Performance Analysis of an Ecological Wastewater Treatment System (Eco-Machine), with Emphasis on Nitrogen Transformations

Adam T. Miller

The University of Vermont

Follow this and additional works at: https://scholarworks.uvm.edu/hcoltheses

Recommended Citation
https://scholarworks.uvm.edu/hcoltheses/266

This Honors College Thesis is brought to you for free and open access by the Undergraduate Theses at ScholarWorks @ UVM. It has been accepted for inclusion in UVM Honors College Senior Theses by an authorized administrator of ScholarWorks @ UVM. For more information, please contact donna.omalley@uvm.edu.
Performance Analysis of an Ecological Wastewater Treatment System (Eco-Machine), with Emphasis on Nitrogen Transformations

Adam T. Miller

A thesis submitted in partial fulfillment of the requirements for the degree of Bachelor of Science

Environmental Sciences: Ecological Design

Rubenstein School of Environment & Natural Resources

Honors College

The University of Vermont

Advisors:

Eric Roy, Ph.D.

Carol Adair, Ph.D.

Clayton Williams, Ph.D.
# Table of Contents

ABSTRACT .......................................................................................................................... 3

ACKNOWLEDGEMENTS .................................................................................................. 4

LIST OF TABLES AND FIGURES .................................................................................. 5

LIST OF ABBREVIATIONS, DEFINITIONS AND SYMBOLS ......................................... 6

INTRODUCTION .............................................................................................................. 7

  WASTEWATER TREATMENT: LITERATURE REVIEW .................................................. 8
  RELEVANT NITROGEN CYCLE PROCESSES .............................................................. 10

METHODS ....................................................................................................................... 13

  SITE DESCRIPTION: MAKING OF THE AIKEN ECO-MACHINE ............................... 13
  CHALLENGES ............................................................................................................... 16

RESULTS ......................................................................................................................... 17

  FLOW ............................................................................................................................ 17
  TEMPERATURE ........................................................................................................... 17
  pH ................................................................................................................................. 18
  O₂ ................................................................................................................................. 18
  NH₄⁺-N ......................................................................................................................... 18
  NO₃⁻-N ......................................................................................................................... 19
  DIN ............................................................................................................................... 19

DISCUSSION .................................................................................................................... 20

CONCLUSION .................................................................................................................. 25

APPENDIX: SUPPLEMENTARY FIGURES .................................................................... 31
Abstract

This research looked at nitrogen removal in the Aiken Center Eco-Machine at the University of Vermont. The mean (± 1 standard deviation) percent removal of the dissolved inorganic nitrogen (DIN = NH₄-N + NH₃-N + NO₃-N) from the system was 51.35% (± 17.28%). The biggest changes in NH₄⁺ and NO₃⁻ concentrations occurred in the closed aerobic tanks. There was a far larger decrease of NH₄-N than the corresponding increase in NO₃-N signifying that other nitrogen cycle processes were occurring in addition to nitrification. While more research needs to be conducted, it is hypothesized that denitrification and microbial assimilation made the largest contributions to the removal of DIN. The results also show that the variability in environmental conditions and inputs may have hindered the stabilization of pH, the nitrification performance, efficiency of the treatment system, and its overall suitability for greywater recycling. Looking ahead, it will be important to gain a more comprehensive understanding of the nitrogen budget by focusing on the link between microbial community dynamics and nitrogen dynamics, as well as measuring nitrogen losses to the atmosphere and to microbial sludge.
Acknowledgements

I want to thank Dr. Eric Roy for assisting me throughout the research process, from research proposal to research design to lab work and final writing. I am grateful for the learning opportunities I had with Matt Beam in the Eco-Machine laboratory. I thank Dr. Clayton Williams for agreeing to join the advisory committee and Dr. Carol Adair for her help navigating academia as my academic advisor and support writing the thesis.

I need to give special thanks to Matt Jackson for his support as a friend as well as his editing/formatting proficiency and technology support. Last but not least, I want to express my many thanks to my parents, brother, and extended family for their encouragement and feedback. I could never have built the self-confidence needed for this kind of endeavor without their support.
List of Tables and Figures

Figure 1: Eco-Machine process diagram ................................................................. 31

Figure 2: Flow and Temperature graphs ............................................................... 32

Figure 3: pH graphs ............................................................................................. 33

Figure 4: Dissolved Oxygen graphs ................................................................. 34

Figure 5: Ammonium graphs .............................................................................. 35

Figure 6: Nitrate graphs ...................................................................................... 36

Figure 7: Dissolved Inorganic Nitrogen graphs .............................................. 37

Table 1: Statistical tests of significance results .................................................. 38

Figure 8: Eco-Machine photo ............................................................................... 38

Table 2: Eco-Machine Five Number Summaries .............................................. 39

Table 3: Statistical tests by Location ................................................................... 40
List of Abbreviations, Definitions and Symbols

- DIN: Dissolved inorganic nitrogen, sum of NO$_3^-$, NO$_2^-$, NH$_4^+$, and NH$_3$
- DNRA: Dissimilatory nitrate reduction to ammonia
- DO: Dissolved Oxygen
- Eco-Machine: Käthe Seidel Eco-Machine
- gal: Gallons
- Gpd: Gallons per day
- L: Liters
- LEED: Leadership in Energy and Environmental Design
- Location: The individual tank, wetland, or influent pipe being described
- mg: milligrams
- NH$_4^+$: Ammonium form of Nitrogen
- NO$_3^-$: Nitrate form of Nitrogen
- NH$_3^+$: Ammonia form of Nitrogen
- NO$_2^-$: Nitrite form of Nitrogen
- N$_2$O: Nitrous oxide gas (“laughing gas”)
- oxidation-reduction (Redox) reaction: Reaction where electrons are transferred from a donor to an acceptor species
- pH: Potential of Hydrogen; base 10 logarithmic scale from 0 to 14 used to determine acidity/basicity of an aqueous solution
- Train: One of 3 parallel treatment systems that comprise the Eco-Machine
- YSI: Water probes produced by this company
Introduction

