily agricultural watersheds in Central Virginia.

**Table of Contents**



**List of Figures**



**List of Tables**

## 1. Introduction

In assessing water quality and nutrient dynamics of inland freshwater ponds and lakes, dissolved organic nitrogen (DON) is one of the most critical factors to consider as it is directly associated with the growth, development and decay of all aquatic organisms. Although DON is exceedingly important to each and every aquatic ecosystem, the driving factors for DON cycling and for the temporal concentration fluctuations that are often observed have yet to be fully understood in the scientific community (McHale et al. 1999). With each successive study that analyzes seasonal DON variability in inland lentic ecosystems, our collective understanding of DON function and movement throughout these systems is enhanced.

DON and dissolved inorganic nitrogen (DIN) constitute the two chemical categories of compounds that make up the total dissolved nitrogen (TDN) pool. The DIN pool is composed of compounds that do not incorporate nitrogen into a hydrocarbon molecular structure (i.e. inorganic N-bearing molecules) (Howarth, 2009). The three foremost forms of DIN in these systems are ammonium ($NH_4^+$), nitrate ($NO_3^-$) and nitrite ($NO_2^-$), each an exceedingly important component in the biogeochemical cycle of nitrogen. Conversely, the DON pool consists of molecules that incorporate nitrogen into various hydrocarbon-based ('organic') molecular frameworks (including amines, alkaloids and amides). The DON pool can be further subdivided into those compounds of high molecular weight (HMWs) and those of low molecular weight (LMWs). HMWs include nucleic acids essential for carrying and reproducing organisms' genetic code (DNA, RNA), proteins that have essential roles in an array of biochemical processes (including dissolved combined amino acids (DCAAs), enzymes, etc.), and humic substances. LMWs on the other hand include a host of smaller organic nitrogen-bearing compounds, including urea, glycine, peptides, dissolved free amino acids (DFAAs), amino sugars, methyl amides and others (Berman and Bronk, 2003). In most of these inland lentic ecosystems, DON concentrations exceed those of DIN; however, in today's industrialized world (with a growing demand for increased agricultural production), many of these systems are becoming dominated by DIN, generally derived from anthropogenic N inputs such as fertilizers or waste-water effluent (Pellerin et al. 2004). DIN nutrients (typically second only to phosphates in primary production limitation in freshwaters) can be directly utilized by phytoplankton, therefore large inputs of DIN can expedite algal growth rates often associated with harmful algal blooms (HABs) that dominate eutrophic systems (Pace 2015, Eutrophication). Alternatively, in waterbodies with relatively undisturbed watersheds, anthropogenic DIN (from synthetic fertilizers, manure, etc.) is removed from the equation, therefore DON dominates the TDN pool.

Although it is known that systems dominated by anthropogenic pollution show characteristically higher DIN concentrations, it is relatively unknown what effects this has on DON concentrations (World Resources Institute, 2016). In researching DON flux with respect to land development in a series of northeastern U.S. rivers and streams, Pellerin et al. (2004) found that DON, unlike DIN, was not closely correlated to urban or agricultural land use, despite large amounts of anthropogenic N inputs. These findings are corroborated by Johnson et al. (2009); however, this raises the question – what *is* responsible for varying DON flux? Variability in DON flux has been linked to various watershed characteristics in certain areas. For example, in the Arbutus Lake watershed of the Adirondack State Park of upstate New York, McHale et al. (1999) found that DON variability was linked to soil type, forest type, and even neighboring bodies of water. The analysis of DON/DIN dynamics is of increasing importance in our rapidly industrializing world, and further scientific insight is necessary for the continuous enrichment of our understanding of water quality and nutrient dynamics as well as of the biogeochemical nitrogen cycle as a whole.

This study sought to explain the seasonal variability in DON concentrations in the pond of Timbercreek Farm (TCF), a semi-permaculture livestock farm in Charlottesville, Virginia. Because anthropogenic N inputs have been proven to be increasingly damaging to inland aquatic environments, some American farmers have recently been converting to other more sustainable farming practices. One such practice is referred to as permaculture (which comes from a combination of the words 'permanent' and 'agriculture'), involving farming methods that seek to imitate natural systems in order to remain self-sufficient (Mollison 1986). Permaculture farms assimilate plants, animals, landscapes and humans into synergetic relationships, the products of one element serving the needs of the others (Cruz and Osentowski, 2015). While TCF uses no synthetic nitrogen fertilizers, the nutrient dynamics within the farm (and therefore the pond) are still ultimately impacted by anthropogenic nitrogen inputs (i.e. feed, purchased livestock) and agricultural cycling of N (i.e. manure).

Regarding the seasonal variability of DON in the pond, I hypothesized that while inflow DON concentrations would not vary much over time, outflow concentrations would increase from spring to summer, and decrease again from summer to spring. This was based on the assumption that primary production rates tend to increase in the summer as the epilimnetic photic zone (the top-most thermal stratification layer in lakes that receives the most sunlight) of the water body lengthens, and that increased primary production generally results in increased DOC, and therefore turbidity and DON, concentrations (Pace 2015; Eutrophication). Additionally, I hypothesized that during storm events, DIN and DON concentrations would temporarily increase (due to increased flushing into the system from the perimeter of the pond).

In order to put this study into a broader context, and in order to verify that increasing degrees of agriculture are in fact linked to higher DIN concentrations, 9 other ponds around

Albemarle County (each in a watershed with a varying degree of agricultural activity) were sampled for DON and DIN. Another point of reference for the measurements collected at TCF came from the Environmental Protection Agency's National Lake Assessment (NLA) of 2007 (EPA 2007).

## **2. Methods**

### *2.1 TCF Site Description*

Surface water samples were collected from inflow and outflow streams of the pond at TCF, a semi-permaculture livestock farm in Charlottesville, Virginia. Although this farm currently operates under permaculture farming methods, this alternative farming operation has only been in existence since the year 2011. Before TCF was established, the land was used primarily as a horse farm but was also used to cultivate small yields of crops (an operation that has been practiced for over a century).

DON and DIN data were analyzed at two stream sites – one inflow site before the central pond (Site H) and one outflow site after the pond (Site J) (Figure 1). The western side of the pond is characterized by a very steep hill leading down to the water, while the eastern pond border is heavily forested with a relatively flat slope. Site H is relatively free of tree cover, while stream outflow Site J is located in a densely forested part of the farm. No livestock other than cattle ever roamed near the edges of the pond, and cattle movement around the pond was often variable.

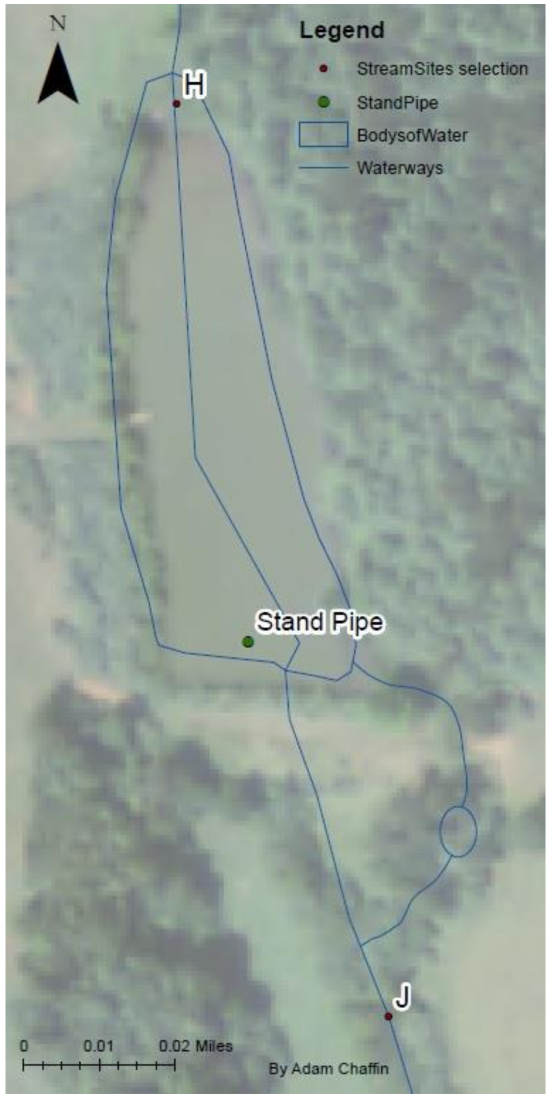


**Figure 1 – TCF Study Sites**: Map of the two stream sample sites of this study – inflow Site H and outflow Site J.

*2.2 Sampling Methods*

In order to obtain a holistic view of the seasonal variability of surface water DON at TCF, samples were collected from April 6, 2015 to April 4, 2016. Polyethylene Nalgene sample bottles were cleaned with DI water and acid washed (1% HCl) prior to use. Two samples from each site were collected weekly over the summer (and bi-weekly in the fall and spring) in the Nalgene sample bottles, and each was then transported to the laboratory on ice, vacuum filtered (pore size = 0.45 μm) to remove any particulate matter, and refrigerated in order to preserve the total dissolved nitrogen until further chemical analysis was performed.

