2017

# THE EFFECT OF CHRONIC NUTRIENT ADDITION FROM WASTEWATER ON FOREST ECOSYSTEMS AT THE RICE RIVERS CENTER

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A thesis submitted in partial fulfillment of the requirements for the Degree of Master of Science in Environmental Studies at Virginia Commonwealth University

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# Acknowledgements

I would like to express gratitude to Joseph Robinson, Richie Dang, and Skye Whitlow for their field assistance. Also, William "Mac" Lee for lab analysis, Jeff Atkins and Chris Gough for project design and Scott Neubauer for his committee commitment. Finally, my advisor Paul Bukaveckas for his time and guidance throughout the thesis process.

# Abstract


## THE EFFECT OF CHRONIC NUTRIENT ADDITION FROM WASTEWATER ON FOREST ECOSYSTEMS AT THE RICE RIVERS CENTER

By Michael Beck, M.S.

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Wastewater application to land can be a useful tool for mitigating impacts of nutrient enrichment on aquatic systems. A land application treatment system at VCU's Rice Rivers Center in Charles City County, VA provided an opportunity to study the impact of wastewater addition on the biogeochemistry of forests representative of the Mid-Atlantic Coastal Plain. Nutrient concentrations in throughfall and leachate were measured at Treatment and Control sites to assess differences in nutrient deposition and retention. Wastewater amended plots from the Walter L. Rice education building received 20-fold (N) and 6-fold (P) higher inputs relative to Control plots and plots located at the Virginia Department of Game and Inland Fisheries building.  Despite higher inputs, leaching losses of P from the Rice Treatment plots were comparable to Control plots, indicating that the land-based application system effectively mitigated  wastewater loads.  Leaching losses of nitrate were two-fold higher from Treatment plots relative to Controls, suggesting a potential N saturation effect and a reduction in N retention capacity of the treated forest.  Nitrogen effects on vegetation were indicated by lower root biomass and greater root N content among Treatment plots relative to Control plots. Overall, these results suggest that wastewater–amended plots near the Rice education building receive appreciably higher nutrient inputs and could, therefore serve as a model system for assessing effects on forest ecosystems.

# Introduction

The discharge of municipal wastewater into surface waters deleteriously impacts aquatic ecosystems (Smith, et al., 1999). These impacts are due to wastewater being enriched with nitrogen, phosphorus, and other pollutants. An alternative to surface water discharge is the application of treated wastewater to land. This method has been utilized since the 1960's to alleviate water pollution problems, recharge groundwater reservoirs and boost terrestrial plant production (Sopper 1971, Kiziloglu et al., 2008). One of the first large scale wastewater re-use systems was implemented in the 1960's at State College, Pennsylvania, and is still in use today. Sprinkler irrigation systems distribute wastewater to a mixed oak - red pine forest and a cropland site (Sopper 1971). The initial study analyzed soil samples for the first 3 years of system implementation and determined there was no significant accumulation of nitrogen, but a significant increase in phosphorus and sodium in the soil. Further, analysis indicated that the forest vegetation was utilizing nutrients in wastewater as indicated by increased tree growth and herbaceous ground cover (Sopper 1971).

Another wastewater re-use study described the ability of wastewater to improve soil fertility without detrimental effects on plants. It was determined that wastewater application increased yield and foliar nutrient content in crops (Kiziloglu et al., 2008). The long-term land application of wastewater is generally thought to have positive effects in agriculture settings, but these effects are more difficult to determine in forests because of differing species and soil structure (Chakrabarti, 1995). The re-use of wastewater effluent for irrigation has also been investigated. A study in southern Australia indicated that secondary treated wastewater could be successfully used to irrigate tree plantations. However, the buildup of sodium in the soil had adverse effects on some species (Stewart et al., 1984). The study concluded that increased growth by select species was due to additional water rather than an increase in nutrients. Overall, these studies show that wastewater application generally stimulates plant production, though the efficiency of nutrient retention relative to the fraction lost through runoff, was not determined.

Nitrogen is an essential nutrient for plant growth and reproduction. Along with phosphorus, nitrogen is a limiting nutrient in ecosystems because of its high demand and low availability in its reactive forms ($NO_2$, $NO_3$, and $NH_3$). A number of ecosystem-scale studies describe the effects of N addition using natural or experimental gradients in N deposition (Aber et al., 1998, Lovett et al., 2004). These studies may be useful for predicting effects of elevated levels of nitrogen from wastewater application (Alobaidy et al., 2010). Understanding the effects of wastewater disposal on forests may be advantageous in addressing broader concerns regarding the effects of anthropogenic nitrogen deposition (Kaiyue1 et al., 2016). Increases in N deposition and atmospheric $CO_2$ are two of the largest anthropogenic environmental perturbations (Galloway et al., 2008). The combustion of fossil fuels results in the emittance of nitrogen to the atmosphere. $NO_x$ emissions from fossil fuel energy production have increased from the 1800s, but leveled off recently because of reductions in emissions from developed countries (Cofala et al., 2007). Nitrogen deposition varies across the United States with the central Virginia region having lower deposition (2.5 – 3.2 kg $ha^{-1}$) than the Midwest (> 4.0 kg $ha^{-1}$) and the Northeast (3.0 – 5.0 kg $ha^{-1}$; NADP, 2015). Regional differences in N deposition may influence the capacity of forests to process N inputs from wastewater application. Nitrogen addition has the potential to boost forest productivity, increase foliage and root nutrient content, but also increase leaching losses (Mohammad et all., 2007). The extent to which these effects occur at a given site are determined by background N availability, plant community composition, soil type and other factors that influence N cycling in forests (Lovett et al., 2000, Lovett et al., 2013). Therefore, predictions of forest responses to elevated N deposition require regional-scale studies which consider differences in both rates of deposition and plant community composition.

Studies on wastewater application have largely focused on agricultural settings in assessing the benefits to tree production, crops, etc. What is needed is to understand these effects from an ecosystem perspective which considers not only plot-scale responses, but also a landscape context that considers N inputs, export and storage. Storage of nutrients within forests is directly related to the amount of nutrients entering the system, in addition to, vegetation uptake, denitrification, and sorption to soils (Lovett et al., 2013). Nitrogen addition via deposition and wastewater application has the potential to alter foliar and root nutrient composition. Nave et al. (2009) showed that fine roots and leaves can contain as high as 6 g $ha^{-1}$ of N and be two of the largest storage components in annual forest nutrient budgets. Zak et al. (2016) reported that in northern hardwood forests, experimental N deposition increased N stored in canopy leaves, but had limited influence on fine roots. In addition, there have been a number of studies that describe the effects of increasing nutrients on plant uptake and export (Magill et al., 2004, Jabloun et al., 2015). Mineral nutrients are usually obtained from the soil through plant roots, and differing levels can affect plant uptake. Plants are known to show increased lateral root growth and density in response to deficiency of N and P. When plant nutrient demands are met, plants devote resources to above ground growth (USDA N.A.S.S., 2013). Aber et al. (1998) described the concept of nitrogen saturation, which occurs when vegetation can no longer absorb added N, increasing N leaching losses. The cumulative effects of N deposition at moderate to high levels were shown to have negative impacts on biomass production and overall tree health in temperate forest systems. There is evidence that not all sites move toward saturation at the same rate, a process that is highly dependent on the initial concentration and nitrogen limitation (Aber et al., 1998). Thus, prior studies suggest that site characteristics like the permeability of the soil, and the severity of N limitation, ultimately determine how quickly N saturation is attained in response to increasing N inputs (van Groenigen et al., 2015).

Alteration of a systems ability to store nutrients directly affects export via leaching losses (Tung et al., 2009). Leaching, the loss of water soluble nutrients from the soil, can cause detrimental effects to downstream habitats such as streams, rivers and wetlands. Leaching losses are an important indicator of an ecosystems response to nutrient addition. Variability in leaching losses can be attributed in part to seasonal changes of temperature and precipitation. In temperate climates, losses are expected to be greater during the winter dormant season because plant uptake and evapotransporation in summer minimize leaching losses (Kreyling et al., 2015). In addition, Jabloun et al. (2015) demostrated that climate change, especially increases in precipitation, enhance N leaching from the soil. If the addition of nutrients remains constant throughout the year, as would be expected from wastewater application, higher leaching losses should occur in winter when plant and microbial activity is reduced (Tung et al., 2009) thus providing evidence that there is seasonal variation in patterns of nutrient retention and leaching.

The VCU Rice Rivers Center provides an opportunity to study the effects of wastewater N and P addition on forests within the Mid-Atlantic region. Inadequate draining soils across the site (Permit and Soil Report, 2015), required the Walter L. Rice education building (RICE) and Virginia Department of Game and Inland Fisheries regional headquarters (VDGIF) to use a land application wastewater disposal system. In this system, the waste is treated on site and sprinklers disperse effluent into the surrounding forest, as opposed to a standard septic system and leach field. The features of this system provide an opportunity to study the effects of wastewater addition on coastal plains forests, thereby improving our understanding of the effects of N addition on these forests.

### *Objectives*

The primary objective of this study was to examine the effects of wastewater effluent on N and P retention, root biomass and chemistry of forest plots at the VCU Rice Rivers Center. Spray fields associated with the RICE and VDGIF wastewater systems provided an opportunity to compare wastewater-amended plots (hereafter, Treatment) to plots receiving only background atmospheric deposition (Control). Data collection entailed measurements of N and P inputs to the plots inclusive of background deposition and wastewater spray. Nutrient losses from the plots were quantified by collecting samples of leachate using lysimeters (underground soil water collectors). Water samples from throughfall were collected for one year beginning February 2016, and for leachate, starting in May 2016. I also compared fine root biomass and N content to assess forest responses to nutrient additions. Data arising from this study will contribute to a larger effort to characterize differences in wood production and C sequestration among these plots (data collected by Chris Gough and Forest Ecology class).

### *Hypotheses*

It was hypothesized that N and P inputs would be higher in the wastewater application plots relative to the controls. Also, it was hypothesized that the VDGIF plots would receive greater nutrient inputs relative to the RICE plots. These predictions were based on the VDGIF facilities having a greater weekday resident population (~8 individuals) compared to the Rice education building (~4 individuals). In addition, it was hypothesized that nutrient concentrations in soil leachate (lysimeter samples) would be lower in comparison to above ground (throughfall samples) due to microbial and plant uptake. Seasonal differences in leaching were expected due a reduction in plant and microbial activity in the dormant season. It was anticipated that treatment plots would have a higher N concentration in root tissue samples due to greater N availability from wastewater inputs.

## Methods

### *Site Description*

The study was performed at the VCU Rice Rivers Center in Charles City County, VA. A total of 10 plots were established within the forest, where deciduous and evergreen conifers dominate. The

Rice Rivers Center property contains two wastewater treatment systems established in 2008. Four plots (2 Treatment, 2 Control) were located on the west side of the Rice Rivers Center property near the education building (RICE). Six additional plots (3 Treatment, 3 Control) were located in the northeast of the property at the VDGIF building (Figure 1). The total annual precipitation for 2016 was 132.8 cm, somewhat higher than the 100-year average (110 cm; NOAA Regional Climate Center Station at Hopewell, VA). The soils at both sites are silty loam topsoil with silty clay beneath (Permit and Soil Report 2015). Organic material was prevalent in the top 20 cm. Each plot was established on a 0.05 hectare of forest with one effluent sprayer at center for treatment plots.