People are demanding more from their wastewater treatment because there is an imbalance between where resources are located and where they are needed (Magid et al., 2006). Furthermore, recycling of wastewater treated on-site could help meet non-potable water demand. However, to achieve this, the removal of wastewater nitrogen is critical to avoid odor and pollution concerns. The same nitrogen and phosphorus that pollutes waterways in one place are critical nutrients for agriculture and soil health in another. Every community has different wastewater treatment needs based on their population size, density, economy, and desired treatment goals. For instance, The University of Vermont Rubenstein School of Environment and Natural Resources has an interest in learning better wastewater management practices through experimentation and providing learning opportunities for students, has a LEED Platinum certified building to house a facility in, is located in an urban setting, and has the money to fund a significant infrastructure project that meets these goals (WBDG, 2017). In contrast, the Arava Institute for Environmental Studies is located in the desert in southern Israel. As an educational nonprofit, they have similar educational goals as UVM but are located in a very different climate, a rural community, and so their wastewater treatment system reflects their different needs (Little, 2014).

As a result of agricultural use of fertilizers, the biogeochemical cycling of nitrogen and phosphorus is often interrelated (Schlesinger & Bernhardt, 2013). Both of these nutrients can become pollutants in great quantities when they leach off of agricultural fields. These nutrients are commonly found in natural and synthetic fertilizers, animal waste, and food waste (Gruber & Galloway, 2008). Research needs to be investigated into the ability of ecologically designed wastewater treatment facilities to replace municipal wastewater facilities by using the concept of
the biorefinery in which resources are recovered while producing clean water (Harrison et al., 2016). Nitrogen cycling has been very well studied in soil environments and can be a starting point for this research (Fuller, 2005). Centralized municipal wastewater systems are the standard for wastewater treatment in the US, but do have some drawbacks (Quijano et al., 2017; Meinhardt, 2005), and decentralized treatment is also common. Decentralized wastewater treatment can include ecological wastewater treatment systems (Kadlec & Wallace, 2008).

**Wastewater Treatment: Literature Review**

Wastewater treatment systems are end-use ways to reduce the pollutant load on the environment (Rashidi et al., 2015). They provide a critical municipal function by helping to protect local water security and quality. When designed and operated correctly, ecologically designed wastewater systems have advantages over traditional wastewater treatment plants (Paranychianakis et al., 2006; Shao & Chen, 2015; Shao et al., 2013). They have a greater visual appeal than large-scale municipal plants as they use principles of biomimicry and ecological design to treat the waste. However, ecologically designed wastewater treatment systems are a relatively new phenomenon. As a result of their reliance on natural processes and reluctance to use strong chemicals, operating an ecologically designed system can be more susceptible to system crashes, where the nitrifying microbial populations can no longer tolerate the changed mesocosm environment. While conventional wastewater treatment plants also use biology that fluctuates in performance, it is a different environment that has more years of research to back up decision-making (Batstone et al., 2015). When Shao and Chen did an energy and water analysis comparing conventional to ecologically designed systems, they found that conventional systems were better able to conserve water while ecological ones had improved energy performance. It is
therefore important to learn more about the operational performance of innovative ecological wastewater treatment systems such as the Aiken Eco-Machine.

The benefit of utilizing natural processes to reach a desired outcome is also a challenge in creating reliable design (Grose, 2014; Ross et al., 2015). The Eco-Machine was designed to have triplicate parallel treatment trains that allow for statistically significant experimentation. Wastewater treatment system operators require consistent operation in order to comply with environmental regulation and serve their community function. Therefore, they need to understand the relevant natural processes that drive water treatment if they are to apply these principles to their specific circumstances when the need arises (Reddy & DeLaune, 2008). Ecologically designed treatment systems provide a challenge for designers and operators because the natural processes don’t allow for a one-size-fits-all solution. The purpose of this research was to add to the existing knowledge about operating an ecologically designed wastewater treatment system. Emphasis was placed on the nitrogen transformations since that was an existing area of concern, relating to treatment stability, nutrient removal, and water recycling.

When designed in a way that harmonizes the engineered functionality of the system with the biophilic aesthetic, ecologically designed wastewater treatment systems can provide a unique architectural feature that also reduces the load on municipal treatment systems (Dou et al., 2017). They can also be built in areas where municipal systems are not feasible such as in rural communities or areas where significant infrastructure is not possible (Paranychianakis et al., 2006). Municipal treatment systems should not be automatically written off as they are also home to many of the same nitrifying and denitrifying microorganisms as the ecologically engineered ones (Ju & Zhang, 2015). They focus on performance and do so at the occasional expense of aesthetics. However, both types of systems are susceptible to biological crashes. It is
useful here to give some perspective on the performance of other ecologically designed wastewater treatment systems. In 2003, Dr. John Todd discussed the performance of a wastewater system called the Advanced Ecologically Engineered System (AEES) in South Burlington, Vermont (Todd et al., 2003). This system measured a 93% reduction in TKN (organic N + ammonium), a 98% reduction in ammonia, a net increase in NO$_3^-$, and an overall 84% reduction in total nitrogen. A prior paper by Todd and Josephson from 1996 looked at a Living Machine in Providence, Rhode Island. They had an almost complete removal of NH$_3$ (Todd & Josephson, 1996). A third study by Lansing and Martin in 2006 of dairy wastewater at The Ohio State University found that NH$_4^+$ reduced from 52 mg N/L to 0.07 mg N/L and NO$_3^-$ increased from 0.2 mg N/L to 0.53 mg N/L (Lansing & Martin, 2006).

Relevant Nitrogen Cycle Processes

The main nitrogen cycling process focused on in this paper is nitrification. However, there is a traditional sequence in wastewater treatment to remove nitrogen by transforming ammonium into nitrate through nitrification and then turning that into nitrogen gas through denitrification thereby removing the nitrogen from the water (Osborne et al.). Assimilation, nitrogen fixation, and ammonia volatilization are also mentioned directly or indirectly.

