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Viability of Wetland Crops for Use in Treatment Wetlands: Nitrogen Removal from Water and Production of Food

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VIABILITY OF WETLAND CROPS FOR USE IN TREATMENT WETLANDS:

NITROGEN REMOVAL FROM WATER AND PRODUCTION OF FOOD

A Thesis

by

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ABSTRACT

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Treatment wetlands are used to treat wastewater from a variety of sources, but their functionality depends on the macrophytes present therein. To better understand the viability of wetland macrophytes both as sources of food and as agents of nitrogen removal from wastewater, this study quantified plant growth, food production, and nitrogen removal capacity of three common wetland crops as well as three locally dominant graminoid species in a variety of relevant ecological contexts. All six plant species and a control were grown over a ten-week period in three related experiments: (1) under three moisture regimes, (2) with or without competition with *Lemna minor*, and (3) under three water cycling regimes. We used repeated measures ANOVAs to examine differences in effluent nitrogen levels among treatments and permutational ANOVAs to evaluate effects of treatments on biomass. We found significant differences between species and across treatments in the macrophytes' filtration and food functions.

DEDICATION

I dedicate this work to my family, friends, and all of my former educators.

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CHAPTER I

INTRODUCTION

Global Needs and Human Population

To adapt to the current and future challenges humanity faces, it is critical that we as a species promote sustainability in all our endeavors. Preparing proper pathways forward that can ensure the highest quality of life for as many people for as long as possible is no small task, and we must consider not only our greatest obstacles, but also the unintended impacts of our attempts to meet this end. To improve human wellbeing now and in the future, a primary focus must be the management of resources. This is foundational to the success of all industries and essential to meeting our basic biological needs. There is a legitimate call for urgency, because the way we manage these resources is fundamentally dependent upon our population, and it is rising rapidly. As it stands now, the worldwide human population is at roughly 7.5 billion, by 2050 that number is projected to rise to 9.8 billion, and by 2100 we can expect a population of more than 11 billion (United Nations, 2017).

The growth that we have experienced during the last century, and that which is to come in the next, is due primarily to technological innovations across fields that directly impact human wellbeing, such as energy production and distribution, medicine, food and agriculture, housing, access to education, etc. Because of these advances, globally, life expectancy has drastically risen (United Nations, 2017), infant mortality has sharply declined (United Nations Population

Division, 2017), and poverty rates have dropped substantially (Roser & Ortiz-Ospina, 2018; World Bank, 2018). While these metrics do indicate marked improvements for the human population at large, the fact remains that there are still large numbers of people incapable of meeting many of their basic needs. There are around 705 million people worldwide that earn no more than \$1.90 US per day who are considered in extreme poverty (Bourguignon & Morrisson, 2002; Roser & Ortiz-Ospina, 2018; World Bank, 2018), or 10% of the total population. Therefore, it is important that we push to make meeting these needs more possible by increasing accessibility to necessities.

Water

While there are myriad resources to consider in developing a sustainable future, one of, if not the most essential of these, is water - in particular, freshwater. Freshwater is a finite resource, comprising only 3.46% of all water on earth. Of that freshwater, 68.7% (24 million km^3) is locked up in glaciers, and another 30.1% (10.5 million km^3) exists as groundwater, leaving the final 1.2% (4.35×10^5 km^3) as atmosphere, biosphere, and surface water. Moreover, of that surface water, only 24% (1.05×10^5 km^3) – 0.29% of all freshwater or 0.014% of all water on Earth – is found in lakes, river, swamps and marshes (Gleick, 1993). Given the scarcity of freshwater, in tandem with its necessity for human civilization and heavy use for domestic and municipal purposes, agricultural irrigation, and industry, it is in humanity's best interest to conserve this vital resource. However, over the past century, as the demand for freshwater has grown, and so too has the rate at which we consume it; in the last decade, the global rate of freshwater consumption was nearly 4,000 km^3/year (Flörke et al., 2013; Ritchie & Roser, 2018).

It should be noted that these metrics consider freshwater taken from both ground and surface waters, as well as consumption across industrial, agricultural, and municipal uses. Water consumption is not homogeneous throughout the world because some regions have more available water (Ritchie & Roser, 2018). Availability is affected by many factors, such as precipitation patterns, evaporation rates, substrate quality, temperature, and the existence of aquifers. For example, many countries in northern Africa and the Middle East are water scarce, meaning their water availability is less than 1000 m^3/year per capita (Pimentel et al., 1997). In the case of Egypt, due to extremely low precipitation and high evaporation, per capita water availability has been as low as 40 m^3/year (Pimentel et al., 1997). In the US, certain regions are more stressed than others, but because of groundwater use, river diversion, and dams, many otherwise uninhabitable places flourish. While this has many short-term benefits, prolonged and excessive withdrawals can deplete aquifers thereby leading to large scale crop failures or municipal water crises (Pimentel et al., 1997).

Agriculture, Water, & Nitrogen Fertilizer

Of the three main uses, agriculture is the largest freshwater consumer, representing approximately 70% of freshwater consumption worldwide, although the percentages range significantly between countries (Ritchie & Roser, 2018). Agricultural water use is heavily dependent on the types of crops being grown, as each species has specific requirements for proper development. Livestock production requires substantially more water, because feed, like hay or grain, must be grown to provide nutrition to the animals plus the water they need to drink to survive. Of all food production, beef consumes the most water by far; in the US, 1 kg of beef requires up to 100 m^3 of water to produce, most of which is used to grow feed (Rosegrant et al., 2009). Demand for beef and other meats are rising worldwide, given that more people are being brought out of poverty, likely resulting in the producers of animal stock increasing the supply in response, thereby further stressing water availability. Increased meat production also requires more arable land to grow feed for these animals. Nonetheless, modern agricultural techniques have been able to reduce global hunger rates substantially over the past 50 years, which saw a doubling of production over that time (Godfray et al., 2010).