*2.3 Chemical Analysis*

The Lachat Automated Ion Analyzer, a four-channeled spectrophotometer, was used in order to quantify the various dissolved nitrogen concentrations of each sample; however only the $NH_4^+$ and the $NO_2^-/NO_3^-$ channel were used for the purposes of this study. Each channel of the Lachat utilizes a different monochromator lens in order to selectively irradiate each sample. The subsequent absorption of each sample is then measured and converted into concentration measurements automatically by the computer program.

A persulfate digestion method was used in this study to quantify TDN (Wells 2014). Because TDN consists of DIN and DON, by chemically oxidizing all of the nitrogen species to nitrate ($NO_3^-$), an accurate reading of the total dissolved nitrogen can be obtained. Furthermore, DON concentrations were determined by subtracting the already measured DIN concentrations from the TDN concentrations (Equation 1).

$$\mathrm{DON} = \mathrm{TDN} - \mathrm{DIN} \qquad \text{(Equation 1)}$$

5.0mL of each refrigerated water sample were combined with 0.5mL of persulfate oxidizing agent in 5mL glass ampules. A series of organic standards (diluted urea and glycine, of known concentration, with added persulfate oxidant) were also added to the queue of samples in order to determine whether or not the digestion was successful. Additionally, persulfate blanks (water with persulfate oxidant) were added to each TDN analysis in order to quantify the efficiency of the digestion (by observing deviations from the ideal 100% digestion). Upon the addition of the persulfate oxidant, each ampule was immediately sealed using a propane flame and metal tongs, subsequently being placed in an autoclave (where they were subjected to high temperature (120°C) and pressure (12psi) for 40 minutes). After cooling, the samples were removed from the autoclave, extracted from the glass ampules and diluted with nanopure water (1mL sample to 5mL water) in order to ensure that the TDN concentrations were within the detection boundaries of the Lachat calibration curve. Samples were then run through the Lachat Automated Ion Analyzer in order to obtain nitrate (and therefore TDN) concentrations.

*2.4. Points of Comparison*

A series of 9 ponds around Albemarle County were sampled between 3/18/16 and 3/26/16 (see Figure 2). Similar field collection strategies were used at each of these ponds as were used at Timbercreek, although duplicate samples were collected only from the outflow stream of each pond and not from the inflow. DIN and TDN were measured using the same laboratory methods discussed above. The percent agriculture of the watershed of each pond that was sampled was visually estimated using GIS land-use maps and digital elevation data (from Albemarle County's GIS service). Agricultural land in this study included both cropland and pasture, and conventional farming methods were not distinguished from alternative farming methods. To confirm the degree of agriculture at each pond site, a short historical agricultural land-use survey was sent to each pond owner.

**Figure 2. 9 Alternate Ponds in Albemarle County** – These photos depict the alternate ponds that were sampled around Albemarle County, including: A. Pond (#1) of Rick and Margaret Haupt (Afton), B. Pond #2 of Rick and Margaret Haupt (Afton), C. Pond of Brooks Marshall (Charlottesville), D. Pond of Art Bulger (Charlottesville), E. Pond of Karen Rice (Gordonsville), F. Dell Pond (Charlottesville), G. Bellair Farm pond (Charlottesville), H. Morven Farm pond (Charlottesville), and I. Neighboring pond of Innisfree Farm (Crozet).

As an additional point of reference, data from the EPAs National Lake Assessment were analyzed with respect to Timbercreek pond. In the EPAs nationwide lake survey (NLA 2007), 1,326 lakes were tested across the United States for over twenty different water quality parameters. For the purpose of this study, this data set was limited to the 24 lakes found in the state of Virginia, and for these 24 lakes, total dissolved nitrogen, ammonium and nitrate/nitrite concentrations (all expressed in mgN/L) were analyzed, as well as their relationships to DOC and turbidity.

*2.5. Storm Data*

The 48 hours surrounding a mild storm event on 9/28/15 were analyzed via the automatic collection of 24 inflow and 24 outflow water samples by an ISCO water sampler. Additionally, stage data from pressure transducers at the inflow and outflow sites was converted (via an established rating curve) to create a discharge hydrograph.

## 3. Results

*3.1. Inflow and Outflow Dissolved Nitrogen*

In the inflow stream (Site H), dissolved $NH_4^+$ concentrations were consistently low over time (reaching a year-round maximum of 0.0491 mgN/L, or 14% of TDN), and although dissolved nitrate and nitrite dominated the dissolved nitrogen profile throughout the course of this study, it is worth noting that DON concentrations sharply increased from June to July (Figure 3, Figure 8, Table 1).

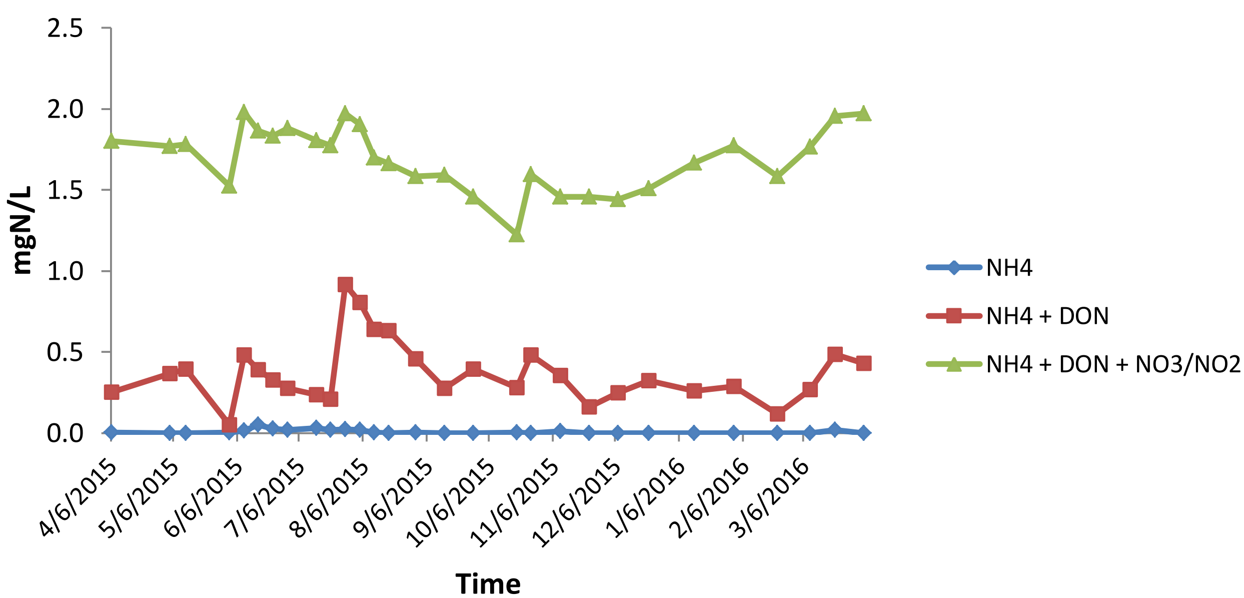


**Figure 3. Dissolved Nitrogen Concentrations (mgN/L) at Inflow Site H** – Over time, inflow waters were consistently dominated by nitrates and nitrites, however a notable increase in DON concentrations was observed from June to July. The top green line represents the TDN of the inflow stream.