Nitrification is a dissimilatory process by which primarily chemoautotrophic bacteria convert ammonium (NH$_4^+$) into nitrate (NO$_3^-$) (Madigan et al., 2015). This process requires an aerobic environment for the bacteria to metabolize the ammonium because of the role of O$_2$ as the electron acceptor. In the nitrification reaction, NH$_4^+$ is converted into NH$_2$OH with oxygen. Then, NH$_2$OH is converted to nitrite, and nitrite converted to nitrate.

The Nitrosomonas and Nitrobacter genera are mostly responsible for the nitrification processes. The nitrification process is highly sensitive to pollutants because of its reliance on a
select number of genera. Pollutants affect microorganisms differently based on their type, concentration, and ability to biodegrade (Fuller, 2005). Microorganisms involved in nitrogen cycling are sensitive to a range of environmental pollutants as well as environmental factors such as pH. Almost all pollutant classes studied had a negative impact on nitrifying communities including hydrocarbons, halogenated compounds, and metals. Chemoautotrophic bacteria are the predominant nitrifying community but methane-oxidizing bacteria, heterotrophic bacteria, and fungi can also be involved in the process (Reddy & DeLaune, 2008). For the chemoautotrophs, Nitrosomonas oxidizes ammonium to nitrite and Nitrobacter oxidizes nitrite to nitrate.

Nitrification is restricted to the aerobic zones of the water column, the soil-floodwater interface, and the aerobic root zone. Nitrification potential is determined by measuring the accumulation of nitrate over time. The number of Hydrogen ions released in the simplified nitrification equations is understood \((\text{NH}_4^+ + 1.5 \text{O}_2 \rightarrow \text{NO}_2^- + 2 \text{H}^+ + \text{H}_2\text{O}; 2\text{NO}_2^- + \text{O}_2 \rightarrow 2\text{NO}_3^-)\) (Reddy & DeLaune, 2008). This results in 2 hydrogen ions for each \(\text{NH}_4^+\) molecule added. It is also known that the nitrifying bacteria are adapted to a specific pH range. Nitrobacter and Nitrosomonas require calcium carbonate or a similar buffer in order to thrive in a wastewater treatment system. Normally, the generation of hydrogen ions acts as a limiting factor in the nitrification process. The bacteria metabolize \(\text{NH}_4^+\) until the hydrogen byproducts decrease the pH to a point where the bacteria can no longer convert ammonium into nitrate. After a length of time, the pH increases from natural buffers in the environment resulting in a continuation of nitrification. The goal of a wastewater treatment process is to speed up nitrification and encourage the most treatment in the least amount of time. This can best be accomplished by buffering the water with sodium bicarbonate. They are also sensitive to salinity, temperature,
redox potential, oxygen availability, and ammonium availability. The optimum temperature is between 25 and 35°C in pure culture or 30-40°C in soil (Vymazal, 2007).

Since its first description in 1872, our understanding of nitrification has come a long way. We are continually learning more about the process, and at an ever-increasing rate as a result of new technologies and approaches that open up new ways of understanding the microorganisms behind this process. For example, in the past ten years, we have discovered a novel Archaean species involved in nitrification (Monteiro et al., 2014). Furthermore, the importance of the 16s rRNA fragment was only understood since the 1990s.

The nitrogen cycle is completed when a fourth set of specialized bacteria denitrify the nitrate back into the atmosphere as N₂ gas through a series of intermediate nitrogen oxide products. Nitrification supports denitrification by supplying the heterotrophs with nitrate, which is utilized as an electron acceptor. Reddy and DeLaune provide a summarized list of the many genera that have been known to be involved in nitrate respiration (Reddy & DeLaune, 2008).

Stabilizing pH is understood to mean adjusting the water pH to mitigate the acidification effect of nitrification through the addition of a quantity of sodium bicarbonate based on the current volume of influent and concentration of NH₄⁺ within. The system performs well if it is efficient with its resource and energy use and reliable in its ability to create clean non-potable water from greywater that can be recycled back into the building and engage the public with natural processes. The objective of this research was to answer the following questions in order to support the iterative design process:

• Is DIN being removed during treatment?
• What transformations are occurring?
• And where are these transformations occurring?
• Can we increase Dissolved Inorganic Nitrogen Removal by stabilizing pH?
It was hypothesized that DIN removal was similar to other systems, the transformations occurring followed the traditional nitrify-denitrify sequence. It was further believed that this sequence would occur primarily in the open aerobic tanks because the roots of the floating plants provide anaerobic surface area for denitrification to occur. Lastly, it was thought that the addition of sodium bicarbonate would help microbial function by limiting drops in pH caused by nitrification.

**Methods**

**Site Description: Making of the Aiken Eco-Machine**

The Käthe Seidel Eco-Machine in the Aiken Center is an ecology-based treatment system developed by Dr. John Todd and his student, Matt Beam. Over several decades, Dr. Todd investigated designed ecosystems and summarized a set of 12 design principles that he found to be the most important for the newly emerging ecological engineering discipline (Todd & Josephson, 1996). Most of these concepts are designed to maximize the diversity of the organisms that process the wastewater. The concepts can, when stated in a general way, seem obvious, but within each category, there is a significant amount of detail. These concepts include topics such as having high microbial, animal, and mineral diversity, a minimum number of subecosystems, a cellular design of the mesocosms, solar-based photosynthetic foundations, and biological exchanges beyond the mesocosm. These general principles are used in order to maximize the growth, metabolic activity, and self-organization of the organisms and communities within the system. An ecologically engineered waste treatment mesocosm is home to a unique assemblage of microorganisms (Todd & Josephson, 1996).

Matt Beam developed the ecological wastewater treatment system, known as the Eco-Machine, at the University of Vermont as a master’s thesis (Beam, 2010). Dr. John Todd guided
the design of the system to meet the 12 principles of ecological design. Together with the Greening of Aiken program that modernized the aging building, the Eco-Machine helped make the Aiken Center a LEED Platinum certified building. The Eco-Machine went through several design phases before it reached its final form. The 15 mesocosm environments, including 3 closed aerobic, 9 open aerobic, and 3 wetlands are housed on the right-hand side of the main entranceway to the Aiken Center. A wall of glass windows and information panel allow visitors to view and learn about the management of the waste stream in the environmental building. The Eco-Machine is adjacent to the solarium, a high-ceiling room with wall-to-wall windows and a continuation of the wetlands and plants that give the room the feel of a greenhouse.