However, the methods used to achieve this increased production are not sustainable and have created negative environmental externalities that are being felt across the world that threaten to reduce future production (Hanjra & Qureshi, 2010; Rockström et al., 2007). Many food producers have had to focus on achieving the highest yields as possible rather than developing sustainable practices. As a result, large scale energy- and water-intensive industrial farming has become the norm in developed countries like the United States. The U.S. has 915 million acres (370 million ha) of farmland, roughly 2.1 million farms and ranches, occupying 40.5% of the country's total land area, and 42.6% of that is considered cropland, with 50% of all crops produced being corn or soybean (USDA, 2014). Popular production methods such as monoculture, in which a single crop is grow year after year, as well as flood irrigation both lead to soil degradation (Diacono & Montemurro, 2010; Rosegrant et al., 2009), to combat this issue producers must use large amounts of fertilizers to account for the lack of soil nutrients. Between 2015 and 2016, 97% of corn producing land, or 83 million acres (33.6 million ha), received nitrogen fertilizer at a rate of 145 pounds per acre (162.5 kg/ha), which totals 12.2 billion pounds (5.5 billion kg) of nitrogen applied in a single year (USDA, 2017). In the case of US soybean production between 2016 and 2017, for 31% of dedicated soybean cropland, or 25.6 million acres (10.4 million ha), nitrogen was applied at 18 pounds per acre (20.2 kg/ha), for a total of 468.3 million pounds (212.4 million kg) (USDA, 2018). These are the rates of nitrogen application for only two crops in only one year, however the use of nitrogen fertilizers has been a common practice in the US for around a century (Galloway et al., 2003).

This century of heavy nitrogen application was triggered, in large part, by the Haber-Bosch process invented in 1910, which converts N_2 – a non-reactive form of nitrogen and the most abundant molecule in the atmosphere – into ammonia, NH_3, a biologically reactive form of nitrogen which can be used as a fertilizer. This process and its widespread adoption is, in large part, responsible for the dramatic rise in population since the beginning of the 20th century, which was then 1.6 billion, by enabling a world-wide boost in agricultural production thanks to the increased availability of nitrogen fertilizer (Smil, 1999). However, the rate of production of reactive nitrogen has begun to reach a point of excess. Since 1970 there has been a 120% increase in reactive nitrogen production (Galloway et al., 2008), and this compounds with the fact that only half of the nitrogen fertilizer applied is actually taken up by crops (Cao et al., 2018). As a result, vast amounts of unused fertilizer run off into nearby water bodies that eventually make their way to the ocean, causing damage to ecosystems and human health in the process.

Water Pollution, Eutrophication, and Disease

One of the greatest impacts made on aquatic ecosystems by the presence of excess nitrogen is eutrophication. Water bodies subjected to high nutrient inputs undergo dramatic changes, marked by a spike in a phytoplankton, such as algae blooms, which subsequently decomposes, thereby greatly increasing biological oxygen demand (BOD), commonly resulting in hypoxic and even anoxic conditions often leading to the death of submerged plants, oxygen sensitive invertebrates, and fishes. The effects of this process impact food webs and ecosystem processes, and can destroy once reliable fisheries, with dire implications for those that depend on these systems– especially those in poverty or subsistence fishermen, who lose access to valuable sources of protein (Rosegrant et al., 2009).

While waterborne diseases are rare in most developed countries, that does not imply that we have solved the issues of water pollution and eutrophication. Some 41% of US freshwater bodies have been listed as “poor” quality in terms of nitrogen (US EPA, 2016), and a major concern – apart from the ecological ones discussed above – is the potential adverse human health effects brought on by drinking water high in nitrate, such as cancer or reproductive complications (Galloway et al., 2008). If nothing is done to properly manage nutrient pollution in our waterways, it is possible that people living in developed countries like the US could experience mortally harmful consequences of unmitigated water pollution. Fortunately, we know that the most effective solution for treating a water body that has undergone anthropogenic eutrophication is to decrease the nutrient input into that system(Lewtas et al., 2015), as such there is a need to find solutions for mitigation.

Statement of Problem

There are a lot of people on earth currently and many more to come. We all need certain resources like food and water to survive. We have found ways to increase our production so that there can be enough food for everyone. However, the prevailing techniques used to achieve this level of production, such as monoculture, water-intensive irrigation, and unfettered application of fertilizer, have set the stage for a looming ecological disaster. This heavy-handed use of fertilizer is having serious negative effects on our already overburdened supply of fresh water, thereby making much of it undrinkable, unsightly, and/or uninhabitable for many of the things we eat. These impacts are felt most strongly by the poorest among us but may be experienced by all of us if things do not change. The aquatic ecosystems that provide so many services for us are being destroyed by our mismanaged and sometimes counterproductive efforts to feed the world at seemingly all costs. There is a pressing need to develop ways to absorb the excess fertilizer that is lost as agricultural runoff before it reaches our rivers, lakes, and coastlines.

Solution: Constructed Wetlands

The good news is that others have recognized these challenges, and many have found answers in natural systems. Fortunately, there is a type of ecosystem that already exists which performs the exact task that we are looking to replicate. So, since the Earth has provided a blueprint, hopefully we can now follow the instructions and yield the same results.