The wastewater effluent applied to these plots goes through a 3-stage, 6-step treatment process advertised to remove 70% of Nitrogen through the use of bacteria providing nitrification and denitrification (Figure 2). The first stage is primary treatment where the solids are settled out in a separation tank. Next, the effluent is run through biological treatment modules MicroFAST® and EZ-Treat. Finally, the effluent is passed through tertiary treatment with UV disinfection before being pumped to the sprayers. Similar wastewater treatment systems are present at both the RICE and VGDIF buildings. Each system is designed to accommodate up to 3,407 L $d^{-1}$, requiring1,015 $m^2$ of land for dispersal (www.biomicrobics.com). The effluent spray at each plot had a max distance of 12.6 meters from the spray nozzle, and directly influenced an area of 498.8 $m^2$. Spray events occurred at varying time intervals depending on quantity of waste produced from the facilities. Each event lasted 5 ± .5 minutes and distributed 79 ± 5 L of effluent over the treatment plots, applying about 0.2 mm of effluent per event.

*Quantification of Water and Nutrient Fluxes*

Nitrogen and phosphorus inputs to the soils were measured using throughfall collectors. Nutrient losses from the study plots were measured by collecting leachate using lysimeters. Samples of throughfall and leachate were collected at 2-week intervals with greater frequency dependent on rainfall. One throughfall collector was installed at each plot. These collectors provided samples of rainwater and, for treatment plots, a combined rainwater and wastewater sample. Each device contained a funnel having a diameter of 20.32 cm attached to a 1000 mL Nalgene bottle with a total collection area of 324.3 $cm^2$. To quantify soil leachate losses, zero-tension lysimeters were installed at a soil depth of 25 cm. One lysimeter was installed per plot and each lysimeter was connected to three 10.16 cm collector disks (total collection area of 243.2 $cm^2$; Figure 3). Both the throughfall and lysimeter collectors were within 3 meters from plot center. Sampling took place from February 2016 to February 2017 for throughfall, and from May 2016 to February 2017 for lysimeters. During each sampling event, the volume of water in throughfall and leachate was measured and a 50-mL water sample was obtained for N and P analysis. Precipitation data for the sampling period was obtained from the NOAA Regional Climate Center database for a station located in Hopewell, VA (9 km from study plots). Based on concentration and measured volume of collectors, nutrient fluxes were calculated for each plot to determine inputs (throughfall) and outputs (leaching). Nutrient fluxes were expressed as kg $ha^{-1}$ for comparability to similar studies (e.g. Nave et al., 2009). The percentage of nutrients retained was calculated as the difference between throughfall and lysimeter fluxes divided by throughfall flux.

*Analytical Methods*

Samples were collected, preserved and processed according to standard protocols used by the VCU Environmental Analysis Lab. Individual samples collected from the same plot in a given month were combined based on volume to make a composite sample for that month. Samples were analyzed for Total Nitrogen (TN), Total Phosphorus (TP), Nitrate/Nitrite ($NO_x$), Ammonia ($NH_3$) and Phosphate ($PO_4$) using a SKALAR SanSystem Auto Analyzer. A total of 230 samples were analyzed: (1 throughfall x 13 months) + (1 lysimeter x 10 months) x 10 plots.

Fine root samples (roots < 2 mm diameter) were collected from all plots to assess differences in N content between Control and Treatment plots. The collection took place by driving a 6.35 cm diameter hollow post to a depth of 40 cm to extract roots and soil. The extracted cores were then divided into 0-10 cm, 11-20 cm, 21-30 cm and 31-40 cm depth increments. A total of 5 cores were collected from each plot during December 2016-January 2017. Soil samples were run through a coarse and fine sieve (35 mesh) to extract the roots. The roots were dried, weighed, ground and run on a Perkin-Elmer Model 2400 CHN analyzer to determine elemental concentrations of N and C. Estimates of root biomass along with % Nitrogen and % Carbon were used to derive areal estimates of root C and N (as g $m^2$).

*Statistical Analysis*

Multiple statistical analyses were performed using 2-way ANOVAs to partition variation in throughfall, lysimeter fluxes and percent of nutrients retained, based on treatment, month and their interaction term. A 2-way ANOVA was also used to partition variation in root chemistry based on treatments, depth and their interaction term. In addition to the ANOVA analysis, linear regressions were used to relate root mass, grams of carbon, grams of nitrogen, C:N ratio and % Nitrogen to soil depth within Treatment and Control plots. The interaction of treatment and depth was used to determine whether treatment effects differed with depth. A significant difference was attributed if the p-value was less than the significance level of 0.05. Analysis was performed using R statistical software v 3.3.1 and Microsoft Excel 2013.

*Data management*

As a VCU Rice Rivers Center collaborator, the final research paper and datasets are hosted on an online data repository according to the VCU Rice Rivers Center Data Management Plan

# Results

## *Water Fluxes*

Seasonal variation in the rate of leaching at RICE and VDGIF locations was positively correlated with precipitation and throughfall. Rainfall was the major determinant of the volume collected as throughfall and leachate. During this study, monthly precipitation ranged from 24.9 cm $mo^{-1}$ in May 2016 to 1.3 cm $mo^{-1}$ in February 2017 (Figure 4 & 5). The amount of throughfall was significantly positively correlated with the amount of precipitation at both RICE ($R^2 = 0.75$, $p < 0.001$) and VDGIF ($R^2 = 0.82$, $p < 0.001$). There was no significant difference in throughfall volume between control and treatment plots ($p > 0.05$) based on the results of the two-way ANOVA. At RICE, throughfall explained a smaller proportion of variation in leachate ($R^2= 0.49$, $p = 0.02$) compared to precipitation ($R^2= 0.75$, $p < 0.001$). Similar but non-significant differences were observed at VDGIF between throughfall and leachate ($R^2= 0.32$, $p = 0.09$). Over the period of study, the average monthly precipitation was 11 ± 2.6 cm. Average monthly throughfall was similar at the two sites (RICE = 6.9 ± 1.2 cm; VDGIF = 6.7 ± 1.2 cm), whereas, leachate was lower at RICE (5.7 ± 1.7 cm) relative to VDGIF (10.6 ± 2.8 cm).

## *Nutrient Concentrations*

Significant differences were found in nutrient concentrations between Control and Treatment throughfall samples collected at RICE, but not VDGIF. At RICE, throughfall concentrations were significantly higher (all $p < 0.001$) in Treatment plots relative to Controls (Figure 6). $NO_x$ exhibited the greatest difference between Treatment and Control plots (means = 3.1 ± 0.8 mg $L^{-1}$ and 0.15 ± 0.06 mg $L^{-1}$, respectively). Throughfall treatment plots at RICE also exhibited significantly higher concentrations of $PO_4$ (0.99 ± 0.06 mg $L^{-1}$) TN (7.5 ± 1.5 mg $L^{-1}$) and TP (1.07 ± 0.15 mg $L^{-1}$) relative to control plots ($PO_4$ = 0.22 ± 0.09 mg $L^{-1}$, TN = 1.7 ± 0.5 mg $L^{-1}$, TP = 0.3 ± 0.10 mg $L^{-1}$).

At RICE, differences in lysimeter concentrations between control and treatment were also significant ($p < 0.05$) for all but $NH_3$ ($p = 0.08$) and TP ($p = 0.10$). The differences in lysimeter concentrations between Control and Treatment plots varied across months with the largest differences observed in May, January and February. At VDGIF, lysimeter Treatment plots for $NO_x$ exhibited significantly higher concentrations at ($2.07 \pm 0.66$ mg $L^{-1}$) versus Control plots ($1.02 \pm 0.28$ mg $L^{-1}$), respectively (Figure 7).

*Nutrient Fluxes and Retention*

Nutrient fluxes varied 10-fold across months as a result of variable throughfall concentrations and rainfall. For all constituents, fluxes were largely driven by the volume of inflow and outflow since volume exhibited a greater range of variation than did concentration. Peak inputs and output of nutrients occurred in months of high precipitation (May, September, and January; Figure 8; Figure 9). There was up to a ten times difference in Treatment throughfall fluxes of $NO_x$, $PO_4$, TN, and TP relative to Control plots at RICE (all $p < 0.001$; Figure 9; Table 1). Significant differences were not observed at VDGIF (Table 2). The influence of the wastewater effluent on throughfall and lysimeter fluxes varied significantly by month at the RICE plots for all N and P fractions ($p < 0.001$; Table 1), but this interaction effect was not related to a consistent seasonal trend (e.g., summer vs winter). At VDGIF, there was a significant month effect, but no Treatment effect or interaction effect for throughfall or lysimeter fluxes (Table 2). $NO_x$ exhibited the greatest difference between Treatment and Control plots at RICE, $2.24 \pm 0.71$ $^{-1}$ and $0.04 \pm 0.01$ kg $ha^{-1}$ $mo^{-1}$, respectively. In addition, there was a significant difference in leachate fluxes between treatments for TN and TP ($p < 0.05$). For Treatment plots at RICE, losses were less than 10% of inputs via throughfall for $PO_4$ (input = $0.65 \pm 0.12$, output = $0.025 \pm 0.01$ kg $ha^{-1}$ $mo^{-1}$) and TP (input = $0.70 \pm 0.12$, output = $0.04 \pm 0.02$ kg $ha^{-1}$ $mo^{-1}$). $NO_x$ leachate output was observed to have over a 50 % reduction over input throughfall at $2.25 \pm 0.71$ and $0.80 \pm 0.36$ kg $ha^{-1}$ $mo^{-1}$, respectively. A similar reduction was observed with TN where input = 5.1 ±

1.4 and output = 1.4 ± 0.50 kg ha $^{-1}$ mo $^{-1}$. $NH_3$ output leachate showed a reduction of close to 50 % on an annual basis but the was highly variable between months. For RICE control plots and all VDGIF plots, there was an opposite trend with $NO_x$ measuring up to 12x the amount leaving the system then entering by throughfall (0.85 ± 0.27 versus 0.07 ± 0.02 kg ha $^{-1}$ mo $^{-1}$). A similar trend of greater outputs then inputs was observed at these plots for TN. Whereas, $NH_3$ and $PO_4$, show a decrease in leachate relative to throughfall inputs (Figure 10). Wastewater amended plots at RICE received 20-fold (N) and 6-fold (P) higher inputs relative to Control plots and plots located at VDGIF. Despite higher P inputs, retention at the RICE Treatment plots were comparable to Control plots (both > 90%). Leaching losses of $NO_x$ were two-fold higher from RICE Treatment plots relative to Controls but the Treatment plots retained 64% relative to -600% for the Control plots. $NH_3$ retention was similar across all plots at both sites. The greater loss of $NO_x$ from the control plots is correlated with the reduction in $NH_3$ leachate seen at the plots (Figure 10).

*Root Analysis*

Depth was a significant factor in accounting for variation in % N, root mass, carbon and nitrogen mass and C:N (Table 3). Analysis of the roots collected at RICE and VDGIF showed significant differences with Treatment plots having a higher % N in deeper roots (>10 cm) and lower root mass in shallow soils (<20 cm) versus controls. At RICE, the two-way ANOVA indicated significant differences between treatments in both % N ($p < 0.05$) and root mass ($p = 0.01$). However, at VDGIF, a significant difference was only found in root mass ($p = 0.01$). At RICE, % N in shallow roots (< 10 cm) for control and treatment was 0.65 % and 0.67 %, respectively, versus 0.45 % and 0.35 % in roots at 40 cm. As % N decreased with depth, a greater ratio of carbon to nitrogen was observed. In addition, root mass decreased with depth in both treatment and control plots by up to 88% from 10 to 40 cm (Figure 11; Figure 12). Greater N content was measured in the shallow roots (10 cm), which decreased substantially from > 1 to < .5 g $m^2$ at 20 cm (Figure 11; Figure 12).