In the outflow stream (Site J of Figure 1), TDN steadily decreased in the summer and increased in the winter (Figure 3). In the summer, outflow DON concentrations increased as DIN concentrations steadily decreased. This trend was reversed in the winter (Figure 4).

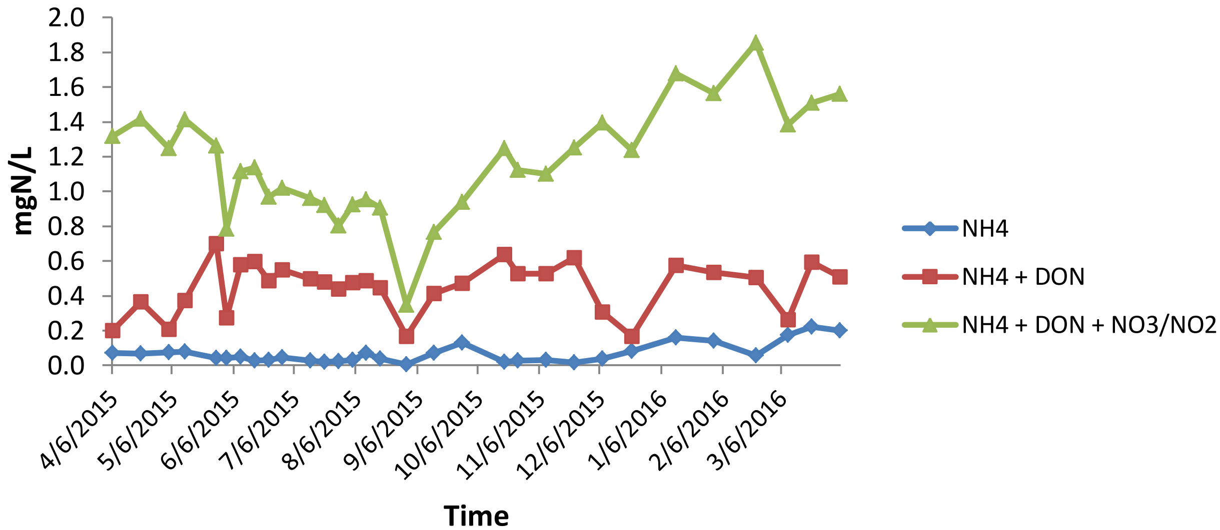


**Figure 4. Dissolved Nitrogen Concentrations (mgN/L) at Outflow Site J** – A decrease followed by an overall increase in TDN was observed over time. While aqueous ammonium concentrations remained low (reaching a year-long maximum of 0.221 mgN/L), DON concentrations increased from 4/6/15 to 9/6/15 (while $NO_3^-/NO_2^-$ concentrations decreased) and DON concentrations decreased from 9/6/15 to 4/4/16 (while $NO_3^-/NO_2^-$ concentrations increased again).

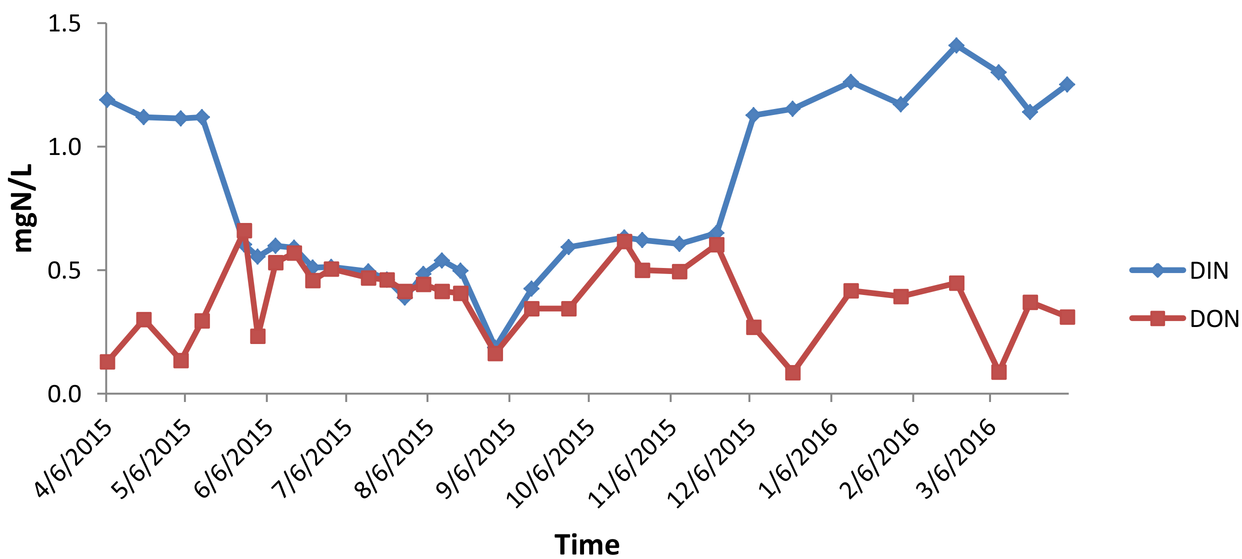


**Figure 5. DON vs. DIN at Outflow Site J** – In mid-spring, outflow DIN concentrations greatly exceeded those of DON. The gap between DIN and DON concentrations completely closed from spring to summer but opened up again in the fall and in the following spring.

**Table 1. Seasonally Variable DON Concentrations (mgN/L)** – This table depicts the DON measurements at both the inflow stream site and at the outflow stream site from 4/6/15 to 4/4/16. Also included are is the %DON of TDN for each measurement.

| Sample Date | Inflow DON (mgN/L) | %DON of TDN | Outflow DON (mgN/L) | %DON of TDN |
|---|---|---|---|---|
| 4/6/2015 | 0.0475 | 3% | 0.129 | 10% |
| 4/20/2015 | - | - | 0.299 | 21% |
| 5/4/2015 | 0.468 | 29% | 0.134 | 11% |
| 5/12/2015 | 0.341 | 18% | 0.295 | 21% |
| 5/28/2015 | - | - | 0.66 | 52% |
| 6/2/2015 | 0.302 | 16% | 0.231 | 29% |
| 6/9/2015 | 0.259 | 14% | 0.53 | 47% |
| 6/16/2015 | 0.204 | 11% | 0.569 | 49% |
| 6/23/2015 | 0.189 | 11% | 0.458 | 47% |
| 6/30/2015 | 0.892 | 45% | 0.506 | 50% |
| 7/14/2015 | 0.787 | 41% | 0.468 | 49% |
| 7/21/2015 | 0.635 | 37% | 0.461 | 50% |
| 7/28/2015 | 0.632 | 38% | 0.415 | 52% |
| 8/4/2015 | 0.457 | 29% | 0.442 | 48% |
| 8/11/2015 | 0.275 | 17% | 0.415 | 44% |
| 8/18/2015 | 0.394 | 27% | 0.407 | 45% |
| 8/31/2015 | 0.276 | 23% | 0.163 | 47% |
| 9/14/2015 | 0.483 | 30% | 0.343 | 45% |
| 9/28/2015 | 0.344 | 24% | 0.345 | 37% |
| 10/19/2015 | 0.159 | 11% | 0.617 | 49% |
| 10/26/2015 | 0.248 | 17% | 0.5 | 45% |
| 11/9/2015 | 0.324 | 21% | 0.494 | 45% |
| 11/23/2015 | 0.258 | 15% | 0.603 | 48% |
| 12/7/2015 | 0.286 | 16% | 0.268 | 19% |
| 12/22/2015 | 0.118 | 7% | 0.085 | 7% |
| 1/13/2016 | 0.267 | 15% | 0.416 | 25% |
| 2/1/2016 | 0.469 | 24% | 0.394 | 25% |
| 2/22/2016 | 0.43 | 22% | 0.448 | 24% |
| 3/9/2016 | 0.367 | 21% | 0.088 | 6% |
| 3/21/2016 | 0.393 | 22% | 0.371 | 25% |
| 4/4/2016 | 0.25 | 14% | 0.31 | 20% |

DON was a significant component of the total dissolved nitrogen pool of the TCF pond throughout the course of this study (averaging 21% of TDN in the inflow and 35% of TDN in the outflow, year-round) (Table 1).