Figure 1 is a useful guide for understanding the spatial arrangement and flow direction. The water from toilets and any other drains in the building (#0 in Figure 1) is sent outside, first to a grinder (#1) to break up debris and then to a septic tank (#2) where solids can settle out. The equalization tank (#3) stores water to ensure that the Eco-Machine receives a steady supply of greywater. The water returns to the building and enters the Eco-Machine in the closed aerobic tanks (#4). There is a sample point before the closed aerobic tanks. After the closed aerobic tanks, the water flows into 3 open aerobic tanks (#5, 6, and 7). The water is then sent to the wetlands (#8). After the wetlands, the water from the three replicate trains recombines and flows into treated water storage (#9). The framework for this research occurred between the influent sampling point and the wetlands before water recombination. Following water storage, the water is sent through a filter (#10) and UV disinfection (#11).

Data were gathered between November 2016 and March 2017. No data were collected from the middle of December through the middle of January between semesters. Samples were collected from the Influent, Closed Aerobic, three Open Aerobic, and Constructed Wetland sites.
from each of the replicate Trains (A, B, and C) approximately three times each week (Figure 1). Samples were analyzed using two YSI electronic probes. One of these probes is calibrated to detect nitrate (NO$_3$) and the other measures ammonium (NH$_4$) and ammonia (NH$_3$).

Temperature, dissolved oxygen, and conductivity were also measured with these probes. The daily flow of water through the system was logged each day with system software. There were several occasions where the flow of wastewater had to be stopped to fix a leak or clear a clog. Overall, the target flow rate was 500 gallons per day ($\sim$1900 L) and it averaged around 400 gpd with a standard deviation of $\pm$ 76.2 gpd ($\sim$1500 L). This methodology was developed in accordance with previous procedure from the first few years of the Eco-Machine’s operation.

Before the samples were taken, NO$_3^-$, and NH$_4^+$ probes were calibrated using a 1 mg/L solution and a 100 mg/L solution. Temperature was calibrated to room temperature with a mercury thermometer.

After the samples were collected, the average and standard deviation were calculated for % removal of dissolved inorganic nitrogen for the first 2/3 and the last 1/3 of the treatment period. During the last 1/3 of treatment, from March 20 until April 28 the input of sodium bicarbonate was adjusted in an effort to improve nitrification performance. Then, the data were analyzed with a Kruskal-Wallis nonparametric test (one-way ANOVA) as well as a Dunn-Bonferroni post hoc test to identify significant changes in NH$_4^+$ and NO$_3^-$ concentrations along the units (i.e., tanks) within the treatment train. The average of all three trains was used in these statistical tests. Non-parametric tests were used because the distributions were not assumed to be normal.

The one-way ANOVA on ranks determined, at an alpha level of 0.05, if at least one mean is statistically different from the others. The two hypotheses were:

- Ho: no difference among trains A, B, and C
  - Ha: statistical difference among trains A, B, and C
• Ho: no difference along each treatment train
  o Ha: statistical difference along each treatment train

The post hoc test identified points in the treatment train sequence where significant
changes in the measured N parameters occurred. This test identified specific differences among
and within the trains as follows:

• Ho: no difference between A-B, A-C, and/or B-C
  o Ha: statistical difference between A-B, A-C, and/or B-C

• Ho: no difference along each treatment train
  o Ha: statistical difference between 3-4, 3-5, …4-7, etc. along each treatment
  train

Challenges

Several challenges were faced while working with a wastewater system in an office
environment at a University. The office environment meant that there was a reduced amount of
carbon inputs via solid waste. The denitrifying organisms require an external source of carbon
since they don’t photosynthesize. Occasionally, the grinder station would clog or the system
would be turned off for other maintenance procedures and the flow would take a few weeks to
get back to the target rate of 500 gal/day. The campus building is not used as intensely at night,
on weekends, or during school breaks and it was also found that the trains are not exact replicates
as result of their placement relative to the windows altering their temperature and other
environmental parameters.
Results

Flow

Flow showed a decrease throughout the sampling period. There were several times that
the flow was zero when the system was turned off for system cleaning. These data points were
removed. The average flow while the system was operational was 431.04 gallons/day. The
median was 461 gal/day with a standard deviation of 76.21 gal/day, a minimum of 41 gal/day,
and a maximum of 570 gal/day (Figure 2).

Temperature

The temperature varied by time of year and by train as trains B and C are further from the
windows and train A is close to the window (Figure 1). The maximum temperature reached in
the system was 19.25°C in the open aerobic tank 6A on February 17, 2017. The minimum
temperature reached was 14°C in the influent on February 6, 2017. The average (±1 standard
deviation) temperature for the entire system throughout sampling was 16.61 ± 1.07°C and the
median was 16.84°C. The train A maximum was 1.05°C warmer than the train B maximum and
0.9°C warmer than the train C maximum. The train minimums were between 14.05°C in train C
and 14.5°C in train A. The train averages varied between 16.25°C in train C and 16.87°C in
train A. The train C median was 16.45°C and the train A median was 16.98°C warmer with train
B median in between at 16.65°C. In general, the temperature increased from the beginning in the
Influent through the wetlands (Figure 2). Statistical analysis with the dunn Bonferroni post-hoc
test showed that temperature was significantly (p<0.05) warmer in A than in B and C, which did
not differ (Table 1).
**pH**

The water became more acidic as it moved through the treatment. The minimum pH of 4.99 was achieved in wetland B on November 23, 2016. The maximum of 8.43 was reached in the final open aerobic train C on March 20, 2017. The average pH was 6.19 and the median was 0.05 lower. By train, the average pH was most acidic for train B at 6.02, with train C at 6.08 and train A at 6.14. The median pH for train C was 5.88, which was also lower than train B at 5.89 or A at 5.96 (Figure 3). The one-way Anova only showed a difference between trains A and B. As a result of the data being collected, the amount of sodium bicarbonate was adjusted during the last 1/3 of the monitoring period.