# Discussion

*Volume and Precipitation*

Effluent spray provided minimal additional water inputs to Treatment plots. The lack of a significant difference between Control and Treatment collection volume is likely due to the limited amount of effluent released at the spray plots. Based on the documented flow rate of the sprinkler and the length of time of the spray events, it is estimated that each spray event released about 0.16 mm of effluent. The amount of variation in throughfall between replicate collectors within a month was 1.2 cm mo $^{-1}$ implying that the 4.8 mm mo $^{-1}$ added by the effluent sprinklers was small relative to the spatial variability in throughfall within the forest. Precipitation during the sampling period was highly influential to the volume collected from both throughfall and leachate. Recovery of the throughfall water was often dependent on the density of the overhead canopy where a denser canopy leads to a lower recovery (Kimmins, 2017). The percentage recovery of throughfall to precipitation was similar between RICE and VDGIF at 59 % and 57 %, respectively. Kimmins (2017) presented comparable recovery of throughfall from precipitation ranging 49 – 70 % but concluded it can be highly variable depending on the forest overstory. Leachate volume percentages presented by Radulovich et al. (1987) stated collection efficiency as high as 46 % of throughfall, whereas, RICE collection efficiency was 83 % and VDGIF over 100 %. The higher collection efficiencies over those stated by Radulovich et al. (1987) suggest that there are hydrological characteristics of the soil at our sites that contributed to the variation. Different characteristics like the location of preferential flow paths and level of the water table in relation to the depth of the lysimeters, can have a cumulative effect on the volume of water making it to the collectors in relation to the throughfall received (Van Der Heijden et al., 2013). In addition, the high-water yield in the lysimeters relative to precipitation and throughfall is a concern as it suggests that the lysimeters may not be working properly to capture leachate. The soils at the Rice

Rivers Center have low permeability and this may result in pooling of water during events. As a result, if the sub-surface water level rises above the collection depth, lateral filling may result in the over-estimation of output volume. Despite this, our estimates of N retention are reasonable based on prior studies (Wollheim et al., 2005).

*Concentrations*

The results from the nutrient concentration analysis of the throughfall samples suggest that the wastewater spray effluent had significant impacts on throughfall concentrations at RICE, with little to no influence at VDGIF. $NO_x$, $NH_3$ and TN throughfall concentrations at both sites were compared to the annual National Atmospheric Deposition Program range for the region (Table 4). Based on these values, concentrations at RICE Treatment plots were elevated at least 2X over controls. However, these concentrations were similar to James River WWTP discharges of $NO_x$ (1 – 13 mg $L^{-1}$) and TN (7 – 22 mg $L^{-1}$). In contrast, the throughfall collected from VDGIF treatment plots closely match that of natural nitrogen deposition received at the sites. Nitrogen deposition at our plots ranged from 0.1 – 1.6 mg $L^{-1}$ which is comparable to the regional NADP range of 0.4 – 0.8 mg $L^{-1}$ (Table 4).

A reduction in lysimeter concentrations at RICE plots were observed providing evidence that storage and uptake are taking place. This is in contrast to VDGIF where there was a 5-fold increase in lysimeter concentrations of $NO_x$ over throughfall (0.66 mg $L^{-1}$ and 0.14 mg $L^{-1}$, respectively). The increase in $NO_x$ leachate concentrations over throughfall at VDGIF treatment plots, suggests some $NO_x$ entering the plots is not being captured in the throughfall collectors and is contributing to leachate by other processes. Biological fixation, mineralization and nitrification are all processes that can provide inputs of nitrogen to the system and are known to be heterogeneous throughout relatively small areas (< 1 ha; Johnson et al., 2005). Leachate samples at RICE are trending about double that of the control leachate. This suggests that the system is effective at reducing the concentration of nutrients leaving the system but the outputs at the treatment plots are still elevated over controls. In addition, leachate values were compared to a report by the USGS analyzing shallow ground water concentrations across the Mid-Atlantic. Overall, RICE concentrations of $NO_x$ and $PO_4$ fall within the range of published values (Wentz et al., 2011). In contrast, VDGIF concentrations are more similar to control leachate values and are reflective of the low concentration received in the throughfall (Table 5).

*Fluxes and Retention*

Flux data show that effluent and precipitation influence the amount of material, entering, stored and leaving the system. The difference between throughfall and leachate fluxes of N and P observed at RICE suggests some retention of N and P is occurring. Leachate nutrient fluxes reflected a decline at RICE compared to throughfall for $NO_x$, $PO_4$, TN and TP. It is also important to note that fluxes were highly variable throughout the year, and in some months treatments were 10x higher compared to controls. VDGIF plot losses of $NO_x$ and TN were higher than inputs by throughfall. In addition, RICE control and all VDGIF plots were acting as a net source for $NO_x$ with negative retention as high as -1214 % between inputs and outputs (Figure 10). The greater loss of $NO_x$ from the VDGIF treatments and all control plots is likely influenced by the conversion of $NO_x$ from $NH_3$ since the reduction in $NH_3$ is proportional to the increase in $NO_x$ on an annual scale and can account for about 60 % of the difference.

The nitrogen flux values measured at our plots were compared to levels of deposition determined by the National Atmospheric Deposition Program of ($0.3 \pm 0.1$ kg ha$^{-1}$ mo$^{-1}$). RICE treatment plots received about ($5 \pm 1.4$ kg ha$^{-1}$ mo$^{-1}$) of nitrogen or about 17x that of regional deposition averages and about 6x that of our control plots at ($0.8 \pm 0.2$ kg ha$^{-1}$ mo$^{-1}$). A study by Aber et al. (1998) based in the Harvard Forest, utilized different levels of nitrogen addition with the highest being 12.5 kg ha$^{-1}$ mo$^{-1}$. Significant effects were only measured at this level, including increased leachate losses, a decrease in C:N ratios and nearly a doubling in root nitrogen concentration. Based these differences, we can determine that our plots were considerably less fertilized then the highest application rates of some studies but considerably more fertilized then by natural deposition only. Also, control plots at our sites measured higher flux rates compared to the regional average, a possible consequence of the $NO_x$ producing industrial facilities located across the river in Hopewell, VA.

RICE treatment plots were found to retain a greater amount of nutrients compared to controls, but the percentage of nutrients retained between individual months were highly variable. RICE treatment plots were also found to be the only plots that had a positive retention percent for $NO_x$, whereas, the other plots were observed to be net sources of $NO_x$. The significantly higher $NO_x$ inputs at RICE treatments plots were likely the contributing factor to this difference. The high percentage of retention for $PO_4$ and TP at both sites suggest that a large portion of incoming P is being sorbed in soils in addition to uptake by vegetation. Lovett et all., (2000) describe wastewater effluent as comparatively rich in nitrogen, providing more N then P to the system. The added nitrogen from the effluent at our plots is likely contributing to enhance tree growth by partially alleviating N limitation observed in most forest ecosystems. When comparing our results to other studies focused on retention efficiency, these studies show that N retention in forests is around 70 ± 20 % (Wollheim et al., 2005) . These results are comparable to the $NO_x$ retention seen in our RICE plots of 64 ± 15%. RICE treatment plots have shown to have higher losses of $NO_x$ by mass but also a higher % of $NO_x$ retained compared to VDGIF.

*Root Analysis*

Analysis of roots found at RICE Treatment plots revealed elevated levels of nitrogen and lower root mass. Root responses to treatment differed with depth. The largest differences in % Nitrogen were observed below 20 cm. This result coincides with nitrogen distribution trends found in soils by Aber et al. (1998), that stated as the upper layers of soils become saturated, the additional N is able to move lower and become available to deeper roots. In our study, the largest difference between treatments in nitrogen concentration of roots was from 20-40 cm. Whereas, roots at 10 cm contained similar amounts of nitrogen suggesting that the difference seen at the lower depths is a result of excess nitrogen from effluent making it to lower depths and thus available to be absorbed. Roots at RICE showed evidence of nitrogen saturation at the 10 cm level, this aspect, along with the root mass differences at depth are reflective of common plant/ root morphology in high nitrogen environments (Fahey et al., 1994). Tung et al. (2009), indicated that there is a lag in the response of roots to changes in biomass and N concentration which can take months to years before it can be significant. Aber et al. (1998) described no changes in fine root biomass through 4 years of nitrogen addition but root nitrogen concentrations nearly doubled and fine root biomass increased significantly after the exclusion of fertilizer from a previous fertilized plot. Unfortunately, our study only had root samples from 1 point during the winter of 2016 -2017 taken 9 years after the installation of the system and there was no way to account for possible changes from pre and post application.

Significantly less root mass was measured at both sites in treatment plots at the 10 cm depth. This result correlates with a plants tendency to devote more resources to above ground growth instead of roots when nitrogen needs are met (Agricultural Service, 2013). The reduction in root mass below 10 cm is also explained well in literature as most tree roots are found in the top 18 cm of soil as these conditions provide the most favorable growing conditions with access to nutrients and water (Crow, 2005). These results provide evidence that the Treatment plot roots are responding to the elevated nutrient levels.

*Conclusion and Application*

Precipitation during the sampling period was highly influential on volume collected and thus nutrient fluxes. It was determined that throughfall and leachate were interconnected with amount of throughfall dictating leachate volume. There was no seasonal difference between summer and winter in nutrient outputs through leachate. In addition, effluent spray impacted concentrations at RICE but not VDGIF, although the effluent spray contributed minimally to overall volume at both sites. Therefore, if future studies look at forest responses to wastewater, the potential effects can be attributed to differences in nutrient loads not water loads. The throughfall concentrations observed at RICE are comparable to local WWTP concentrations, whereas VDGIF is more reflective of natural deposition. Most of the added N and P to the treatment plots at RICE was retained, indicating that the forest was effective at preventing downstream transport of the nutrients. Overall, the forest in the RICE treatment plots was effective at reducing nitrate concentrations, but the leachate was still enriched over control. In addition, roots at RICE showed evidence of nitrogen saturation in the upper layers of soil. There are unanswered questions associated with the large differences in nutrient concentrations of throughfall between RICE and VDGIF treatment plots. During the sampling period, there were multiple spray events observed at RICE but not VDGIF, these spray events are how the wastewater effluent reaches the treatment plots. The low throughfall fluxes measured at VDGIF are surprising given the higher values measured at RICE where the building occupancy is lower. While the daily occupancy of RICE may be lower, frequent large events at the Rice Education Building might be responsible for the greater wastewater load at this site. Even so, it would be useful to conduct dye studies at VDGIF and RICE to ascertain pathways and distribution of effluent discharge as there may be substantial performance differences between the two systems that need to be farther evaluated. The data arising from this study, with the addition of DBH and foliar N content, will contribute to a larger effort to characterize differences in wood production and C sequestration among these plots. Based on results of the study, in addition to, observations of spray events at the sites, one can conclude that the RICE system is functioning as expected with consistent spray observed and elevated levels of nutrients measured in throughfall with a significant reduction in leachate concentration. This suggests that these types of systems are viable in the Mid-Atlantic forests found at the Rice Rivers Center and are an improvement over direct discharge into a waterbody. However, with the difference seen between our two sampled systems, it is imperative that at least bi-annual testing of leachate and spray application is essential to verify performance specified by the manufacture.