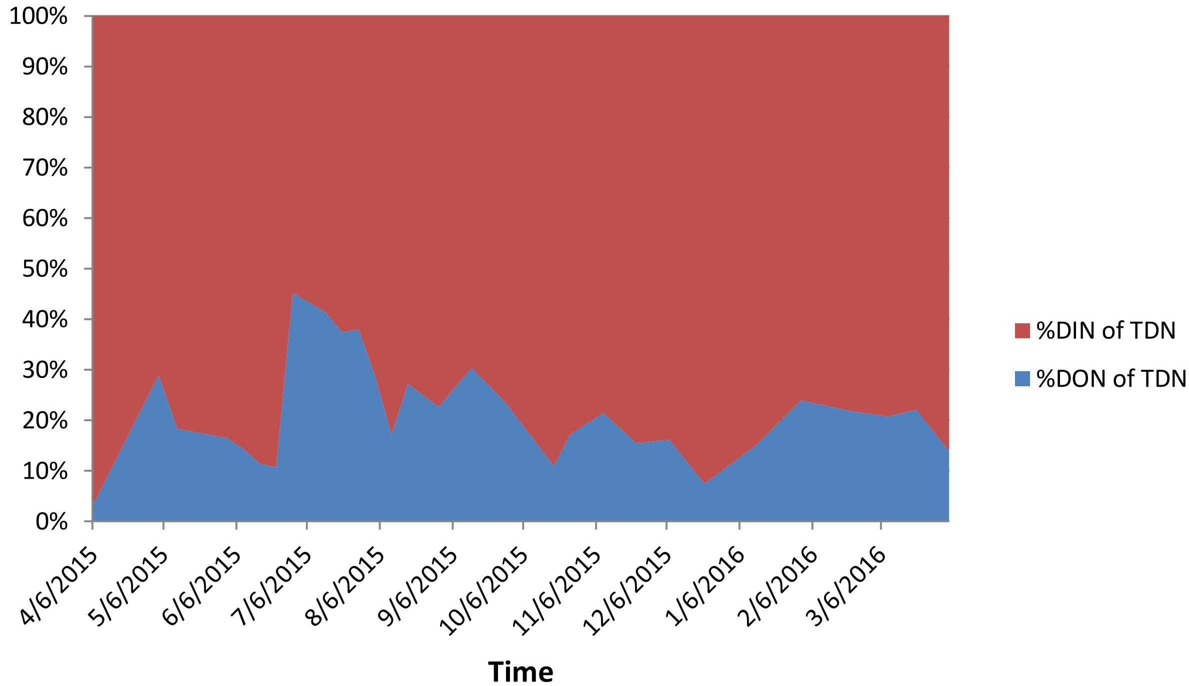


**Figure 6. Inflow (Site H) %DON and %DIN of TDN** – DIN consistently dominated the dissolved nitrogen profile in the inflow stream, despite a temporary jump in %DON from June to July of 2015.

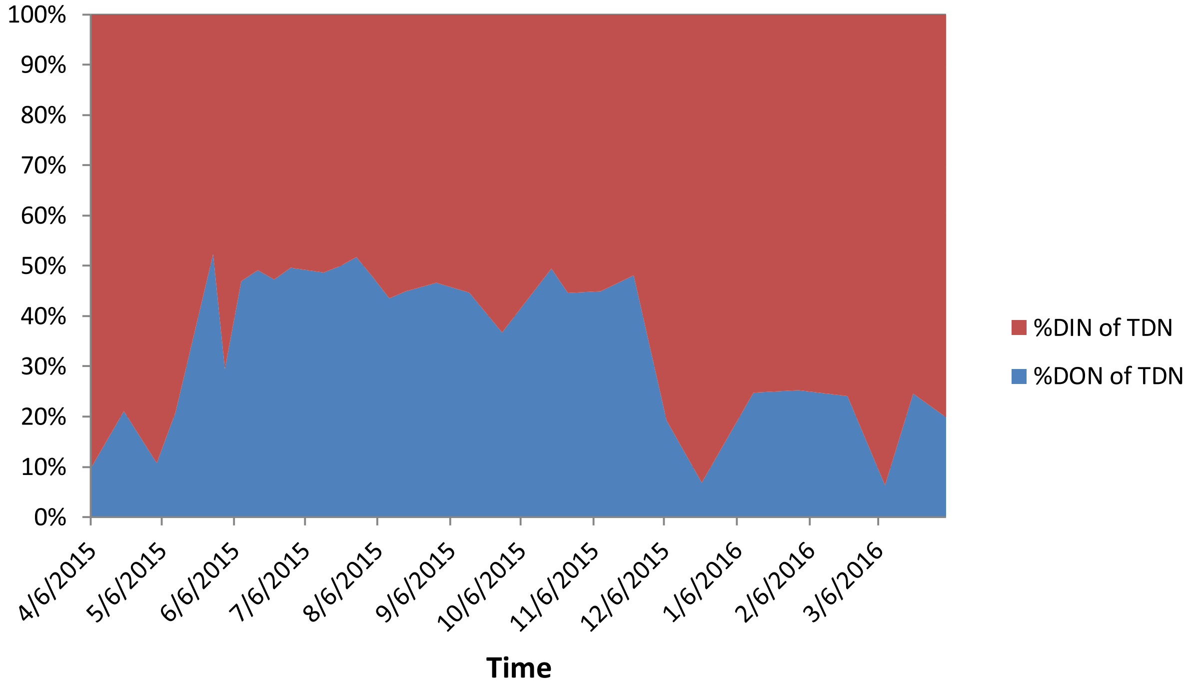


**Figure 7. Outflow (Site J) %DON and %DIN of TDN** – Seasonal analysis of the data reveals that %DON became a much larger component of the TDN pool in the summer and fall of 2015 (as compared to the spring of 2015 and to the winter and spring of 2016).

In comparing DON concentrations between the inflow and the outflow throughout the year, there was a fair amount of inherent variability in DON concentrations. Having said that, experimental DON measurements between the inflow and outflow were fairly equivalent (both the inflow and the outflow hovering around 0.40 mgN/L) except for a major jump in inflow DON concentrations in late June (Figure 8).

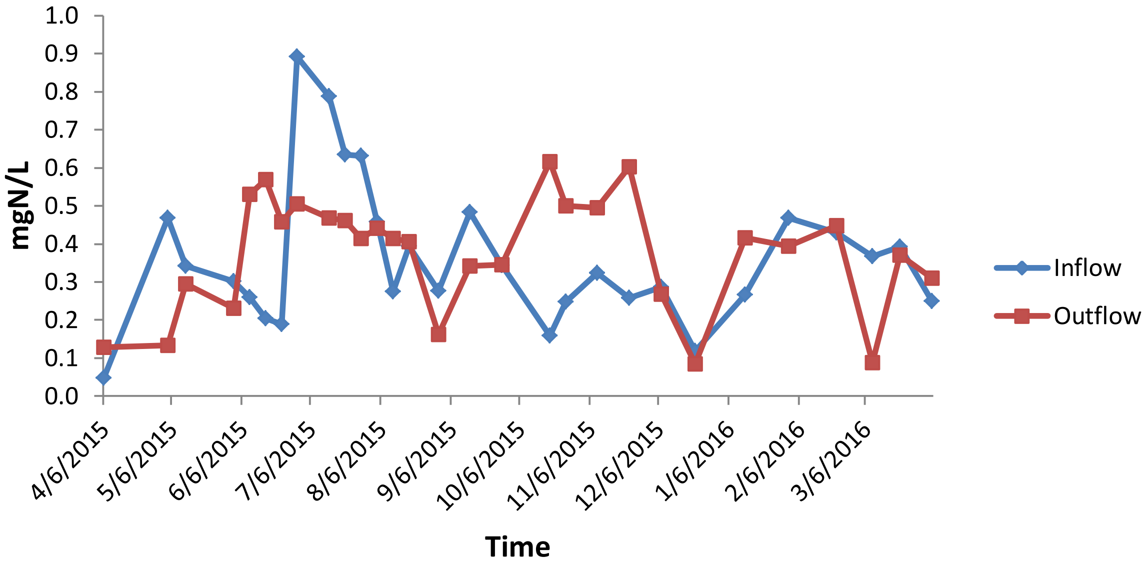


**Figure 8. Spatial Variability in DON (Inflow vs. Outflow)** – DON concentrations of the inflow were fairly equivalent to those of the outflow throughout the year, save a drastic temporary increase in inflow DON concentrations in late June.