**O₂**

The dissolved oxygen increased overall through the treatment process from influent to wetland despite a decline as it entered the wetland. It was often very low in the influent with a minimum of 0.27 mg/L on December 5. The maximum of 9.23 mg/L was reached in the final open aerobic tank of train B on November 7. The average was 4.87 mg/L and the median was almost identical at 4.89 mg/L with a standard deviation of 1.76 mg/L (Figure 4). Dissolved oxygen was statistically different between (A-C, B-C) but not (A-B).

**NH₄⁺-N**

Throughout the 5 months of sampling, the largest reduction in ammonium occurred between the influent and the closed aerobic tanks. A statistically significant difference was found between all of the locations except for between the open aerobic tanks (Table 2). The maximum value was 144.30 mg/L in the Influent on February 3rd and minimum value of 2.50 mg/L on January 20th in the final train C wetland. There was a sharp decrease of NH₄⁺ from
influent to closed aerobic followed by smaller changes throughout the rest of the treatment process (Figure 5).

**NO$_3$-N**

Nitrate production was also most significant between the influent and closed aerobic tanks, but this change was not as significant as the ammonium change. From influent to wetland within each train, the NO$_3$-N was unique in the changes that were statistically significant. There was no significant change from the closed aerobic tanks to the wetlands. Interestingly, there was a significant difference between tanks 5 and 8 with tank 8 having a lower average NO$_3$-N concentration. The maximum was 97.70 on February 27th in the closed aerobic train C. The minimum was 0.70 mg/L in the Influent on November 16th. By train, C had the largest maximum of 182.00 mg/L. Train A had a maximum of 92.00 mg/L and train B was the lowest at 86.40 mg/L. Train C also had the lowest minimum at 4.20 mg/L. Train A had the highest minimum at 8.30 mg/L and train B was a little bit lower at 6.00 mg/L. The highest average NO$_3$-N by train was for train C at 59.77 mg/L followed by train B at 54.65 mg/L and train A at 50.32 mg/L (Figure 6).

**DIN**

Figure 7 illustrates that DIN was reduced by about 70 mg/L from the Influent through the Wetlands. Since the average Influent DIN was about 125 mg/L, that means that (± 1 standard deviation) the mean removal from the system was 51.35% (± 17.28%). The maximum value was 204.20 mg/L on February 3rd in the Influent. The minimum value was 5.00 mg/L in the wetlands on November 16th. By train, the lowest minimum value was in train B at 18.10 mg/L followed by train A at 29.50 mg/L and C at 33.70 mg/L. The lowest maximum value was train C at 91.20 mg/L followed by train A at 111.50 mg/L and B at 128.10 mg/L (Figure 7). In Table 3, the
dissolved inorganic nitrogen showed a similar pattern of significance to that of NH₄-N: significant with the exception of between the open aerobic tanks. During approximately the last 1/3 of treatment, between March 21-April 28, when the sodium bicarbonate was adjusted to stabilize pH, the % removal of DIN was 49.34% (± 9.48%). This is compared to the first 2/3 % removal of 50.79% (± 16.48%).

**Discussion**

Overall, there may have been too much nitrogen in the effluent on a consistent basis (Li et al., 2009). The small size of the constructed wetlands (#8), along with the challenges of being located within an office building, prevented them from providing an anaerobic environment with sufficient carbon for denitrifying microorganisms to thrive. In contrast to the original hypothesis, most of the nitrogen transformations occurred in the closed aerobic tanks. Additionally, adjusting pH did not have the desired effect in the last 1/3 of treatment as the % DIN removal did not increase.

There are many nitrogen processes that were not emphasized in this research. Based on a schematic for nitrogen transformations in wetlands, nitrogen can take the form of N₂ gas, N₂O, dissolved NH₄⁺, NO₃⁻, adsorbed NH₄⁺, NH₃⁺, nitrogen in microbial or plant biomass, or nitrogen in dead organic matter (Reddy & DeLaune, 2008). Some of the nitrogen transformation pathways are nitrogen fixation, nitrification, denitrification, mineralization, biomass uptake, ammonification, immobilization, dissimilatory and assimilatory nitrate reduction to ammonia, dinitrogen fixation, and ammonia volatilization. Several of these pathways function through the exchange of electrons in what is known as an oxidation-reduction reaction. The Eco-Machine has a variety of intentionally designed environments, each with their own environmental parameters. One of the more obvious ways to determine what transformations are taking place is
to differentiate between the aerobic, anoxic, and anaerobic processes. For example, nitrification only occurs in aerobic conditions while denitrification typically (although not always) requires anaerobic environments (including microenvironments). However, several processes such as ANRA and ammonification can occur in either oxygenated or deoxygenated environments. pH is another possible differentiation criterion. Ammonia volatilization is significantly more important above a pH of 7.5. These considerations can complicate the analysis of what transformations are occurring and which ones are significant.

Inorganic nitrogen forms such as ammonium and nitrate are believed to be the predominant forms of nitrogen that are assimilated into microorganisms and taken up into plants as biomass. Studies in the past several decades have found that organic uptake in the form of amino acids and other small organic matter can also be taken up or assimilated, dependent on availability of each nitrogen form, physiological preference, as well as other factors such as temperature (Boczulak et al., 2013). This new research has not found direct quantitative evidence of the importance of this pathway to plant nitrogen nutrition (Näsholm et al., 2009). However, there is an understanding that NO₂⁻ can build up in plant and microbial biomass in human impacted systems such as wastewater and can cause a disruption in the metabolic pathway (Wunderlin et al., 2013).