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Table 1: ANOVA results relating variation in throughfall and lysimeter N and P fluxes to Treatment, Month and their interaction effects for plots located at the Rice building.

| | | Throughfall Flux kg ha $^{-1}$ mo$^{-1}$ | | | | | Lysimeter Flux kg ha $^{-1}$ mo$^{-1}$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Ind Variable** | **Df** | **Sum Sq** | **F value** | **p value** | **$R^2$** | **Df** | **Sum Sq** | **F value** | **p value** | **$R^2$** |
| | *Treatment* | 1 | 48.2 | 116.4 | < 0.001 | 0.27 | 1 | 4.1 | 2.9 | 0.106 | 0.06 |
| | *Month* | 9 | 61.8 | 16.6 | < 0.001 | 0.35 | 9 | 18.3 | 1.4 | 0.244 | 0.29 |
| ***$NO_x$*** | *Treatment:Month* | 9 | 58.8 | 15.8 | < 0.001 | 0.33 | 9 | 12.8 | 1.0 | 0.476 | 0.20 |
| | *Residuals* | 20 | 8.2 | | | | 20 | 28.6 | | | |
| | *Total* | | 177 | | | 0.95 | | 63.8 | | | 0.55 |
| | *Treatment* | 1 | 0.2 | 1.97 | 0.176 | 0.02 | 1 | 0.2 | 1.6 | 0.223 | 0.03 |
| | *Month* | 9 | 6.0 | 8.36 | < 0.001 | 0.62 | 9 | 4.9 | 5.3 | < 0.001 | 0.66 |
| ***$NH_3$*** | *Treatment:Month* | 9 | 1.9 | 2.62 | 0.035 | 0.20 | 9 | 0.3 | 0.3 | 0.951 | 0.04 |
| | *Residuals* | 20 | 1.6 | | | | 20 | 2.0 | | | |
| | *Total* | | 9.7 | | | 0.84 | | 7.4 | | | 0.73 |
| | *Treatment* | 1 | 2.9 | 158.3 | < 0.001 | 0.42 | 1 | 0.002 | 2.2 | 0.156 | 0.06 |
| | *Month* | 9 | 2.0 | 12.2 | < 0.001 | 0.29 | 9 | 0.015 | 2.1 | 0.075 | 0.42 |
| ***$PO_4$*** | *Treatment:Month* | 9 | 1.6 | 9.3 | < 0.001 | 0.23 | 9 | 0.004 | 0.6 | 0.771 | 0.11 |
| | *Residuals* | 20 | 0.4 | | | | 20 | 0.015 | | | |
| | *Total* | | 6.9 | | | 0.94 | | 0.036 | | | 0.58 |
| | *Treatment* | 1 | 185.5 | 222.8 | < 0.001 | 0.27 | 1 | 9.1 | 5.6 | 0.028 | 0.09 |
| | *Month* | 9 | 254.8 | 34.0 | < 0.001 | 0.36 | 9 | 35.3 | 2.4 | 0.048 | 0.35 |
| ***TN*** | *Treatment:Month* | 9 | 241.5 | 32.2 | < 0.001 | 0.35 | 9 | 23.0 | 1.6 | 0.189 | 0.23 |
| | *Residuals* | 20 | 16.7 | | | | 20 | 32.4 | | | |
| | *Total* | | 698.5 | | | 0.98 | | 99.8 | | | 0.68 |
| | *Treatment* | 1 | 3.0 | 188.3 | < 0.001 | 0.41 | 1 | 0.005 | 9.0 | 0.007 | 0.06 |
| | *Month* | 9 | 2.3 | 16.2 | < 0.001 | 0.32 | 9 | 0.044 | 8.0 | < 0.001 | 0.56 |
| ***TP*** | *Treatment:Month* | 9 | 1.7 | 11.7 | < 0.001 | 0.23 | 9 | 0.017 | 3.2 | 0.015 | 0.22 |
| | *Residuals* | 20 | 0.3 | | | | 20 | 0.012 | | | |
| | *Total* | | 7.3 | | | 0.96 | | 0.078 | | | 0.85 |

Table 2: ANOVA results relating variation in throughfall and lysimeter N and P fluxes to Treatment, Month and their interaction effects for plots located at the VDGIF building.

| | | Throughfall Flux kg $ha^{-1}$ $mo^{-1}$ | | | | | Lysimeter Flux kg $ha^{-1}$ $mo^{-1}$ | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Ind Variable** | **Df** | **Sum Sq** | **F value** | **p value** | **$R^2$** | **Df** | **Sum Sq** | **F value** | **p value** | **$R^2$** |
| | *Treatment* | 1 | 0.01 | 0.1 | 0.998 | 0.01 | 1 | 2.50 | 4.7 | 0.037 | 0.05 |
| | *Month* | 9 | 0.18 | 18.3 | < 0.001 | 0.78 | 9 | 19.63 | 4.1 | < 0.001 | 0.40 |
| ***$NO_x$*** | *Treatment:Month* | 9 | 0.01 | 1.2 | 0.304 | 0.04 | 9 | 4.95 | 1.0 | 0.438 | 0.10 |
| | *Residuals* | 40 | 0.04 | | | | 40 | 21.46 | | | |
| | *Total* | | 0.23 | | | 0.83 | | 48.54 | | | 0.56 |
| | *Treatment* | 1 | 0.62 | 1.3 | 0.254 | 0.02 | 1 | 0.01 | 0.1 | 0.712 | 0.00 |
| | *Month* | 9 | 15.20 | 3.7 | 0.002 | 0.39 | 9 | 1.08 | 5.0 | < 0.001 | 0.46 |
| ***$NH_3$*** | *Treatment:Month* | 9 | 4.50 | 1.1 | 0.397 | 0.12 | 9 | 0.28 | 1.3 | 0.261 | 0.12 |
| | *Residuals* | 40 | 18.46 | | | | 40 | 1.00 | | | |
| | *Total* | | 38.81 | | | 0.52 | | 2.33 | | | 0.59 |
| | *Treatment* | 1 | 0.01 | 0.3 | 0.606 | 0.01 | 1 | 0.01 | 2.7 | 0.106 | 0.05 |
| | *Month* | 9 | 0.30 | 2.5 | 0.022 | 0.32 | 9 | 0.12 | 11.3 | < 0.001 | 0.60 |
| ***$PO_4$*** | *Treatment:Month* | 9 | 0.11 | 1.0 | 0.453 | 0.12 | 9 | 0.02 | 2.1 | 0.05 | 0.10 |
| | *Residuals* | 40 | 0.51 | | | | 40 | 0.05 | | | |
| | *Total* | | 0.92 | | | 0.45 | | 0.20 | | | 0.75 |
| | *Treatment* | 1 | 0.68 | 1.6 | 0.216 | 0.02 | 1 | 3.95 | 4.5 | 0.041 | 0.04 |
| | *Month* | 9 | 17.38 | 4.5 | < 0.001 | 0.44 | 9 | 60.51 | 7.6 | < 0.001 | 0.55 |
| ***TN*** | *Treatment:Month* | 9 | 4.33 | 1.1 | 0.369 | 0.11 | 9 | 10.00 | 1.3 | 0.292 | 0.09 |
| | *Residuals* | 40 | 17.10 | | | | 40 | 35.26 | | | |
| | *Total* | | 39.49 | | | 0.57 | | 109.67 | | | 0.68 |
| | *Treatment* | 1 | 0.00 | 0.1 | 0.726 | 0.01 | 1 | 0.01 | 1.6 | 0.211 | 0.06 |
| | *Month* | 9 | 0.55 | 4.4 | < 0.001 | 0.44 | 9 | 0.11 | 10.9 | < 0.001 | 0.65 |
| ***TP*** | *Treatment:Month* | 9 | 0.13 | 1.0 | 0.445 | 0.10 | 9 | 0.01 | 0.7 | 0.673 | 0.06 |
| | *Residuals* | 40 | 0.55 | | | | 40 | 0.04 | | | |
| | *Total* | | 1.20 | | | 0.56 | | 0.17 | | | 0.76 |

Table 3: ANOVA results relating variation in root chemistry of control and treatment plots to Treatment, Depth and their interaction effects for plots located at the RICE and VDGIF.

| | | RICE | | | | | VDGIF | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Ind Variable** | **Df** | **Sum Sq** | **F value** | **p value** | **$R^2$** | **Df** | **Sum Sq** | **F value** | **p value** | **$R^2$** |
| **% N** | *Treatment* | 1 | 0.03 | 5.4 | 0.048 | 0.08 | 1 | 0.08 | 4.5 | 0.051 | 0.12 |
| | *Depth* | 3 | 0.26 | 17.5 | < 0.001 | 0.76 | 3 | 0.25 | 4.6 | 0.017 | 0.38 |
| | *Treatment:Depth* | 3 | 0.01 | 1 | 0.456 | 0.03 | 3 | 0.04 | 0.8 | 0.506 | 0.06 |
| | *Residuals* | 8 | 0.04 | | | | 16 | 0.29 | | | |
| | *Total* | | 0.34 | | | 0.87 | | 0.66 | | | 0.56 |
| **RM** | *Treatment* | 1 | 12999 | 10.5 | 0.012 | 0.07 | 1 | 3768 | 8.4 | 0.01 | 0.02 |
| | *Depth* | 3 | 153845 | 41.2 | < 0.001 | 0.82 | 3 | 146403 | 108.4 | < 0.001 | 0.91 |
| | *Treatment:Depth* | 3 | 9714 | 2.6 | 0.124 | 0.05 | 3 | 3212 | 2.4 | 0.108 | 0.02 |
| | *Residuals* | 8 | 9950 | | | | 16 | 7201 | | | |
| | *Total* | | 186508 | | | 0.94 | | 160584 | | | 0.95 |
| **g of C** | *Treatment* | 1 | 838 | 5.3 | 0.051 | 0.11 | 1 | 60 | 0.4 | 0.524 | 0.01 |
| | *Depth* | 3 | 5286 | 11.2 | 0.003 | 0.67 | 3 | 10398 | 24.4 | < 0.001 | 0.8 |
| | *Treatment:Depth* | 3 | 484 | 1 | 0.432 | 0.06 | 3 | 243 | 0.6 | 0.643 | 0.02 |
| | *Residuals* | 8 | 1261 | | | | 16 | 2272 | | | |
| | *Total* | | 7869 | | | 0.84 | | 12973 | | | 0.83 |
| **g of N** | *Treatment* | 1 | 0.28 | 4.6 | 0.064 | 0.03 | 1 | 0.03 | 0.5 | 0.508 | 0.01 |
| | *Depth* | 3 | 9.25 | 51 | < 0.001 | 0.86 | 3 | 10.91 | 48.6 | < 0.001 | 0.89 |
| | *Treatment:Depth* | 3 | 0.73 | 4 | 0.051 | 0.07 | 3 | 0.07 | 0.3 | 0.816 | 0.01 |
| | *Residuals* | 8 | 0.48 | | | | 16 | 1.2 | | | |
| | *Total* | | 10.74 | | | 0.96 | | 12.21 | | | 0.91 |
| **C:N** | *Treatment* | 1 | 936 | 5.1 | 0.055 | 0.14 | 1 | 103 | 0.8 | 0.392 | 0.01 |
| | *Depth* | 3 | 3876 | 7 | 0.013 | 0.59 | 3 | 5494 | 13.7 | < 0.001 | 0.68 |
| | *Treatment:Depth* | 3 | 234 | 0.4 | 0.743 | 0.04 | 3 | 337 | 0.8 | 0.491 | 0.04 |
| | *Residuals* | 8 | 1481 | | | | 16 | 2135 | | | |
| | *Total* | | 6527 | | | 0.77 | | 8069 | | | 0.73 |