It is well known that wetland biomes provide many important ecosystem services, one of the greatest of these being that wetlands act as natural water filtration systems, especially in terms of nitrogen uptake. Wetlands, both inland and coastal, provide more waste treatment and nutrient cycling (i.e., reduce the effects of eutrophication) than any other type of ecosystem, and those services are valued at an upwards of $200,000 per hectare annually (de Groot et al., 2012). Since the beginning of the 20th century, people have been intentionally using these systems to treat wastewater from many sources, and, in the 1950s, scientists and engineers began to construct artificial wetlands explicitly for this purpose. A constructed wetland (CW henceforth) is a treatment system that uses natural processes involving wetland features, such as plants, soils, and microbial communities, to filter wastewater (US EPA, 2015). As such, one of the most crucial components of a CW is the use of macrophytes, given that they both provide physical structure that allows the removal processes to occur and have shown the greatest abilities to uptake nutrients (Kadlec & Wallace, 2009). Many studies have been conducted on the various designs, proper implementation, and the types of plants used within CWs, but, due to the lack of studies on wetland food crops, especially in south Texas (study site) there is an ongoing need to further examine these macrophytes.

Integrating Constructed Wetlands into Agriculture

A solution to reducing the amount of fertilizers entering major waterways is the integration of CWs into the treatment of agricultural runoff. CWs associated with agricultural lands are often used for *in situ* water treatment, which is a powerful solution that can provide many wetland functions at the farm site itself. For example, wildlife habitat and pollinator benefits are well-recognized, but one currently underappreciated additional provision could be production of food by utilizing wetland food crops. As the human population continues to grow, novel and sustainable solutions to food production must be further explored to meet the accompanying demand. The integration of CWs into agricultural production and agroecosystem design presents several pathways toward more sustainable farming practices.

Focal Plant Species

To better understand both (a) the viability of wetland macrophytes as sources of food production and (b) the capacity of these macrophytes for removal of nitrogen from fertilizer-rich water, in this study we quantify plant growth, food production, and nitrogen removal capacity of three common wetland crops as well as three locally dominant graminoid species in a variety of relevant ecological contexts which will be described in the following chapter. Focal plant species include: wetland crops – *Nelumbo nucifera* (sacred lotus), *Oryza sativa* var. Presidio (a locally-adapted rice cultivar), and *Colocasia esculenta* (taro, AKA elephant ear); local graminoids – *Sorghum bicolor* (grain sorghum, also a crop), *Typha domingensis* (southern cattail), and *Cynodon dactylon* (Bermuda grass).

Nelumbo nucifera **(Sacred Lotus)**

Lotus is a perennial forb that grows in flooded soils, of which virtually every part is edible. Most notably, its rhizome is high in starch and can be eaten raw or cooked and is used in a wide variety of dishes. Petioles may be eaten in salads, the seeds are used to make many food products, including traditional "Moon cakes", and the leaves and flowers are often used as ingredients in tea. This plant is also known for its ability to remove pollutants from wastewaters, though its effect on nitrogen removal has shown mixed results (Kanabkaew & Puetpaiboon, 2004; Liu et al., 2013), making it a good candidate for further study.

Oryza sativa **var. Rex (Rice)**

Rice is an annual grass and a staple cereal worldwide that has been cultivated for thousands of years, primarily in Asia, but now is grown in wetter or irrigated areas globally. Most long-grained varieties are grown in flooded soils, and therefore, may be used in CWs to uptake excess nutrients in runoff. This cultivar, Presidio, has been developed to be grown in relatively hot, dry climates, making it most suitable for this study. This is a relatively new and region-specific variety, little is known about it, with the few studies mentioning it pertaining mainly to rice diseases and planting times (Dou et al., 2016; Mani et al., 2016). Also (1) there has been little work on growing rice in south Texas (or other hot and dry areas), (2) there has been little work on growing rice in treatment wetlands, (3) there has been little work in quantifying rice's N uptake in a treatment wetland contexts, and no work that does so in this region, so there is a need to examine its growth and nitrogen uptake.

Colocasia esculenta **(Taro)**

Taro is an herbaceous perennial forb and a staple in Asia, Africa and Oceania, known primarily for its edible corms and leaves. Much like rice and lotus, taro is often grown in flooded conditions, but it is more commonly grown in non-flooded or "dry-land" conditions (Onwueme, 1999). Known as the "potato of the tropics," this crop is the focus of many dishes. While being a nutritious food, this plant can be very weedy and is considered invasive in parts of the US, mainly because of its growth rate and its ability to outcompete many native species for light (USDA NRCS, 2003) However, its growth rate can be positively utilized in the context of a CW, and this is an area in which more study is needed. The only source pertaining to treatment wetland applications of *C. esculenta* focused on its abilities to treat domestic wastewater (Bindu et al., 2008), but not with agricultural fertilizers, making it an appropriate choice for this study.

Sorghum bicolor **(Sorghum)**

Sorghum is an annual grass and one of the five most important cereal crops worldwide. It is grown as grain for human consumption, animal feed, biofuel, and syrup production. While sorghum is best known for its drought tolerance and ability to maintain high yields in a large range of environmental conditions, it is not commonly found in wetlands, and as such there are very few studies referring to its use as a treatment wetland plant. One recent study focused on the treatment of swine wastewater by a CW using sweet sorghum, found that total nitrogen removal reached as high as 97%, while still maintaining desired yields throughout a variety of moisture treatments (Zhu, Zhu, Shen, & Chen, 2017). The current study is similar to this, however our use of commercial fertilizer may yield different results.