A brief comparison of the boxplots by location of nitrate, ammonium, and dissolved inorganic nitrogen reveals a seemingly missing quantity of nitrogen. There is a reduction of ammonium from influent to the closed aerobic of approximately 80 mg N/L and only a 40-50 mg N/L increase in nitrate between those same tanks. The contribution of ammonia is minimal. Understanding that mass cannot be created or destroyed according to the law of conservation of mass, it is important to discuss where this nitrogen could have gone.
One possibility is that the missing nitrogen assimilated into microbial biomass and accumulated as sludge at the bottom of the tanks or attached itself to the surfaces of the bioballs in the closed aerobic tanks. Another possibility is that the nitrogen denitrified into the atmosphere as nitrous oxide (N₂O) or inert nitrogen gas (N₂). There are several possible tank conditions that could make this pathway possible. Assuming sufficient aeration in the closed aerobic tanks, based on the observed results, there was sufficient oxygen for aerobic bacteria to survive. The presence of aerobic nitrifying bacteria can also be confirmed by the apparent conversion of the majority of NH₄-N to NO₃-N. Small pockets of anoxic or anaerobic conditions could allow for denitrification. Similarly, aerobic denitrification can also occur. In *Biogeochemistry of Wetlands*, Reddy and DeLaune suggest that, at least in the context of wetlands, denitrification and volatilization, also known as biotic and abiotic factors respectively are the main causes of this change ([Reddy & DeLaune, 2008](#)).

Further analyses would have gone beyond the scope of this project. The statistical and graphical data analysis presented here is sufficient to show the locations of the major nitrogen transformations as well as the shifts over time within an individual tank and as a composite system.

An inherent challenge of ecologically-designed wastewater treatment systems is that they are all unique. Each one is made to meet the needs of the specific community including budget, available architectural space, operations personnel, and water treatment/value-added needs. The difficulty with being one-of-a-kind is that operations and maintenance needs have to be discovered on a case-by-case basis. For example, the open aerobic tanks might have had improved nitrification values if they were shallower and had more root surface area as microbial living space.
The challenges associated with operating a wastewater treatment system in an office setting at a University may have negatively impacted the DIN removal. Maintaining a constant flow rate of 500 gallons/day was made challenging because of the imprecise nature of the control mechanism, the weekends, and occasional class break when building occupancy was significantly reduced and consequently flow was close to or at zero, and the times when the system was turned off for cleaning. The Eco-Machine was not designed to process all of the greywater from the Aiken building when it is at regular occupancy. Much of it is sent to the city sewer to be processed at the municipal plant. However, it is known that during storm events, the municipal water treatment facility cannot process the sudden influx of water and ends up dumping overload directly into Lake Champlain. Having a distributed network of wastewater treatment systems could mitigate the load on centralized treatment systems and reduce or eliminate the possibility for untreated stormwater to be transported directly into the watershed.

Environmental parameters such as temperature and pH were shown to influence the nitrogen treatment effectiveness. Influent temperature is a baseline for the water treatment. Being able to manage the temperature throughout the treatment process would allow for optimal treatment considering that the biological and chemical treatment processes are often driven by temperature. It is also important to pay attention to the temperature that the treated water leaves the treatment facility, particularly if it is sent directly into a body of water, because temperature can be a form of pollution in itself. Higher temperature water does not hold as much dissolved oxygen as colder water and can result in an area where oxygen-breathing heterotrophs cannot survive. Fortunately, this was not an issue with the Eco-Machine as temperature did not increase significantly and the only points where oxygen was near zero was in the influent. The design was to either send the water into the sewer or recycled back into the building toilets. Had the
water ever been sent to the toilets, it may have been a good idea to consider temperature as a contributing factor to occupant social perception of clean water. Knowledge that low oxygen levels would be present in the influent is why electrical aeration was added to the treatment system. It is interesting to note that the dissolved oxygen decreased when it entered the wetlands. Additionally, 82% of the electricity used by the Eco-Machine is used to deliver oxygen. Ultimately, there may have been too much oxygen throughout the treatment process to create sufficient space for denitrification.

The most significant change in mean pH occurred between the closed aerobic and first open aerobic tank. This supports the nitrogen change data that shows the majority of the transformation from NH$_4^+$ to NO$_3^-$ occurring in the closed aerobic tank. Considering the sensitivity of nitrifying bacteria to pH, the high and low pH values during treatment show that optimal treatment did not occur at all times. pH was the only parameter studied that did not have statistical significance between trains after running a post-hoc test. The one-way Anova resulted in a value of 0.1074. The last 1/3 of treatment surprisingly did not show an improvement in DIN removal as a result of increasing the pH to a range and stability that is more suitable for nitrification (Figure 3). The lack of a connection between the increasing pH and improving nitrogen removal could be the result of confounding factors. For instance, the sampling might not have been run long enough after the change in sodium bicarbonate input to effectively change the wastewater microbial ecosystem.

It is perplexing that the majority of the system had minimal impact on NH$_4^+$ concentration. It could be that the open aerobic tanks had too small of a surface area to volume ratio to create spaces for the nitrifying bacteria to live. The ammonium statistical level of difference between trains was not as large as that for nitrate.
The NH$_4$-N concentration decreased more than NO$_3$-N concentration increased between the influent and closed aerobic tanks. The very small p-value for difference between trains may have been a factor in the ability of the Eco-Machine to treat the water consistently between trains. NO$_3^-$ increased most from influent to the closed aerobic tanks and decreased slightly from the final open aerobic tanks to the wetlands. The decrease is most likely a result of denitrification, a movement of the nitrogen into the atmosphere as N$_2$ gas.

The reduction in total dissolved inorganic nitrogen, the summation of NH$_3$-N, NH$_4$-N, and NO$_3$-N, signifies that treatment is occurring. As noted in the results section on flow, inconsistencies outside of the realm of wastewater treatment such as school vacations played a role in reducing the consistency of the treatment. Train A was able to remove more DIN than the other trains. Trains B and C were not statistically different from one another. Contrary to the hypothesis, when the pH was adjusted, the DIN removal decreased by a few percent although the large standard deviation makes this result inconclusive.