Table 4: Range of nitrogen concentration from RICE and VDGIF throughfall, National Atmospheric Deposition Program regional ranges and throughfall concentrations from control plots. All constitutes are described in mg $L^{-1}$.

| *Constituent* | *RICE Treatment* | *VDGIF Treatment* | *NADP* | *RICE-VDGIF Control* |
|---|---|---|---|---|
| $NO_x$ | 1.0 – 11.0 | 0.1 – 1.0 | 0.4 – 0.8 | 0.1 – 0.7 |
| $NH_3$ | 0.2 – 2.0 | 0.1 – 4.0 | 0.2 - 0.5 | 0.1 – 4.0 |
| TN | 1.0 – 16.0 | 0.5 – 5.0 | 0.8 - 1.3 | 0.2 – 6.0 |

Table 5: Comparison of lysimeter leachate nutrient concentration ranges from RICE, VDGIF, published USGS groundwater ranges for the Mid-Atlantic and control plots. All constitutes are described in mg $L^{-1}$ .

| *Constituent* | *RICE Treatment* | *VDGIF Treatment* | *USGS* | *Control Leachate* |
|---|---|---|---|---|
| $NO_x$ | 0.5- 4.7 | 0.4 – 1.2 | 1.8 – 2.2 | 0.1 - 1.7 |
| $NH_3$ | 0.1 – 2.4 | 0.1 – 0.7 | *NA* | 0.1 – 1.2 |
| $PO_4$ | 0.01-0.07 | 0.01 – 0.1 | 0.02 – 0.04 | 0.01 – 0.04 |
| TN | 0.1 – 4.3 | 0.6 – 5.4 | *NA* | 0.1 – 1.6 |
| TP | 0.03 – 0.11 | 0.01 – 0.06 | *NA* | 0.01 – 0.06 |


Figure 1: Overlook of sites at the Rice Rivers Center property with plots labeled and the outer ring specifying the area of influence from the effluent sprayers. RICE plots are 1, 2, R1, R2 (bottom left) and VDGIF plots 3, 4, 5, R3, R4, R5 (upper right).

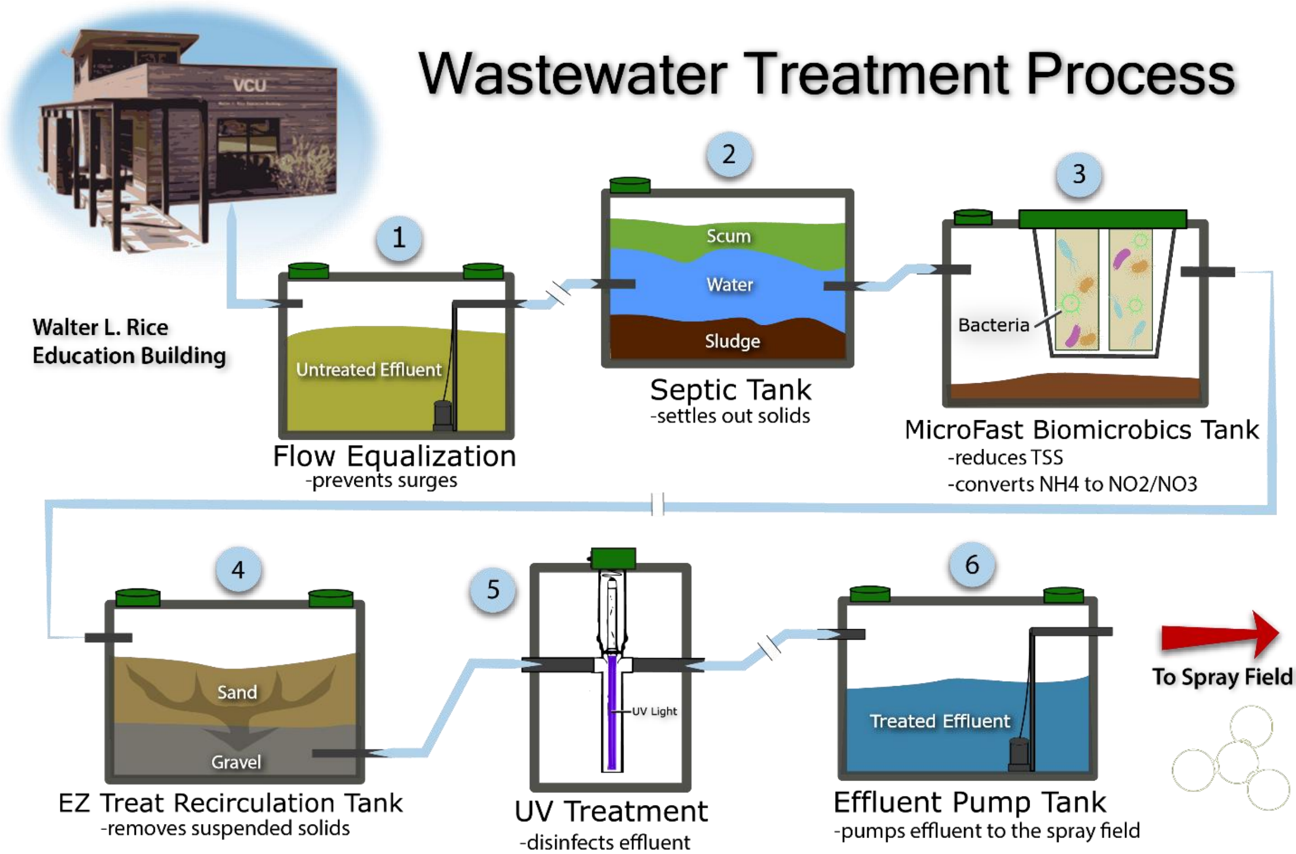


Figure 2: Illustrated wastewater treatment process at the Rice Education and the VDGIF buildings.

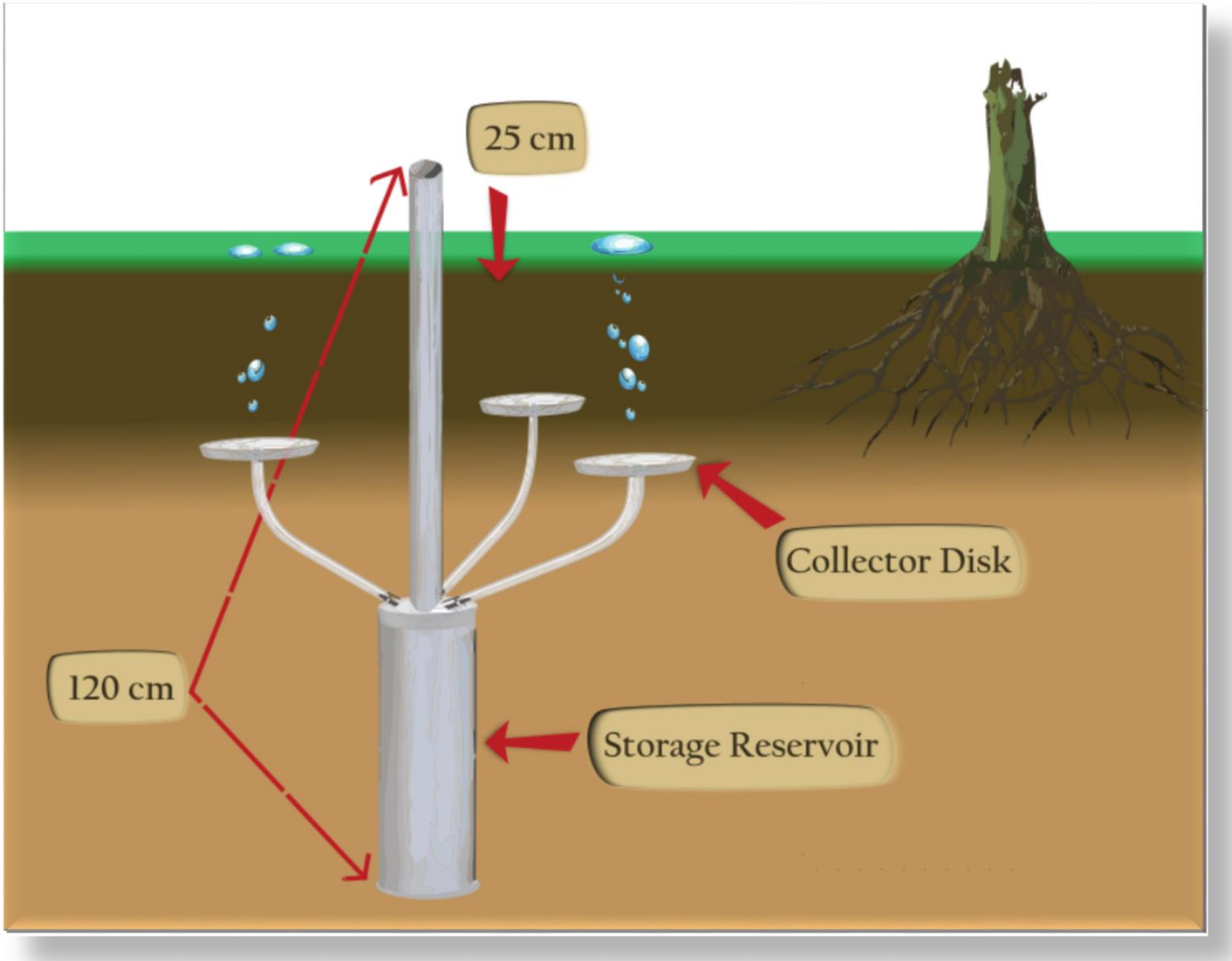


Figure 3: Illustrated example of the lysimeter design with dimensions.