Typha domingensis **(Southern Cattail)**

Cattail is a rhizomatous, herbaceous, perennial graminoid (a rush) and an obligate wetland species. *Typha* species are some of the most commonly found wetland plants across the world. Many parts of this plant are edible at various points in its lifecycle; young shoots emerging from rhizomes may be eaten raw or cooked, young flower stalks can be boiled and eaten like corn, and even the pollen can be used as a flower substitute and is highly nutritious. Cattail has a longstanding reputation as an ideal treatment wetland plant, and as such, myriad studies have tested its viability for wastewater treatment. It has been implemented in CWs designed for wastewater from every major source, i.e. agriculture, industry, etc. and has been found to be highly effective at nitrogen uptake (Calheiros et al., 2009; Ciria et al., 2005; Coleman et al., 2000).

Cynodon Dactylon **(Bermuda Grass)**

Bermudagrass is a sod-forming stoloniferous rhizomatous perennial graminoid (grass) that is well adapted to tropical and subtropical climates due to its extreme drought-tolerance. It is found throughout much of the world and is very common in south Texas. Bermudagrass grows rapidly and is durable, therefore it is frequently used in as a sports turf and lawn grass (Duble, 2018).While not appropriate for human consumption, bermudagrass is a great source for livestock hay and pasture**.** It is one of the most heavily researched grasses in terms of turf and lawn uses, but only a few studies examine its functionality as a treatment wetland species (Andrade et al., 2017; Giannini et al., 2015; Licata et al., 2017; Matos et al., 2010), all of which show bermudagrass to be effective within CWs. However, no research has been conducted in south Texas for this purpose, as such, this study will provide insight into its usefulness in this context.

CHAPTER II

METHODS

Study Site

This study was conducted in the southernmost region of Texas known as the Rio Grande Valley (RGV), more specifically its most southeastern portion, Cameron County. Located in the subtropics, Cameron County is within a climatic transition zone that is semi-arid, and as such it experiences significant seasonal variation in both temperature and rainfall. The average yearly temperature is 23.6°C and the average rainfall is 684 mm per year, with the average minimum temperature being 10.5°C, occurring in January, and the average maximum being 34.1°C in August (Climate-Data.org, 2019). The majority of the rainfall occurs in the fall, peaking in September, however all other seasons are relatively much drier.

Treatments

To quantify both the water filtration (N removal) and the food production functions of our six focal species, we cultivated the six species outdoors in a common garden at the Brownsville Research and Community Garden on UTRGV campus in Brownsville, TX. Plants were reared in full sunlight within 19-liter plastic mesocosms (buckets), which were filled with 3 cm of pea gravel at the bottom and 10 L of coarse sand, which functioned as our sediment. We inoculated the sediment in all mesocosms with an aqueous solution containing live nitrifying bacteria harvested from an established freshwater aquarium filter. Mesocosms were tapped near the bottom with a ball valve so that effluent could be drained.

In three related experiments, the six plant species were all grown (A) in one of three moisture regimes, (B) in a flooded competition scenario with *Lemna minor* (duckweed), or (C) in one of three water cycling treatments. Individual mesocosms were stocked with a variable number of juvenile plants so that the initial plant biomass was as similar as possible across all treatments. For all three experiments, the plant species treatments included: (1) rice, (2) lotus, (3) taro, (4) cattail, (5) Bermuda grass, (6) sorghum, and (7) control with no plants added. The control was necessary to quantify the role that the substrate itself and its microbial community played in filtering water. Throughout the experiment we added water and fertilizer to each mesocosm at the beginning of every week by applying a standard solution, which we then drained at the end of the week before adding a fresh solution. The standard solution added consisted of municipal water and Miracle-Gro Water Soluble All Purpose Plant Food, whose nitrogen content is 20.5% urea and 3.5% ammoniacal nitrogen, mixed to a total nitrogen concentration of 304.33 mg/L (304 ppm). We chose an all-purpose fertilizer, because the use of sand as our sediment meant that we needed to supply other essential nutrients. There are two reasons for the high concentration of our standard solution, (1) we sought to mimic realistic, heavily eutrophicated conditions, and (2) we never wanted our focal species to be nitrogen limited. The volume of standard solution added was based on the treatments described below.

In the moisture regime experiment, water level treatments included: (1) flooded, 5 L of solution added, resulting in 10 cm of standing water above the soil surface at each filling; (2) saturated, 2.5 L of solution added, leaving roughly 1 cm of standing water above the soil surface at each filling; or (3) below surface, 1 L of solution added, where the water table is 10 cm below the soil surface at each filling. In the competition experiment, treatments included: (1) *Lemna minor* (common duckweed, a floating aquatic plant) added to flooded treatments; or (2) control, with no *Lemna* added (the flooded mesocosms in the moisture regime experiment served as controls). In the water cycling experiment, treatments included: (1) 1 cycle, where water is drained from the bottom of the mesocosm and reapplied to the soil surface once per week; (2) 2 cycles, where water is drained and reapplied twice per week; or (3) control, where water is not cycled but is still added and removed weekly (equivalent moisture treatments from the moisture regime experiment served as controls). For the cycling experiment, each plant species was subjected to the moisture treatment from the moisture experiment that we hypothesize will be optimal for its growth, i.e. flooded for cattail and lotus, saturated for grass, rice, and the control, and below surface for taro and sorghum. There we 3 replicates per treatment combination, for a total sample size of n = 126 mesocosms (n = 63 for the moisture experiment; n = 42 for the competition experiment, with 21 shared with the moisture experiment; and n = 63 for the cycling experiment, with 21 shared with the moisture experiment).