*3.2. Storm Events*

The 48 hours surrounding a storm event on 9/28/15 were analyzed with respect to DIN and DON concentrations. Figure 9 shows that the outflow discharge remained unchanged throughout the storm while the inflow discharge steadily increased. Additionally, DIN remained relatively constant at both stream sites while DON varied a fair amount.

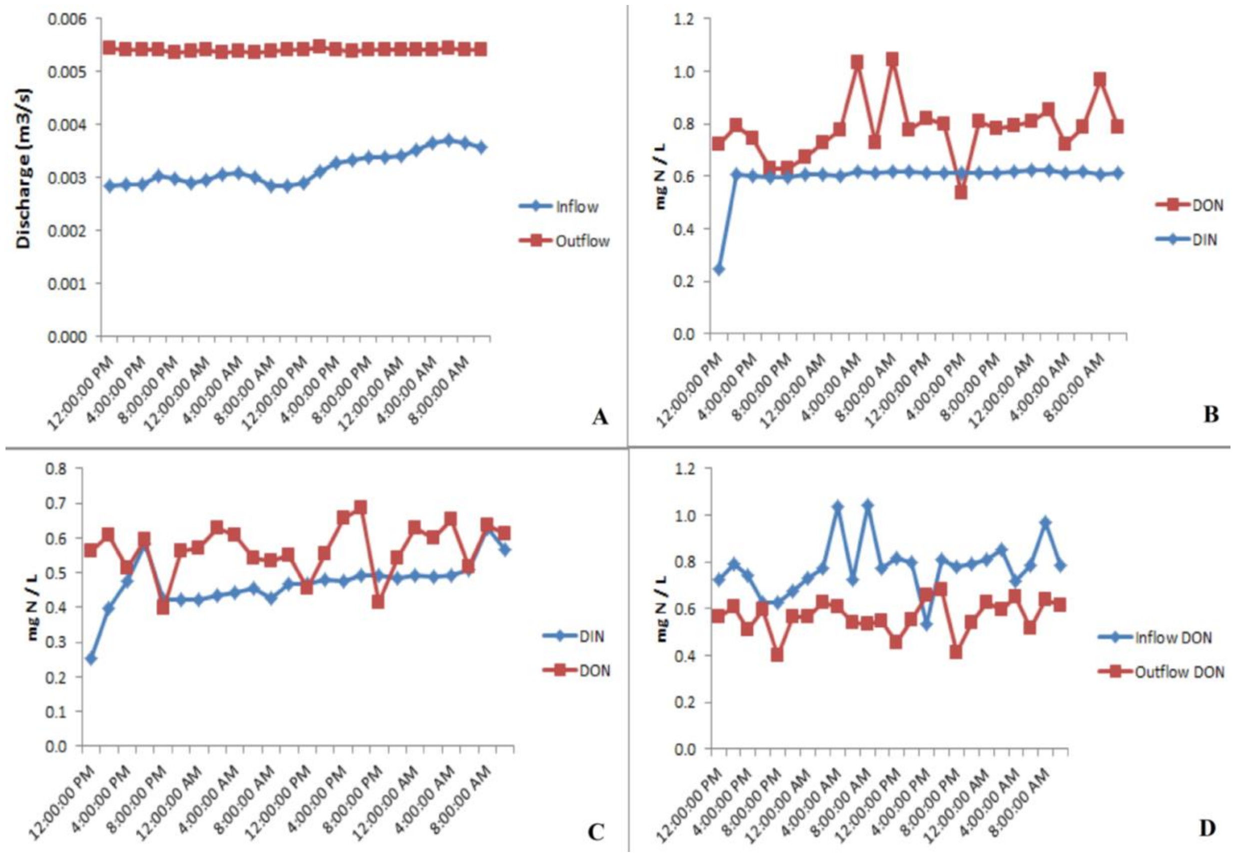


**Figure 9. 9/28/15 Storm Event** – A. This hydrograph shows a static outflow discharge and a steadily increasing inflow discharge. B. Inflow DIN remains constant throughout the storm while DON varies. C. Outflow DIN steadily increases while DON varies. D. Inflow DON remains higher than outflow DON throughout most of the storm, although both vary a fair amount.

### *3.3. Points of Comparison Results*

The DIN/DON analysis of the 9 alternate ponds in Albemarle County (Figure 10) yielded consistently low TDN and DIN concentrations relative to those gathered at TCF (Table 2). Additionally, these 9 ponds exhibited widely variable %DON and %DIN of TDN measurements (Figure 11).

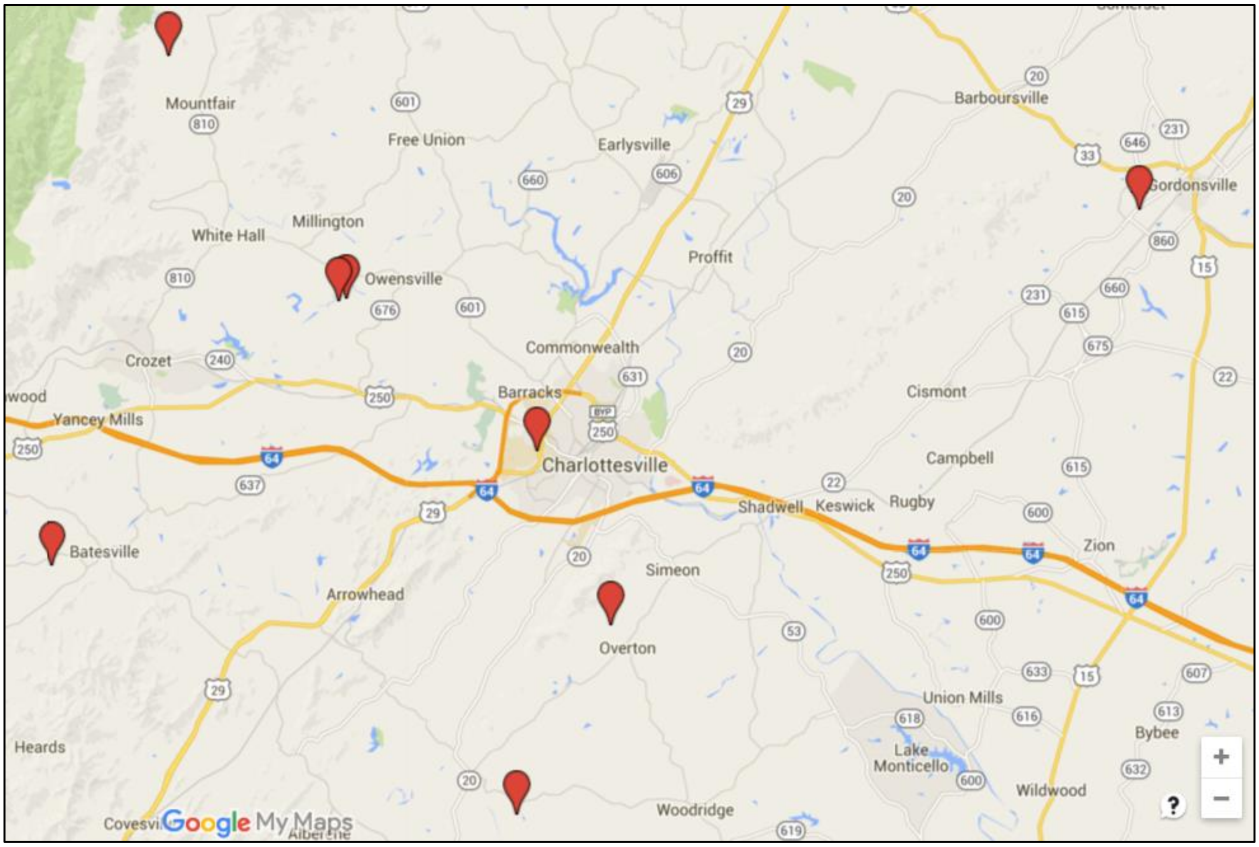


**Figure 10: Map of 9 Alternate Ponds** – This map depicts the 8 properties that contained the 9 alternate ponds that were sampled in this study (the coordinates for which are provided in Table 2).