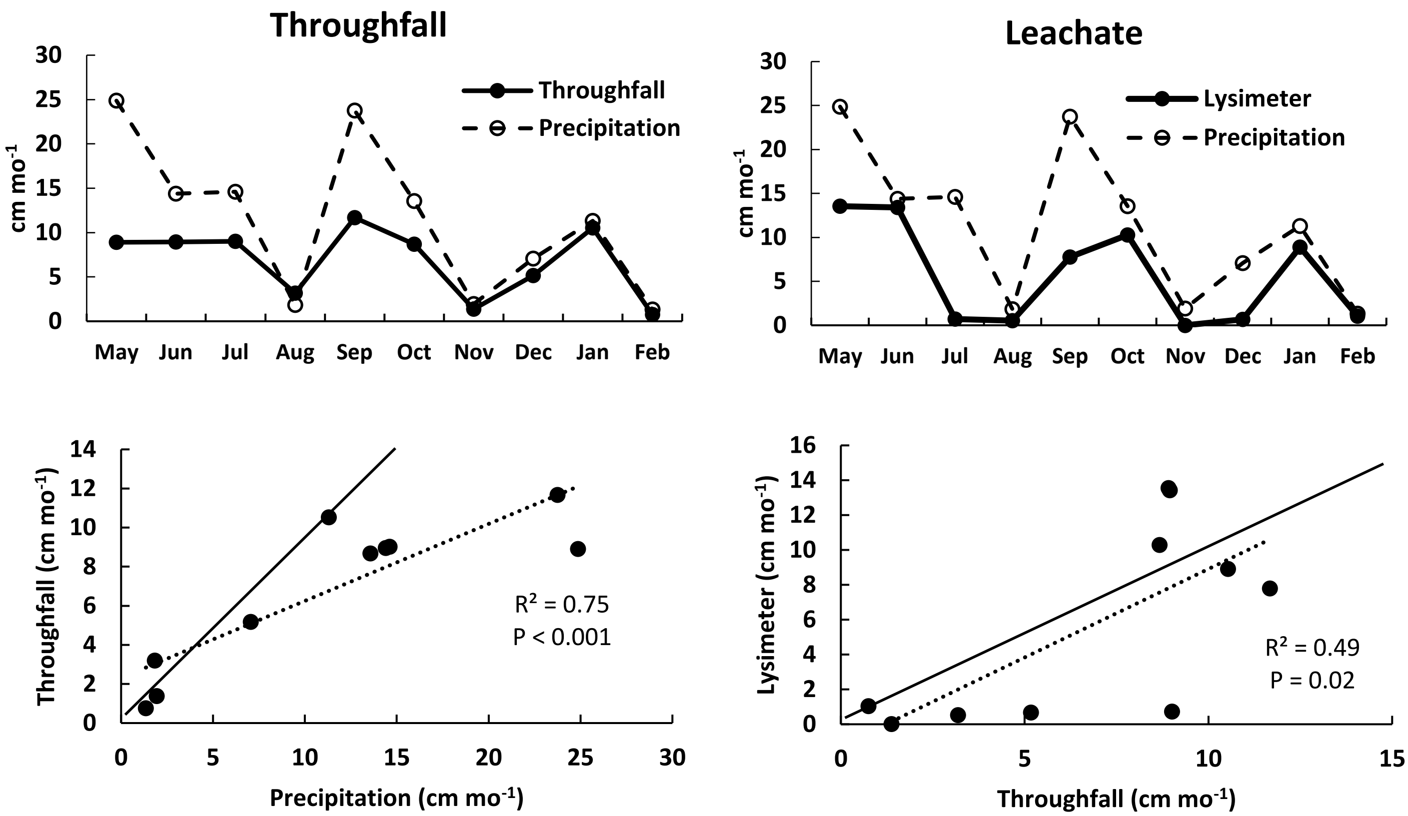


Figure 4: Time series and regression plots showing the monthly mean volume of throughfall and lysimeter control and treatment collectors in comparison to total monthly precipitation at RICE throughout May 2016-Feb 2017. Precipitation data are from the NOAA Regional Climate Center Station at Hopewell, VA.

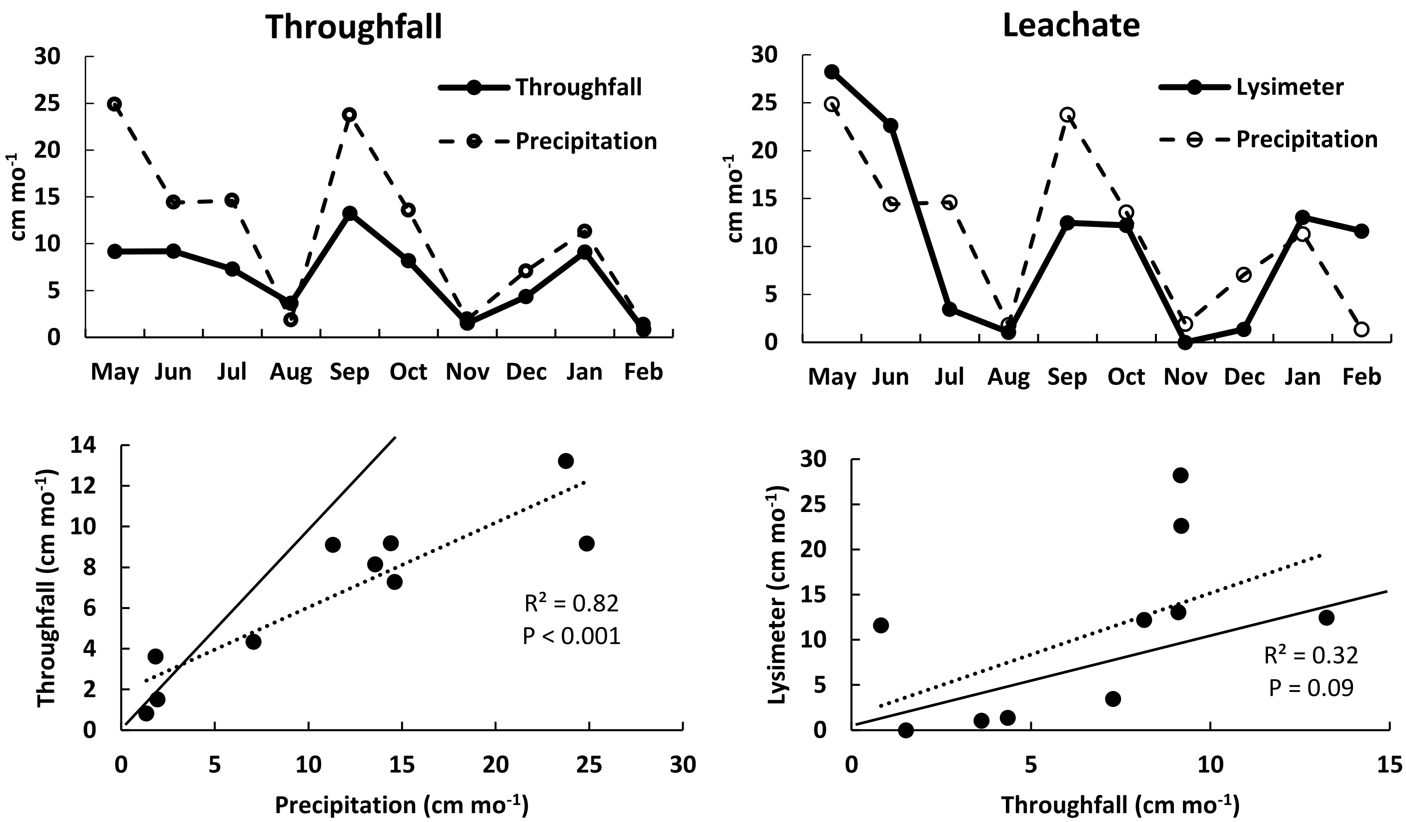


Figure 5: Time series and regression plots showing the total monthly mean volume of throughfall and lysimeter control and treatment collectors across replicate plots in comparison to total monthly precipitation at VDGIF throughout May 2016- Feb 2017. Precipitation data are from the NOAA Regional Climate Center Station at Hopewell, VA.

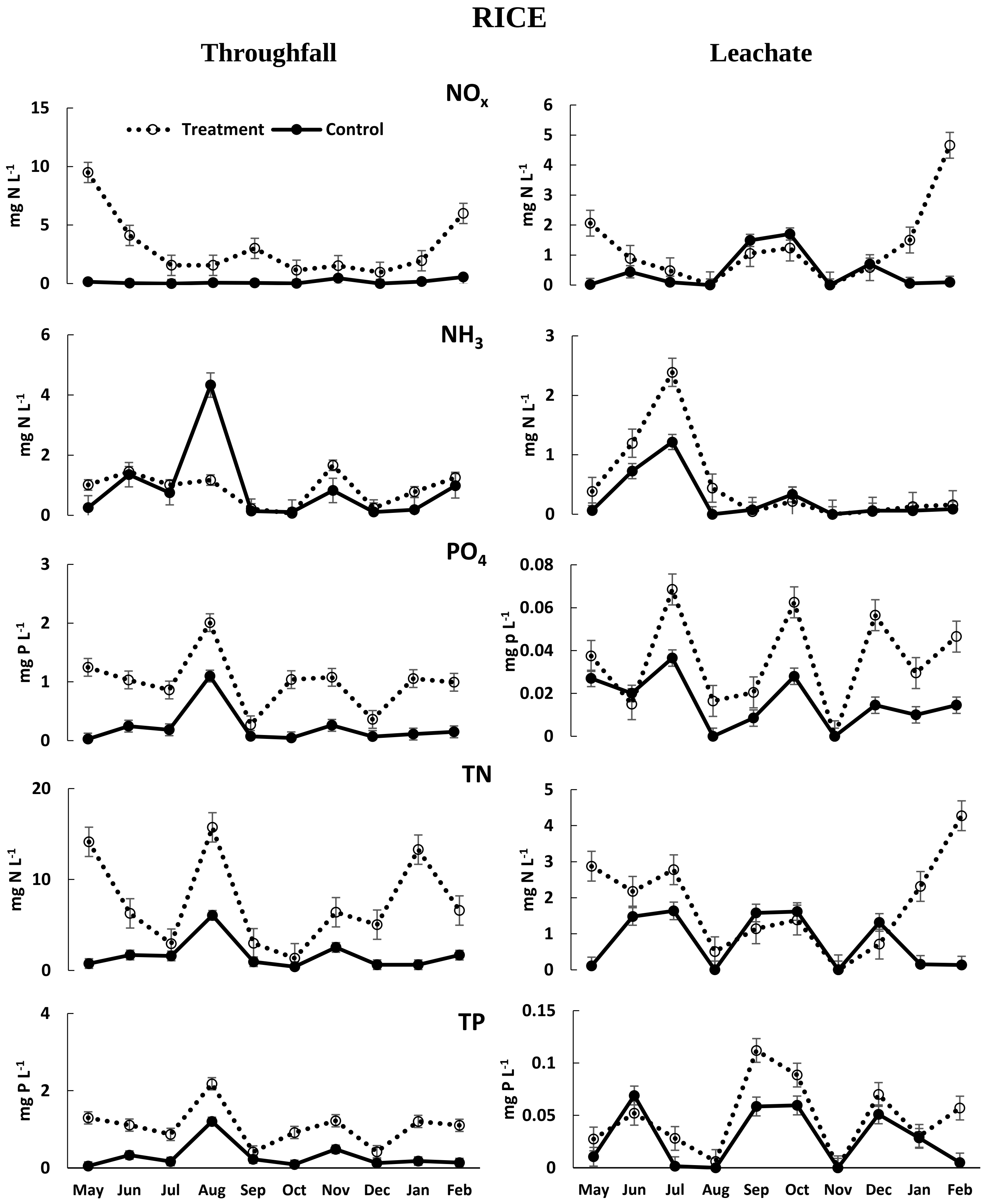


Figure 6: Nutrient concentrations in throughfall and lysimeter samples at RICE Control and Treatment plots throughout May 2016- Feb 2017. Data shown are monthly means across replicate plots with error bars indicating standard error.