Experiments were run simultaneously for 10 weeks, beginning on March 8, 2019 ending on May 18, 2019, with active weekly data collection occurring throughout. At the end of each week, effluent was drained from each mesocosm, and along with that, municipal water at a volume of 2.5 L in weeks 3 – 4, and 5 L in weeks 5 – 10 was added to each mesocosm as a flush to provide ample effluent volume for water quality analysis. The effluent was analyzed before new standard solution was added in the volumes described above. Water quality parameters measured include effluent volume; and temperature, conductivity, pH, and concentrations of ammonium, ammonia, and nitrate (measured using a YSI DSSPro water sonde). Initial wet biomass was measured prior to transplant into mesocosms. After the final data collection, plants were harvested, separated in the following categories for each plant: aboveground biomass, belowground biomass, and edible biomass. Following harvest, plants were dried, and dry mass was recorded, except in the case of edible biomass, which was measured in its marketable condition. Plant survival was surveyed three times per week and all mortality events were recorded. Estimations of nutritional and market value based on produce weights were also made.

Due to high mortality of duckweed in Experiment B and relatively little novel information emerging from Experiment C, results are reported only here for Experiment A, which was the largest and most important of the related experiments.

Analysis

We evaluated the effects of our treatments for plant species and water regime on the focal response variables using repeated measures ANOVAs. For each response variable, we fit a linear mixed effect (LME) model ('lme' function in R), with treatments for species and water regime as fixed categorical factors, week as a continuous time factor, and mesocosm as a random subject factor (which is required for repeated measures analyses). The ANOVA model terms for each response variable included the main effects of each factor and the second-order and third-order interactions among fixed factors (plant species, water regime, and week). For LME models it is necessary to test the effects of the random subject factor by performing a likelihood ratio test to compare models with and without the random effect. Mixed models do not use ordinary least squares methods to estimate model parameters, thus it was necessary to use an alternative parameter estimating approach. In our case, we used restricted maximum likelihood, and we used likelihood ratio tests to derive p-values for each model and individual model terms. For this reason, our test statistics are Chi-squared values, rather than F ratios, and we used the 'pR2' function in R to calculate each model's goodness of fit, specifically Cox and Snell pseudo R^2 values. We then tested the residuals for each model to confirm that we did not violate the assumptions of ANOVA related to normality or homoscedasticity of residuals. When necessary, variables were log or square root transformed to conform to the assumptions of ANOVA. Following the full model testing, we examined each of the significant treatments or interactions individually using least square means post-hoc tests to determine significant differences between levels within each treatment or interaction. All analyses were performed using R version 3.5.

Major response variables include ammoniacal nitrogen within the effluent normalized as a percent of total N added (%), nitrate nitrogen within the effluent normalized as a percent of total N added (%), total N within effluent normalized as a percent of the total N added (%), estimated total N removed (mg), total N removed normalized as a percent of total N added (%), recovered effluent as a percent of water added (%), total dry weight biomass (g), and total wet weight edible biomass (g).

It should be noted that idiosyncratic values arose in various weeks as an artifact of experimental protocol; therefore, it was necessary to exclude certain weeks. Weeks 1 and 2 were removed because of rain and because we began using a different model of data sonde from week 3 onward, and week 5 was excluded because the amount of municipal water used as flush was increased, which resulted in a pulse of ammoniacal N and nitrate from within the sediment that produced artificially high values in the nitrogen response variables.

Rather than looking at solely the ammoniacal N and nitrate concentrations within the effluent, we were able to derive more meaningful results upon considering the *in situ* measurement as a proportion of the total N added at the beginning of the week, hence our emphasis on normalized values based on the amount of N added.

CHAPTER III

RESULTS

Moisture Regime Experiment

Normalized Ammoniacal N remaining in effluent

The values for normalized ammoniacal N were log transformed prior to analysis. All main effects were very significant, all second order interactions were very significant except the interaction of water level and week, and the third order interaction was very significant (Table 1). The whole model was extremely significant (p = 6.9711e-80) and the Cox and Snell pseudo R^2 value was 0.736176.

Table 1

Results of repeated measures ANOVA for normalized ammoniacal N remaining.

Treatment	*Chisq*	*df*	*P*
Species	186.2555	6	< 0.001 ***
Water level	72.9746	2	< 0.001 ***
Week	105.2783	1	< 0.001 ***
Species:Water level	37.7463	12	< 0.001 ***
Species:Week	55.9091	6	< 0.001 ***
Water Level:Week	3.4978	2	0.1739

Species:Water level:Week	26.6305	12	0.0087 **

Note. *Chisq* indicates the chi-squared test statistic values. *Df* indicates the degrees of freedom. *P* values indicate the probability that random values would generate a test statistic greater than those observed.
* indicates $0.01 < p < 0.05$. ** indicates $0.001 < p < 0.01$. *** indicates $p < 0.001$.

When analyzing the percent of ammoniacal N there were significant differences between species (Figure 1, Table 2). Cattail had the lowest percent of remaining ammoniacal N in the effluent and was significantly different from the control and all other species except grass. Grass, rice and taro were not significantly different from one another; however, grass was different from sorghum, lotus and the control. Sorghum showed no significant difference from rice or taro but did significantly differ from lotus and the control. Lotus and control had the highest percent of ammoniacal N remaining in the effluent of all treatments.