**Conclusion**

The ecological design and engineering frameworks can be useful paradigms for planning a better relationship between people and the natural world. As with most scientific endeavors, collecting data is sometimes the only way to test and reiterate how these frameworks can be applied in particular circumstances. The results of this study found that the majority of the dissolved inorganic nitrogen removal occurred in the closed aerobic tank rather than the open aerobic tanks as was initially hypothesized. Nitrogen cycling in wastewater can seem overwhelmingly complicated. For the purposes of this study, only the main processes were discussed including nitrification, denitrification, and assimilation into plant and microbial biomass. Despite having about a 50% reduction in DIN, the full treatment sequence was not
utilized effectively as most of this change occurred in the closed aerobic tanks. In practice, the Eco-Machine never recycled water to the toilets due to concerns about social perception and consistent nitrogen treatment. Increasing the mean percent removal of DIN is important for wastewater treatment because it ensures that nitrogen is effectively removed from the water and into the atmosphere as nitrogen gas. However, when pH was stabilized in the last 1/3 of treatment, the DIN removal did not significantly improve. Future research should further develop the connection between nitrogen exchange with the atmosphere and microbial dynamics with environmental parameters. Additionally, as described in the discussion on flow, there is a need to pay attention to whether the priority should be to maximize treatment stability given changing influent loading or stabilize the influent loading characteristics.
References


doi: [https://doi.org/10.1016/j.chemosphere.2014.10.021](https://doi.org/10.1016/j.chemosphere.2014.10.021)


doi: [https://doi.org/10.1016/j.landurbplan.2014.08.011](https://doi.org/10.1016/j.landurbplan.2014.08.011)


Biological Wastewater Treatment and Reactive Nitrogen.


Appendix: Supplementary Figures

Figure 1. Complete flow diagram of Eco-Machine.

0 Bathrooms
1 Grinder Station
2 Septic Tank
3 Equalization Tank
4 Closed Aerobic Tank
5-7 Open Aerobic Tanks
8 Constructed Wetlands
9 Treated Water Storage
10 Fines Filter
11 UV Disinfection
Figure 2. Top center: Flow (gallons/day) time series; Middle left: Average Temperature time series °C by Location; Middle right: Average Temperature time series °C by Train; Bottom left: Average Temperature °C by Location; Bottom right: Average Temperature °C by Train.
Figure 3. Top: Average pH time series by Location; Middle: Average pH by Location; Bottom: Average pH by Train.
Figure 4. Top left: Average DO (mg/L) time series; Top right: Average DO (mg/L) by Train; Bottom left: Average DO (mg/L) by Train; Bottom right: Change in DO (Wetland-Influent) in mg/L by Train.
Figure 5. Top left: Average NH$_4^+$ (mg/L) time series; Top right: Average NH$_4^+$ (mg/L) by Location; Bottom left: Average NH$_4^+$ (mg/L) by Train; Bottom right: Change in NH$_4^+$ (Wetland-Influent) in mg/L by Train. Nitrogen is measured on an N-basis.
Figure 6. Top left: Average NO$_3^-$ (mg/L) time series; Top right: Average NO$_3^-$ (mg/L) by Location; Bottom left: Average NO$_3^-$ (mg/L) by Train; Bottom right: Change in NO$_3^-$ (Wetland-Influent) in mg/L by Train. Nitrogen is measured on an N basis.
Figure 7. Top left: Average DIN (mg/L) time series; Top right: Average DIN (mg/L) by Location; Bottom left: Average DIN (mg/L) by Train; Bottom right: Change in DIN (Wetland-Influent) in mg/L by Train. Nitrogen is measured on an N basis.
Table 1. Results of Kruskal-Wallis and Dunn-Bonferroni post-hoc tests. Grey shaded boxes are statistically significant at the α level of 0.05.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Dunn-Bonferroni</th>
<th>NH4</th>
<th>Dunn-Bonferroni</th>
<th>NO3</th>
<th>Dunn-Bonferroni</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-B</td>
<td>0.0160</td>
<td>A-B</td>
<td>1.3*10^-4</td>
<td>A-B</td>
<td>0.0497</td>
</tr>
<tr>
<td>A-C</td>
<td>4.7*10^-5</td>
<td>A-C</td>
<td>0.1850</td>
<td>A-C</td>
<td>1.05*10^-13</td>
</tr>
<tr>
<td>B-C</td>
<td>0.3770</td>
<td>B-C</td>
<td>8.2*10^-9</td>
<td>B-C</td>
<td>6.61*10^-7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH</th>
<th>Dunn-Bonferroni</th>
<th>DO</th>
<th>Dunn-Bonferroni</th>
<th>DIN</th>
<th>Dunn-Bonferroni</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-B</td>
<td>0.1490</td>
<td>A-B</td>
<td>1.0000</td>
<td>A-B</td>
<td>7.0*10^-9</td>
</tr>
<tr>
<td>A-C</td>
<td>1.0000</td>
<td>A-C</td>
<td>0.0020</td>
<td>A-C</td>
<td>1.7*10^-8</td>
</tr>
<tr>
<td>B-C</td>
<td>0.2940</td>
<td>B-C</td>
<td>0.0010</td>
<td>B-C</td>
<td>1.0000</td>
</tr>
</tbody>
</table>

Figure 8. Image of Eco-Machine viewed through the window from the Solarium in the Aiken Center (WCAX, 2013).
Table 2. Statistical summary of Eco-Machine parameters as a system and by Train.