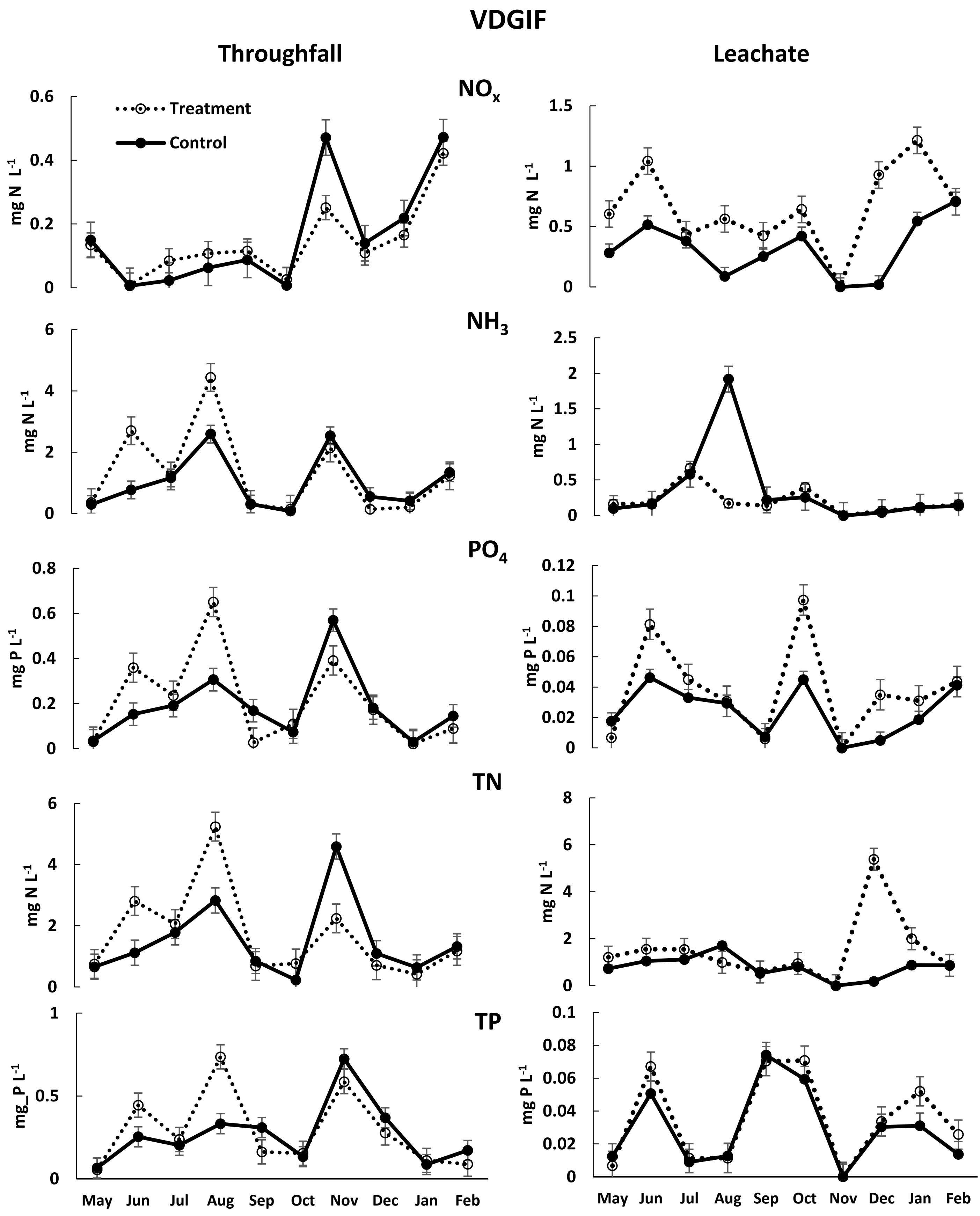


Figure 7: Nutrient concentrations in throughfall and lysimeter samples at VDGIF throughout May 2016- Feb 2017. Data shown are monthly means across replicate plots with error bars indicating standard error.

# RICE

## Throughfall

## Leachate

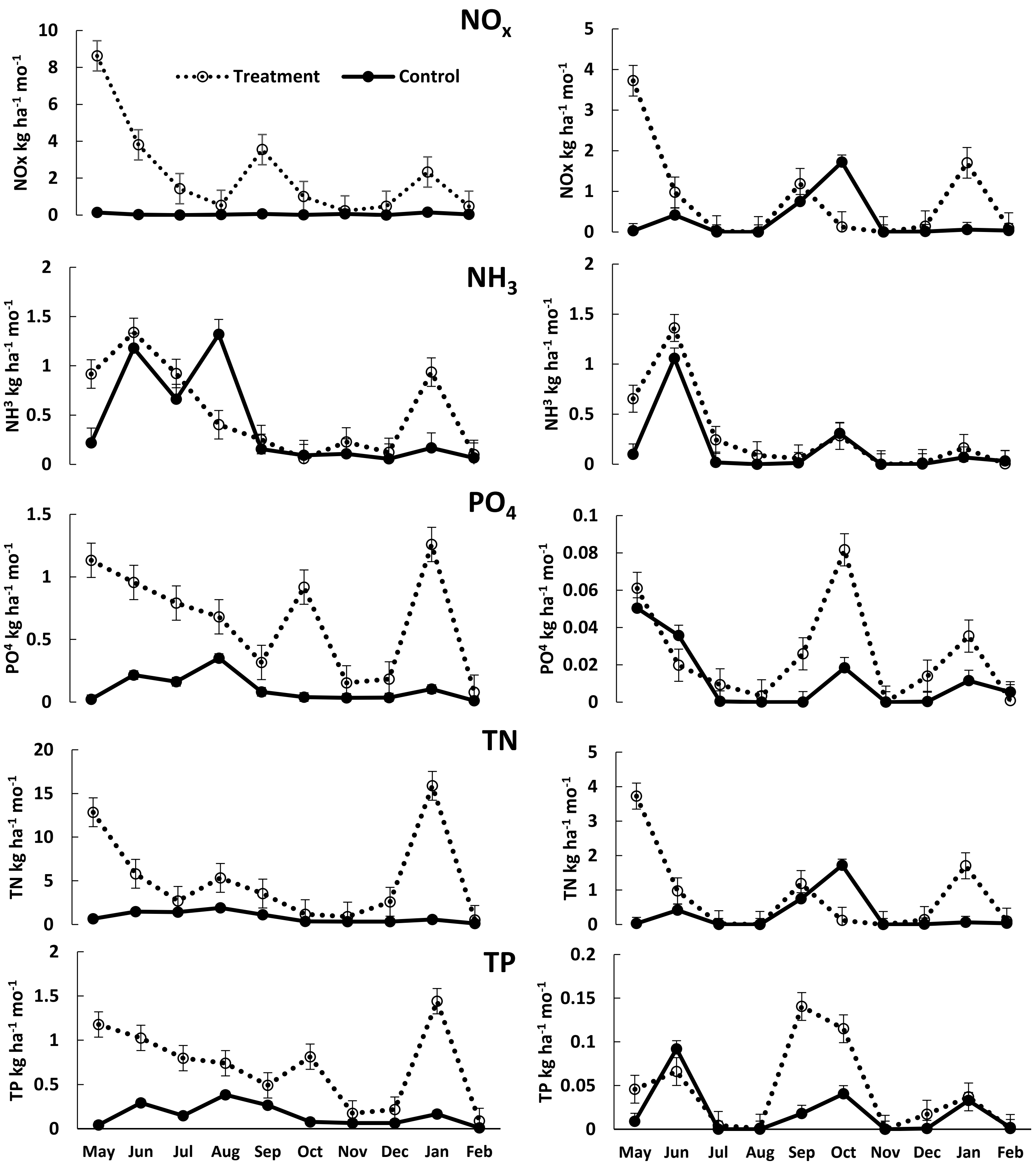


Figure 8: RICE treatment and control plots mean nutrient fluxes from Throughfall (nutrient load) and Lysimeter (nutrient loss) for May 2016 – Febuary 2017 with error bars describing standard error.

# VDGIF

## Throughfall

## Leachate

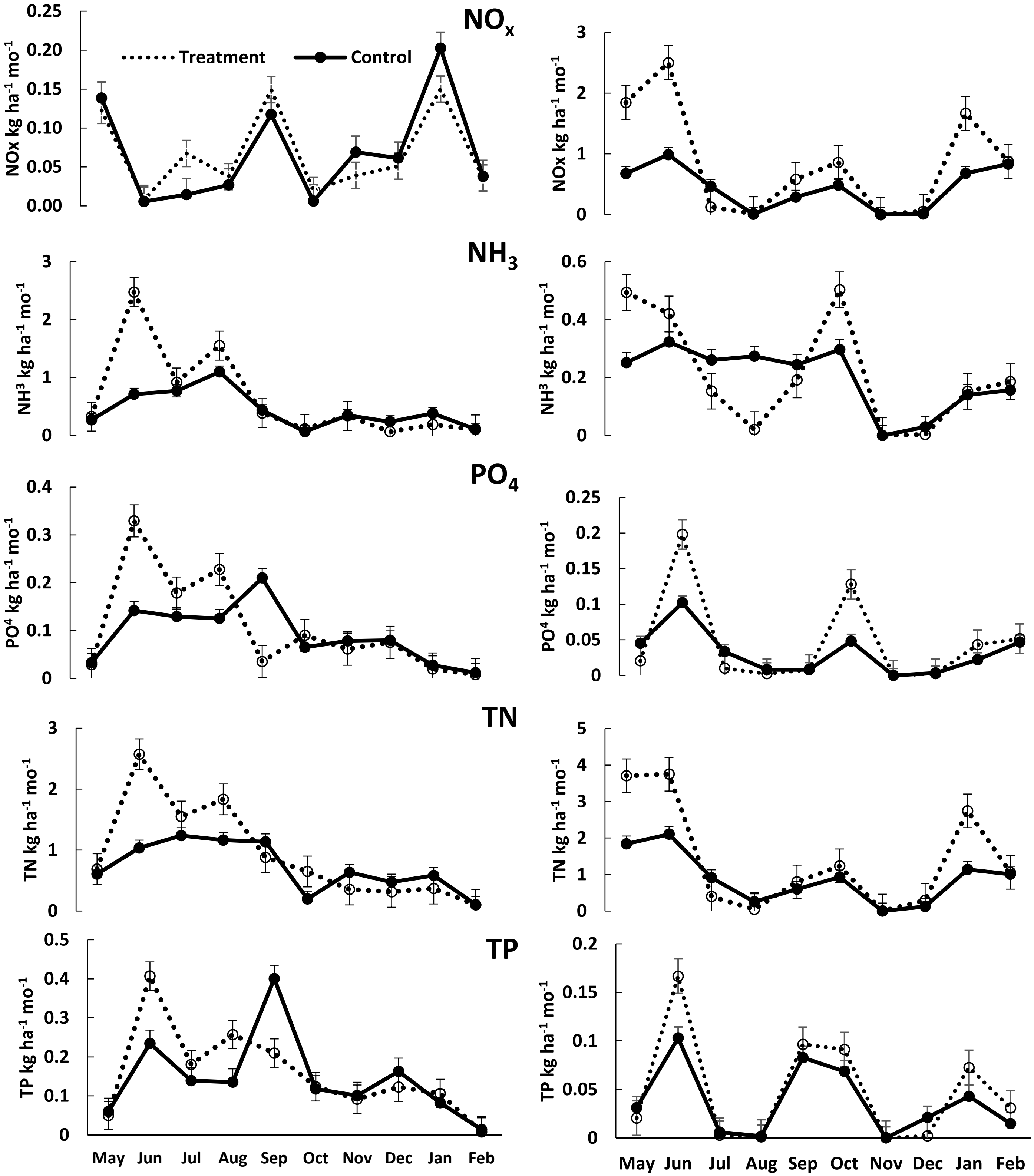


Figure 9: VDGIF treatment and control plots mean nutrient fluxes from Throughfall (nutrient load) and Lysimeter (nutrient loss) for May 2016 – Febuary 2017 with error bars describing standard error.