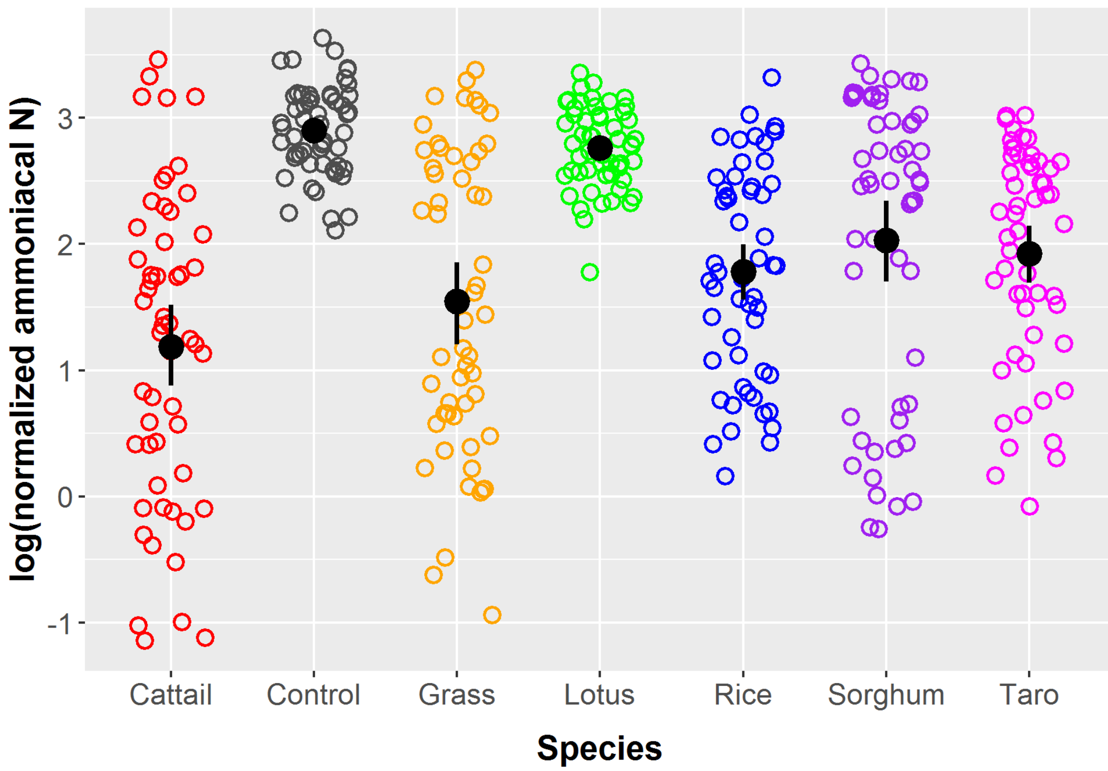

Figure. 1. Normalized ammoniacal N remaining by species. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 2

Results of a least squared means post-hoc comparison test using a Tukey adjustment for normalized ammoniacal N remaining by species.

Species	*lsmean*	*SE*	*Df*	*lower.CL*	*upper.CL*	*group*
Cattail	1.03	0.118	42	0.696	1.36	a
Grass	1.48	0.118	42	1.146	1.81	ab
Rice	1.76	0.118	42	1.426	2.09	bc
Taro	1.88	0.118	42	1.551	2.22	bc
Sorghum	2.01	0.118	42	1.681	2.35	c
Lotus	2.74	0.118	42	2.404	3.07	d
Control	2.86	0.118	42	2.526	3.19	d

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

Variance in percent of ammoniacal N was also explained by the moisture regime they were assigned. There were significant differences between each treatment (Figure 2, Table 3). Across the three treatments the below surface mesocosms had the lowest percent of ammoniacal N remaining, with saturated having the second most remaining, and flood mesocosms had the highest percent of ammoniacal N remaining.

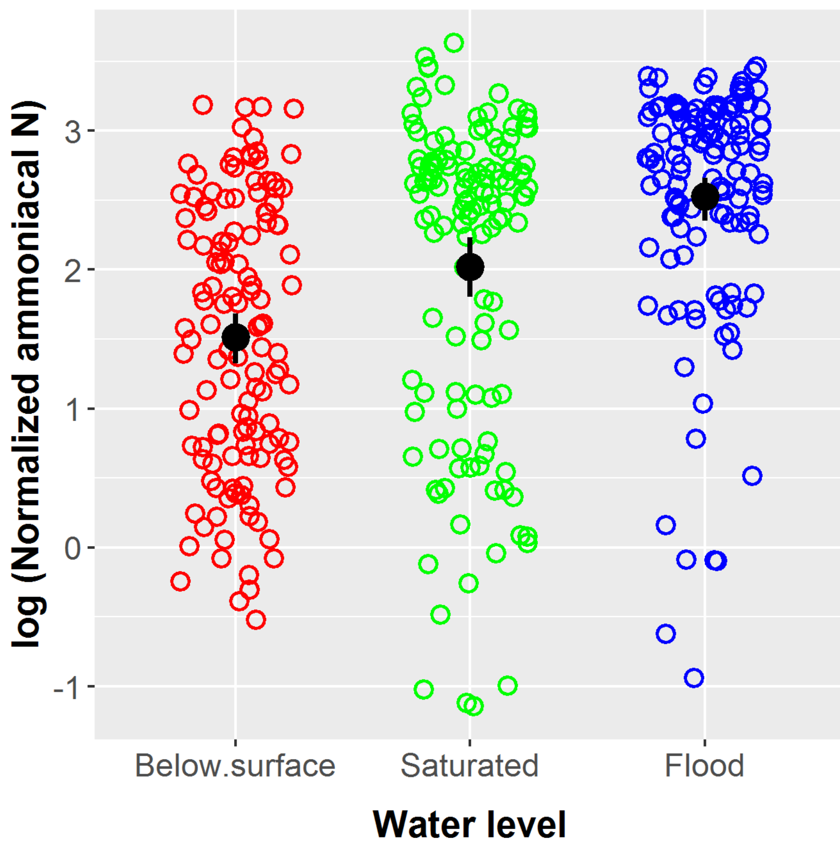

Figure. 2. N*ormalized ammoniacal N remaining by water level.* Colors denote moisture regime. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 3

Results of a least squared means post-hoc comparison test using a Tukey adjustment for normalized ammoniacal N remaining by water level.