**Table 2. Data from 9 Alternate Ponds around Albemarle County** – TDN, DON and DIN concentrations (in mgN/L), as well as %DON and %DIN of TDN, for 9 other ponds around Albemarle County are depicted in the table below (with Timbercreek (3/21/16 outflow sample) given as a reference). Additionally, the watershed of each pond was visually estimated for %Agriculture based on GIS imaging of land-use.

| Approximate % Ag | Sample Date | Latitude, Longitude | Pond Owner | TDN (mgN/L) | DON (mgN/L) | %DON of TDN | DIN (mgN/L) | %DIN of TDN |
|---|---|---|---|---|---|---|---|---|
| 0-25 | 3/18/2016 | 37.991, -78.748 | Haupt (#1) | 0.228 | 0.221 | 97% | 0.00688 | 3% |
| 0-25 | 3/18/2016 | 37.991, -78.748 | Haupt (#2) | 0.212 | 0.171 | 81% | 0.0414 | 19% |
| 0-25 | 3/18/2016 | 38.093, -78.607 | Marshall | 0.332 | 0.325 | 98% | 0.00711 | 2% |
| 0-25 | 3/18/2016 | 38.094, -78.603 | Bulger | 0.117 | 0.117 | 100% | 0.000 | 0% |
| 0-25 | 3/19/2016 | 38.035, -78.510 | Dell | 0.590 | 0.161 | 27% | 0.429 | 73% |
| 0-25 | 3/26/2016 | 38.188, -78.690 | Innisfree | 0.168 | 0.155 | 92% | 0.0126 | 8% |
| 25-50 | 3/26/2016 | 37.894, -78.520 | Bellair | 0.391 | 0.331 | 85% | 0.0603 | 15% |
| 50-75 | 3/26/2016 | 37.967, -78.474 | Morven | 0.451 | 0.223 | 49% | 0.228 | 51% |
| 75-100 | 3/19/2016 | 38.128, -78.214 | K. Rice | 0.835 | 0.324 | 39% | 0.511 | 61% |
| 75-100 | 3/21/2016 | 38.103, -78.564 | TCF | 1.51 | 0.371 | 25% | 1.14 | 75% |

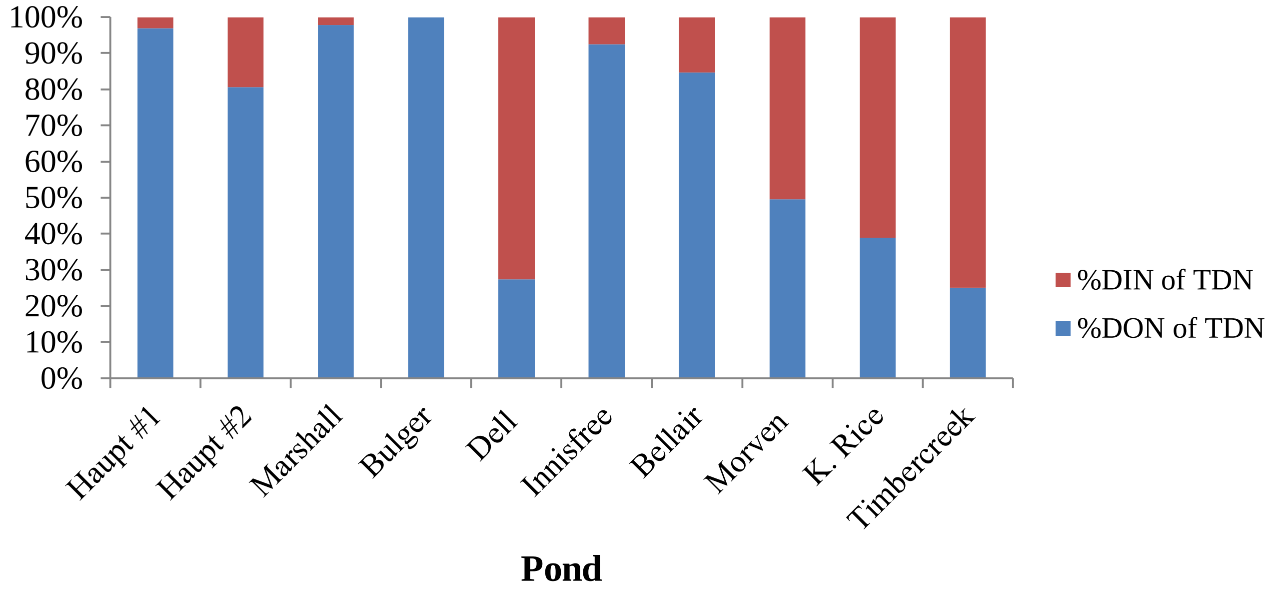


**Figure 11. %DON and %DIN of TDN of 9 Alternate Ponds** – A graphical representation of the data in Table 2 reveals the variability in %DON over these 9 ponds around Albemarle County (with Timbercreek (3/21/16 outflow sample) given as a reference).

As is evident in Figure 12, ponds in watersheds of high %Agriculture had characteristically higher DIN and TDN concentrations.

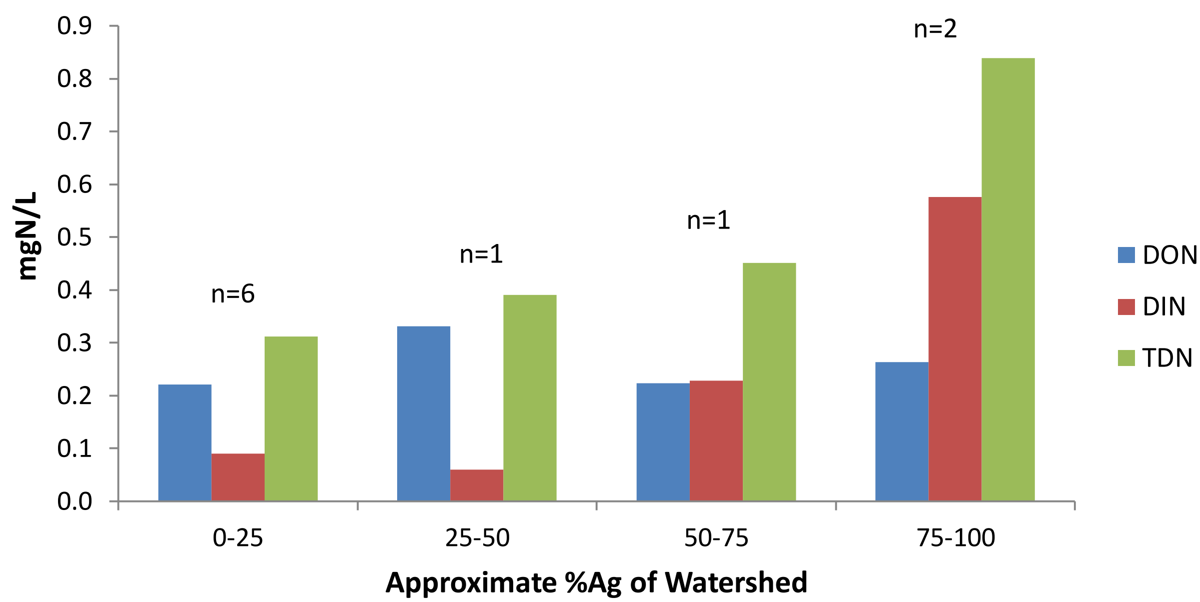


**Figure 12. %Agriculture as a Function of Dissolved Nitrogen Concentration** – TDN and DIN markedly increase with increasing %Agriculture in the watershed, while DON is relatively variable. The variable n represents the number of ponds included in each quartile (Timbercreek being included in the 75-100 bracket).