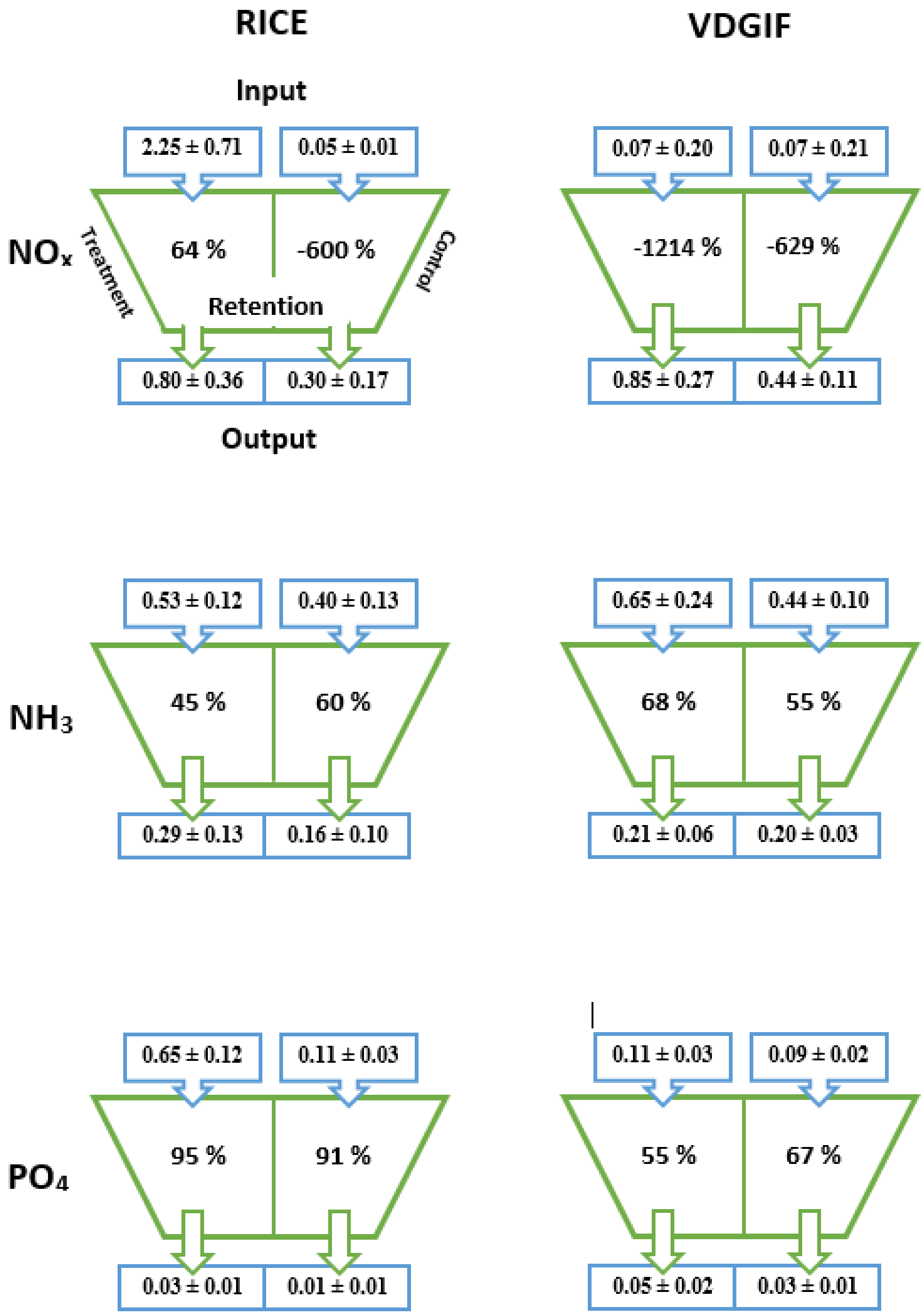


Figure 10: Percentage of nutrients retained by the ecosystem derived from the annual mean quantity of nutrient inputs and outputs to control and treatment plots at RICE and VDGIF from May 2016 – Febuary 2017. Values indicate ka $ha^{-1}$ $mo^{-1}$ and ± indicates standard error.

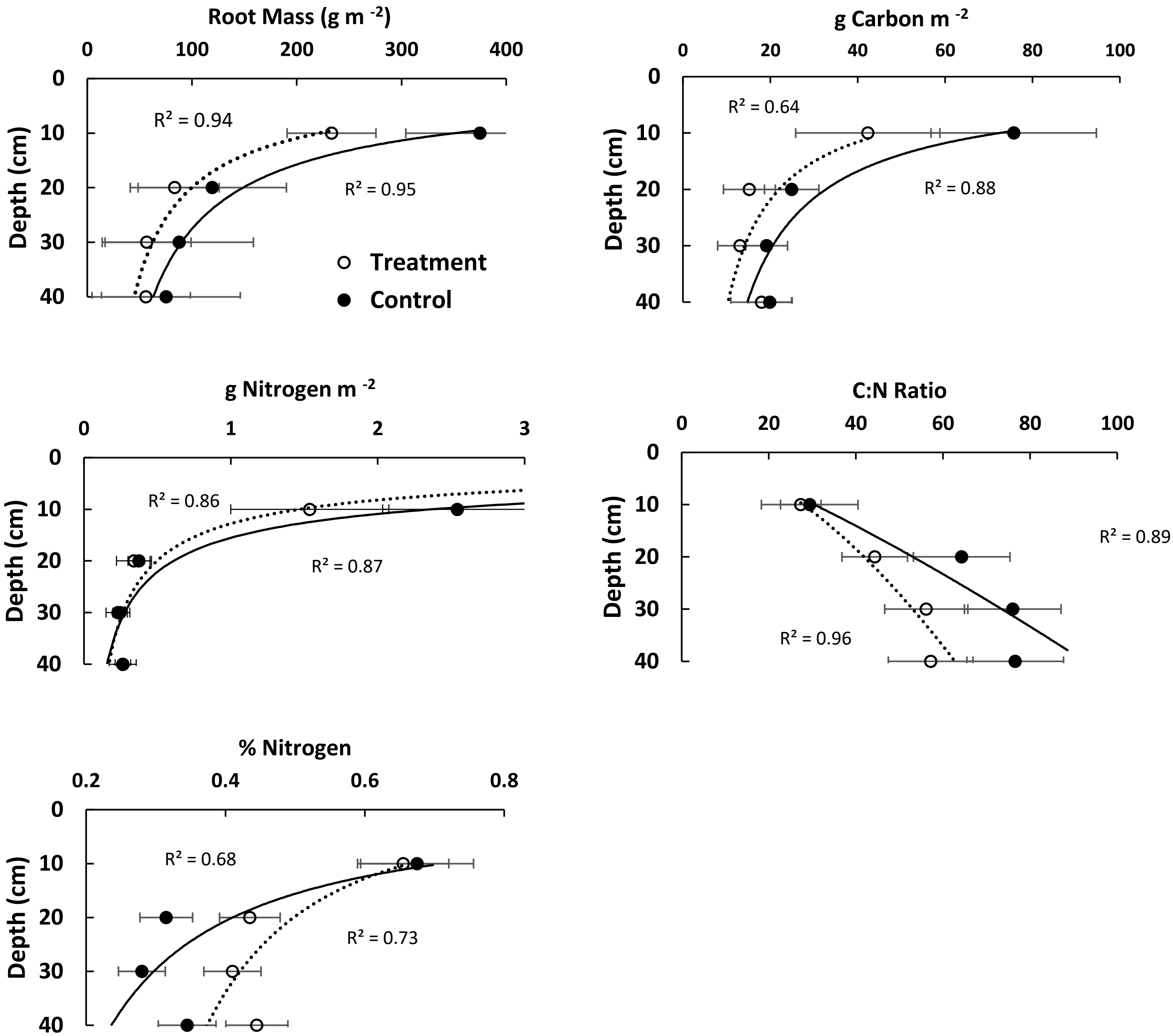


Figure 11: Root mass, C and N in relation to soil depth at RICE treatment and control plots. Data are mean values based on 5 replicate cores taken from Control and Treatment plots. All regressions are statistically significant at $P < 0.05$ with error bars showing standard error.

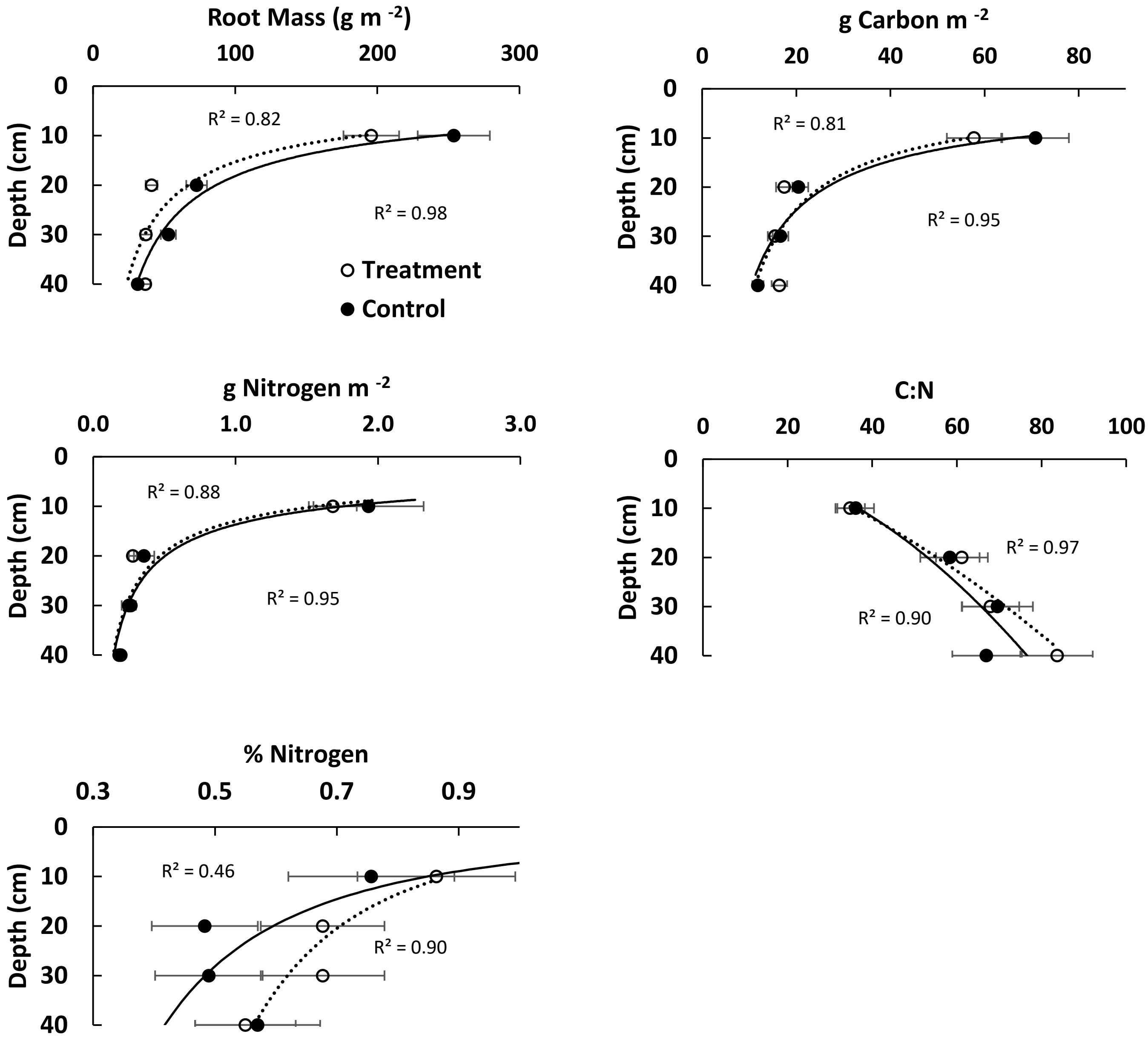


Figure 12: Elemental concentrations of root samples from VDGIF plots in relation to soil depth. Data are mean values based on 5 replicate cores taken from Control and Treatment plots. All regressions are statistically significant at $P < 0.05$ with error bars showing standard error.