Water level	*lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Below surface	1.50	0.0775	42	1.31	1.69	a
Saturated	1.96	0.0775	42	1.76	2.15	b
Flood	2.44	0.0775	42	2.25	2.64	c

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test.

Lower.CL and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

The interaction between the species and water level treatments also explained a significant amount of variance within the model. We saw significant differences in the percent of ammoniacal N remaining between the species and water level interactions (Figure 3, Table 4). Lotus and the control showed no significant differences across each treatment, and they had the highest amount of ammoniacal N remaining of all species except for sorghum in the flood treatment. Cattail had the lowest percent remaining in both flood and saturated of all species, and second lowest in the below surface treatment, as well as being significantly lower than half of all interactions. Excluding lotus and the control, of all water treatments across species, below surface had the lowest percent of remaining ammoniacal N.

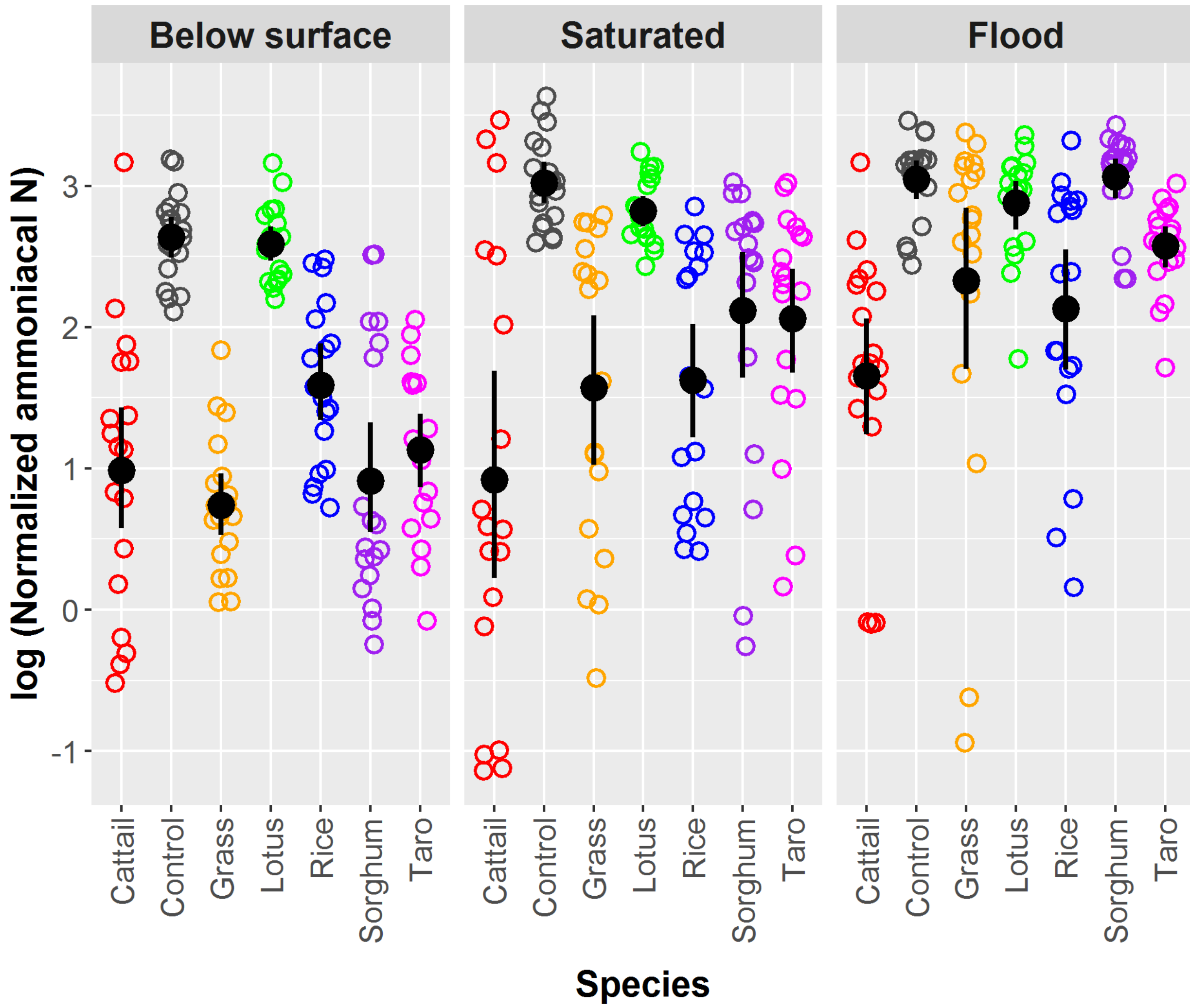

Figure 3. N*ormalized ammoniacal N remaining by species separated by water level.* Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 4

Results of a least squared means post-hoc comparison test using a Tukey adjustment for normalized ammoniacal N remaining by species and water level.