## 4. Discussion

*4.1. Inflow vs. Outflow DON Variability*

In the surface waters of both the inflow (Site H) and outflow (Site J), DON concentrations were consistently low relative to DIN (averaging 21% and 35% of TDN year-round, respectively). Furthermore, DON concentrations were relatively equivalent between the inflow and outflow (averaging 0.364mgN/L and 0.383mgN/L year-round, respectively). Between sample dates 6/23/15 and 6/30/15 however, inflow DON concentrations drastically increased (from 0.189mgN/L to 0.892mgN/L), falling back to normal (~0.30mgN/L) in late July. This was accredited to the major storm event that took place on 6/27/15. A precipitation level of 5.33cm was recorded on 6/27/15, which was the highest amount of rainfall recorded throughout the entire spring and summer of 2015 (accuweather.com, 2016).

*4.2. Storm Events*

The 6/27/15 storm event mentioned in Section 4.1 likely resulted in drastically increased flushing of organic matter (and therefore DON) into the pond, resulting in higher than normal inflow DON concentrations. This explained the inconsistency between inflow and outflow DON in Figure 8.

The 9/28/15 storm event depicted in Figure 9 was concluded to be non-representative of typical storm events, given that (1) the hydrograph does not resemble that of a typical storm event (Pace 2015, River, Stream Ecosystems), and (2) because the DON doesn't markedly increase as it did with the 6/27/15 storm event. Although not representative of a typical storm, the 9/28/15 storm measurements do speak to the inherent variability in DON measurements. While DIN concentrations remained steady (indicating reliable and steady data measurements), the DON concentrations varied a fair amount (indicating a higher degree of inherent variability in the persulfate oxidation method of DON determination). If further experimentation were performed on other storms events (especially those more representative of typical storm events), I would expect to observe a noticeable spike in DON concentrations before returning to normal conditions (similar to what was observed on 6/27/15).

*4.3. Seasonal Outflow DON Variability*

The key seasonal trend observed in the surface waters of the TCF pond was that the outflow DON concentrations increased (while outflow DIN decreased) from spring to fall of 2015, and decreased (while outflow DIN increased) from fall to spring of 2016 – confirming the hypothesis that outflow DON would seasonally increase in the summer months. This seasonal trend was most likely due to biological activity within the pond; however other physical and environmental phenomena could also be contributing to this pattern.

In inland lentic ecosystems across the state of Virginia, winter is typically characterized by low temperatures, de-stratification and vertical mixing of ponds, low primary production rates, and relatively inactive biological assemblages (especially if the water body freezes over, which the Timbercreek pond did in January - February of 2016). Conversely, summer, which tends to be a more biologically active time of year, is generally characterized by higher temperatures, more sunlight exposure, thermal pond stratification, and higher primary production rates. As phytoplankton biomass increases, DIN nutrients ($NH_4^+$, $NO_3^-$, $NO_2^-$) are converted into organismal biomass (DON and dissolved organic carbon, or DOC). Having said this, the biomass associated with the accumulation of phytoplankton (and other aquatic organisms) in the TCF pond was concluded to be the most probable source of the observed seasonal DON variability (DON concentrations rose in the summer and fall of 2015 as DIN concentrations fell, and DON concentrations fell in the winter and spring of 2016 as DIN concentrations rose).

Furthermore, dissolved organic carbon and turbidity data from the NLA study of 2007 (combined with NLA measurements of DON) supported this finding (Figures 13 and 14).

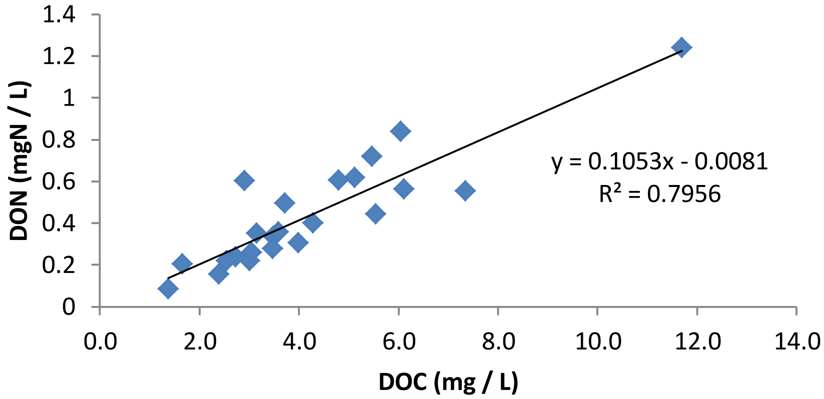


**Figure 13. DOC vs. DON in 24 Virginia Lakes** – DON was observed to have a significant positive relationship with DOC (as exhibited by the R squared value close to 1.0). Note that this figure omits one outlying piece of data, falling at (14.0, 0.5). (EPA 2007)

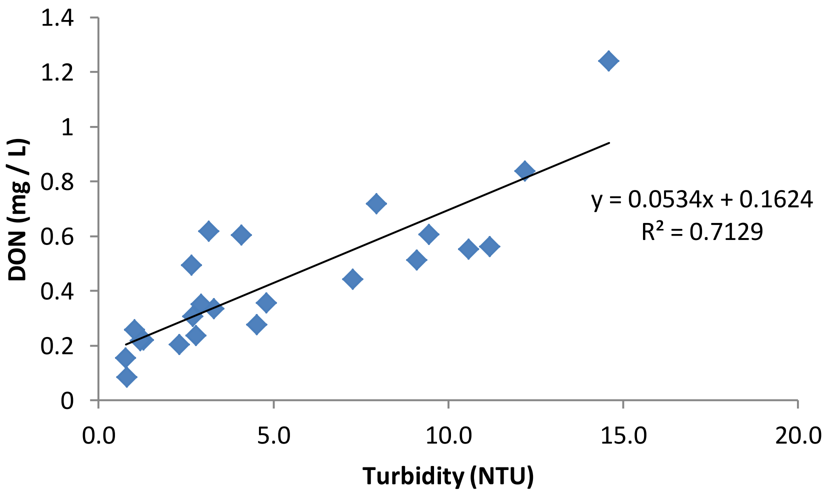


**Figure 14. Turbidity vs. DON in 24 Virginia Lakes -** Similar to with DOC, DON was also observed to share a significant direct relationship with turbidity. Note that this figure omits one outlying piece of data, falling at (16.6, 0.02). Turbidity is given in nephelometric turbidity units, or NTU. (EPA 2007)

**Table 3. NLA Regression Analysis Results** – Notice DON shares a statistically significant relationship with all parameters. Both p-values were far below 0.05, so each relationship was deemed statistical significant.

| **Regression** | **R Square** | **P-value < 0.001?** |
|---|---|---|
| DOC vs. DON | 0.80 | Yes |
| Turbidity vs. DON | 0.71 | Yes |

While the data presented in Figures 13 and 14 are representative of the 24 Virginia lakes in the EPA's National Lake Assessment of 2007, the implications are very relevant to the seasonal DON variability at TCF. Dissolved organic carbon (DOC) is a measure of the dissolved organic material in water, while turbidity is a measurement of the scattering of light due to suspended (in this case organic) particles as a function of depth (Pace 2015, Light). Having said this, DON, DOC and turbidity are all directly related to dissolved, suspended organic material in water. Therefore, the statistically significant relationship between DON and DOC (Figure 13) and between DON and turbidity (Figure 14) suggests that increased DON concentrations at TCF are a result of seasonally elevated concentrations of organic material (planktonic biomass).

Another explanation likely working in tandem with biomass accretion is the amount of tree coverage at each site. The inflow Site H is essentially free of tree cover; however, the outflow Site J is located in a heavily wooded area of the farm. As trees accumulate biomass in the summer, falling leaves and organic debris at Site J could cause higher detritus loads, higher decomposition rates and a higher accumulation of organic matter in the water. Additionally, groundwater could be entering the pond from underneath, potentially contributing to elevated concentrations of DON or DIN (Durand et al. 2011). All of these potential explanations likely tell part of the story as to why DON concentrations temporarily rose in the summer and fall of 2015.