<table>
<thead>
<tr>
<th>System Average</th>
<th>Median</th>
<th>Average</th>
<th>Standard Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>16.84</td>
<td>16.61</td>
<td>1.07</td>
<td>14.00</td>
<td>19.25</td>
</tr>
<tr>
<td>pH</td>
<td>6.14</td>
<td>6.19</td>
<td>0.67</td>
<td>4.99</td>
<td>8.43</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>4.89</td>
<td>4.87</td>
<td>1.76</td>
<td>0.27</td>
<td>9.23</td>
</tr>
<tr>
<td>NH4+ (mg/L)</td>
<td>31.20</td>
<td>32.83</td>
<td>23.31</td>
<td>2.50</td>
<td>144.30</td>
</tr>
<tr>
<td>NO3- (mg/L)</td>
<td>55.92</td>
<td>51.66</td>
<td>17.33</td>
<td>0.70</td>
<td>97.70</td>
</tr>
<tr>
<td>DIN (mg/L)</td>
<td>83.10</td>
<td>83.88</td>
<td>22.63</td>
<td>5.00</td>
<td>204.20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Train A Average</th>
<th>Median</th>
<th>Average</th>
<th>Standard Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>16.98</td>
<td>16.87</td>
<td>1.06</td>
<td>14.50</td>
<td>19.25</td>
</tr>
<tr>
<td>pH</td>
<td>5.96</td>
<td>6.14</td>
<td>0.70</td>
<td>5.16</td>
<td>8.30</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>5.62</td>
<td>5.07</td>
<td>1.76</td>
<td>0.81</td>
<td>7.93</td>
</tr>
<tr>
<td>NH4+ (mg/L)</td>
<td>23.35</td>
<td>24.84</td>
<td>13.41</td>
<td>3.00</td>
<td>59.10</td>
</tr>
<tr>
<td>NO3- (mg/L)</td>
<td>53.95</td>
<td>50.32</td>
<td>15.72</td>
<td>8.30</td>
<td>92.00</td>
</tr>
<tr>
<td>DIN (mg/L)</td>
<td>76.25</td>
<td>75.24</td>
<td>18.67</td>
<td>29.50</td>
<td>111.50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Train B Average</th>
<th>Median</th>
<th>Average</th>
<th>Standard Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>16.65</td>
<td>16.46</td>
<td>1.02</td>
<td>14.15</td>
<td>18.20</td>
</tr>
<tr>
<td>pH</td>
<td>5.89</td>
<td>6.02</td>
<td>0.69</td>
<td>4.99</td>
<td>7.95</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>5.38</td>
<td>5.06</td>
<td>1.76</td>
<td>1.59</td>
<td>9.23</td>
</tr>
<tr>
<td>NH4+ (mg/L)</td>
<td>33.35</td>
<td>30.89</td>
<td>14.35</td>
<td>3.30</td>
<td>67.30</td>
</tr>
<tr>
<td>NO3- (mg/L)</td>
<td>56.40</td>
<td>54.65</td>
<td>13.87</td>
<td>6.00</td>
<td>86.40</td>
</tr>
<tr>
<td>DIN (mg/L)</td>
<td>89.00</td>
<td>85.31</td>
<td>19.42</td>
<td>18.10</td>
<td>128.10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Train C Average</th>
<th>Median</th>
<th>Average</th>
<th>Standard Deviation</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>16.45</td>
<td>16.25</td>
<td>1.78</td>
<td>14.05</td>
<td>18.35</td>
</tr>
<tr>
<td>pH</td>
<td>5.88</td>
<td>6.08</td>
<td>0.72</td>
<td>5.06</td>
<td>8.43</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>5.86</td>
<td>5.36</td>
<td>1.98</td>
<td>0.56</td>
<td>8.38</td>
</tr>
<tr>
<td>NH4+ (mg/L)</td>
<td>25.00</td>
<td>23.58</td>
<td>18.56</td>
<td>2.50</td>
<td>58.30</td>
</tr>
<tr>
<td>NO3- (mg/L)</td>
<td>59.50</td>
<td>59.77</td>
<td>23.97</td>
<td>4.20</td>
<td>182.00</td>
</tr>
<tr>
<td>DIN (mg/L)</td>
<td>80.20</td>
<td>59.77</td>
<td>33.01</td>
<td>4.20</td>
<td>182.00</td>
</tr>
</tbody>
</table>
Table 3. Results of Kruskal-Wallis and Dunn-Bonferroni post-hoc tests. Grey shaded boxes are statistically significant at the α level of 0.05.

<table>
<thead>
<tr>
<th></th>
<th>DIN KW p-value &lt; 2.2*10^-16</th>
<th>NH4 KW p-value &lt; 2.2*10^-16</th>
<th>NO3 KW p-value = 3.252*10^-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIN</td>
<td>Dunn-Bonferroni</td>
<td>Dunn-Bonferroni</td>
<td>Dunn-Bonferroni</td>
</tr>
<tr>
<td>3-4</td>
<td>2.02*10^-6</td>
<td>2.01*10^-4</td>
<td>1.19*10^-7</td>
</tr>
<tr>
<td>3-5</td>
<td>6.64*10^-12</td>
<td>3.48*10^-13</td>
<td>2.68*10^-10</td>
</tr>
<tr>
<td>3-6</td>
<td>1.26*10^-14</td>
<td>9.45*10^-17</td>
<td>3.36*10^-9</td>
</tr>
<tr>
<td>3-7</td>
<td>3.82*10^-17</td>
<td>1.73*10^-18</td>
<td>9.64*10^-8</td>
</tr>
<tr>
<td>3-8</td>
<td>1.27*10^-31</td>
<td>8.07*10^-36</td>
<td>3.59*10^-5</td>
</tr>
<tr>
<td>4-5</td>
<td>0.0277</td>
<td>3.28*10^-6</td>
<td>1.00</td>
</tr>
<tr>
<td>4-6</td>
<td>1.70*10^-4</td>
<td>2.11*10^-10</td>
<td>1.00</td>
</tr>
<tr>
<td>4-7</td>
<td>6.85*10^-7</td>
<td>1.27*10^-12</td>
<td>1.00</td>
</tr>
<tr>
<td>4-8</td>
<td>1.01*10^-4</td>
<td>1.18*10^-38</td>
<td>1.00</td>
</tr>
<tr>
<td>5-6</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>5-7</td>
<td>0.279</td>
<td>0.339</td>
<td>1.00</td>
</tr>
<tr>
<td>5-8</td>
<td>1.91*10^-12</td>
<td>1.52*10^-14</td>
<td>0.0110</td>
</tr>
<tr>
<td>6-7</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>6-8</td>
<td>1.29*10^-8</td>
<td>1.66*10^-9</td>
<td>0.0935</td>
</tr>
<tr>
<td>7-8</td>
<td>6.43*10^-6</td>
<td>1.37*10^-7</td>
<td>1.00</td>
</tr>
</tbody>
</table>