*4.4. Pond Comparisons across Albemarle County, Virginia*

Perhaps the most profound result of this study was the strikingly positive correlation that was observed between elevated TDN concentrations, elevated DIN concentrations, and %Agriculture of a watershed (Figure 11). Because DON remained variable throughout all 9 alternate ponds, DIN was concluded to be the main driver of the elevated TDN concentrations in watersheds of higher %Agriculture. In the end, these data led to the general conclusion that watersheds containing higher degrees of agricultural-related land use have characteristically higher DIN concentrations (corroborating the findings of Pellerin et al. 2004). It is also worth noting that watersheds containing conventional agricultural methods produced nearly the same results as alternative (e.g. permaculture) agricultural methods (with regards to DIN and TDN). Therefore, it is safe to say that the difference between non-agricultural land-use and agricultural land-use is far greater than the difference between conventional agricultural land-use and alternative agricultural land-use (from a dissolved nitrogen perspective).

*4.5. Pond Comparisons across the State of Virginia*

**Table 4. 2007 NLA DON Data for 24 Virginia Lakes** – DON and DIN values for the different Virginia lakes are given in mgN/L. Also listed is the percent DON and percent DIN of the total dissolved nitrogen in each lake. Notice how the average %DON is very high (93%) compared to that of Timbercreek (50%).

| Lake Name | DIN (mgN/L) | DON (mgN/L) | %DIN | %DON |
|---|---|---|---|---|
| Rainey Pond | 0.029 | 0.552 | 5% | 95% |
| Lake Lanier | 0.017 | 0.494 | 3% | 97% |
| Philpott Reservoir | 0.016 | 0.203 | 7% | 93% |
| Lake Burnt Mills | 0.01 | 0.619 | 2% | 98% |
| Lake Bonaventure | 0.01 | 0.238 | 4% | 96% |
| Gatewood Reservoir | 0.019 | 0.258 | 7% | 93% |
| Wheeler's Pond | 0.019 | 0.837 | 2% | 98% |
| Flanagan Reservoir | 0.029 | 0.154 | 16% | 84% |
| Spring Creek | 0.053 | 0.603 | 8% | 92% |
| Beaver Pond | 0.042 | 0.606 | 6% | 94% |
| Jolly Pond | 0.031 | 0.563 | 5% | 95% |
| Waller Mill Reservoir | 0.015 | 0.351 | 4% | 96% |
| Beaver Dam Reservoir | 0.017 | 0.218 | 7% | 93% |
| Harrison Lake | 0.018 | 0.513 | 3% | 97% |
| Little Creek Reservoir | 0.01 | 0.356 | 3% | 97% |
| Holiday Lake | 0.01 | 0.22 | 4% | 96% |
| Diascund Creek Reservoir | 0.022 | 0.442 | 5% | 95% |
| Little River Reservoir | 0.024 | 0.401 | 6% | 94% |
| Wrights Pond | 0.022 | 0.718 | 3% | 97% |
| Elkhorn Lake | 0.017 | 0.084 | 17% | 83% |
| Bald Run Reservoir | 0.034 | 1.241 | 3% | 97% |
| Breckinridge Reservoir | 0.062 | 0.277 | 18% | 82% |
| Manassas | 0.018 | 0.335 | 5% | 95% |
| Lake Anne | 0.023 | 0.306 | 7% | 93% |
| **Average** | 0.024 | 0.447 | 5% | 96% |
| Timbercreek Outflow (Average) | 0.783 | 0.383 | 65% | 35% |

In the 2007 NLA study, the EPA reported dissolved nitrogen data for 24 different Virginia lakes (Table 4) (EPA 2007). The average DON concentration in lakes across the state of Virginia fell in the center of values obtained in this study, which demonstrated that the experimental results of this study were relatively accurate; having said this, the %DON of TDN values of this study were far lower than those of the NLA. This was explained by the simple fact that the TCF land is and has been used for agriculture for over a century. The soils and sediments, and therefore surface waters, of TCF are likely enriched in $NO_3^-$ and $NO_2^-$ from both past and present agricultural practices, whereas none of the 24 Virginia lakes from the NLA were located on agriculture properties (likely resulting in the low %DIN of TDN values relative to those at TCF).

*4.6. Error Analysis*

A few experimental limitations to this study could have adversely impacted the results that were presented in this report. One such limitation came from potential sampling uncertainty; that is, the specific location in the stream from which each sample was taken could have a different nutrient profile than, say, a slightly different location in the same stream (e.g. a few meters upstream). This heterogeneity which is common in our complex natural world is one potential source of error that could have resulted in dissolved nitrogen values that weren't necessarily representative of the stream site as a whole. Additionally, another inherent source of error comes from the moderate-precision of the Lachat spectrophotometer. While the Lachat is capable of yielding highly-reproducible measurements most of the time, this complex machine requires standards and reagents to be prepared almost perfectly, as well as for all the tubing and internal hardware of the machine to be clean from use to use, so it is not out of the realm of possibility that certain Lachat analyses could have produced less precise results. Overall though, the experimental design of this study was sound and I believe the data presented in this paper to be fairly accurate and representative of the pond that was being studied.

*4.7. Future Work*

DON and DIN measurements will continue to be measured from inflow and outflow streams of the TCF pond. A sample storage study will be conducted in order to determine the length of time that water samples can still be deemed 'good' without losing any dissolved nitrogen. Additionally, surface water chlorophyll levels will be quantified in the outflow in order to confirm the seasonal DON variability conclusion of epilimnetic biomass accumulation.

In the end (not just in our laboratory but in laboratories nation-wide), further research should be done on the seasonal and spatial variability in DON in order to advance our ever-growing understanding of both water nutrient subtleties and of inland aquatic ecosystem dynamics.

## 5. References


Berman, Bronk. 2003. Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. Aquatic Microbial Biology. 31: 279-305.

Cruz, Osentowski. What is Permaculture? Central Rocky Mountain Permaculture Institute. www.crmpi.org/What_is_Permaculture_files/permarticle.pdf. Web.

Durand, Breuer, Johnes. 2011. Nitrogen Processes in Aquatic Ecosystems. The European Nitrogen Assessment; Cambridge University Press. 126-142. Web.

EPA. 2007. National Lake Assessment. https://www.epa.gov/national-aquatic-resource-surveys/nla.

Howarth. 2009. Inorganic Chemicals: Nitrogen. Cornell University, Ithaca, NY. Elsevier Inc. 57-64.

Heathwaite. 1993. Nitrogen Cycling in Surface Waters and Lakes. Dept. of Geology; University of Sheffield. 99-136. Web.

Johnson, et al. 2009. The effect of land use on dissolved organic carbon and nitrogen uptake in streams. Freshwater Biology. 54: 2335-2350.

McHale, et al. 2000. Nitrogen solutes in an Adirondack forested watershed: Importance of dissolved organic nitrogen. Biogeochemistry. 48: 165-180.

Mollison, B. 1986. Permaculture: A Designers' Manual.

Pace, M. 2015. Eutrophication. Limnology; Dept. Environmental Sciences; University of Virginia. Powerpoint.

Pace, M. 2015. Light. Limnology; Dept. Environmental Sciences; University of Virginia. Powerpoint.

Pace, M. 2015. River, Stream Ecosystems. Limnology; Dept. Environmental Sciences; University of Virginia. Powerpoint.

Pace, M. 2015. Temperature, Mixing and Stratification. Limnology; Dept. Environmental Sciences; University of Virginia. Powerpoint.

Pellerin. et al. 2004. Role of Wetlands and Developed Land Use on Dissolved Organic Nitrogen Concentrations and DON/TDN in Northeastern U.S. Rivers and Streams. Limnology and Oceanography. 49: 910-918.

Wells. 2014. Standard Operating Procedure for Determination of Total Nitrogen. National Atmospheric Deposition Program, National Trends Network, Central Analytical Laboratory.

World Resources Institute (WRI). "Sources of Eutrophication." May, 2016. <http://www.wri.org/our-work/project/eutrophication-and-hypoxia/sources-eutrophication>.

## 6. Acknowledgements

A huge thank you to Jim Galloway, Laura Cattell Noll, Aaron Mills, Meg Miller and Matias Hahn for helping out so much with this project!

Also a big thanks to Rick & Margaret Haupt, Brooks Marshall, Art Bulger, Emily Salle at Morven Farms, Peter and Nich Traverse at Innisfree Village, Jamie at Bellair Farm, and Karen Rice for volunteering to help advance this study through the sampling of their ponds.