Species	Water level	*lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Cattail	Saturated	0.717	0.205	42	0.0559	1.38	a
Grass	Below surface	0.757	0.205	42	0.0961	1.42	a
Cattail	Below surface	0.881	0.205	42	0.2201	1.54	a

Sorghum	Below surface	0.988	0.205	42	0.3270	1.65	ab
Taro	Below surface	1.106	0.205	42	0.4447	1.77	abc
Cattail	Flood	1.491	0.205	42	0.8295	2.15	abcd
Grass	Saturated	1.533	0.205	42	0.8721	2.19	abcde
Rice	Below surface	1.602	0.205	42	0.9409	2.26	abcde
Rice	Saturated	1.623	0.205	42	0.9622	2.28	abcde
Taro	Saturated	2.002	0.205	42	1.3406	2.66	bcdef
Sorghum	Saturated	2.049	0.205	42	1.3883	2.71	bcdef
Rice	Flood	2.055	0.205	42	1.3935	2.72	bcdef
Grass	Flood	2.147	0.205	42	1.4863	2.81	cdef
Taro	Flood	2.546	0.205	42	1.8847	3.21	def
Lotus	Below surface	2.555	0.205	42	1.8941	3.22	def
Control	Below surface	2.604	0.205	42	1.9429	3.27	ef
Lotus	Saturated	2.788	0.205	42	2.1269	3.45	f
Lotus	Flood	2.869	0.205	42	2.2076	3.53	f
Control	Saturated	2.979	0.205	42	2.3182	3.64	f
Control	Flood	2.996	0.205	42	2.3346	3.66	f
Sorghum	Flood	3.006	0.205	42	2.3451	3.67	f

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

The interaction of species and week explained a significant amount of variance within the model. All species, except cattail in week 3 and 4, showed a decrease in the ammount of ammoniacal N remaining in the effluent as the weeks went by (Figure 4).

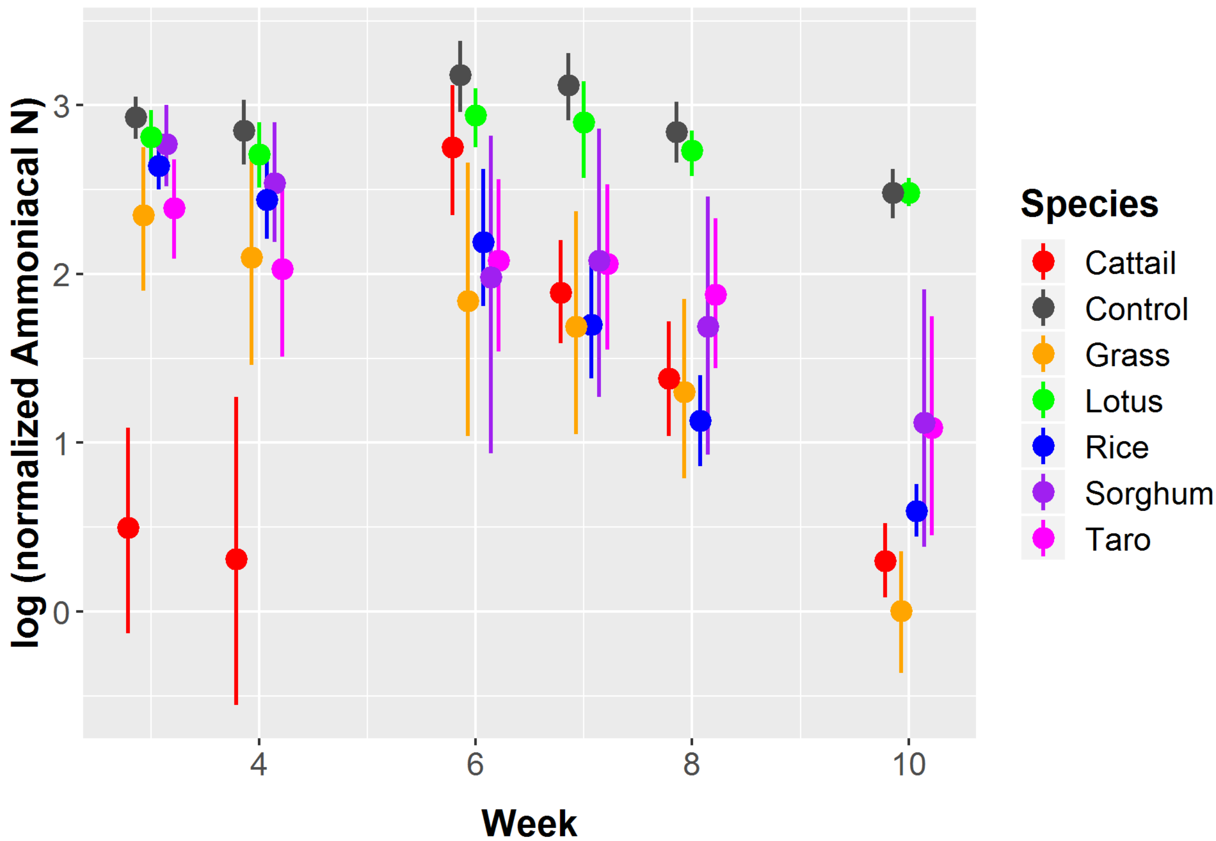

Figure 4. Normalized ammoniacal N remaining by species across weeks. Color denotes species. Colored points represent the arithmetic means of all values for a species in a given week. Error bars represent a bootstrapped 95% confidence interval. Week values are integers, points are jittered to improve clarity.

Normalized Nitrate N remaining in effluent

The values of normalized nitrate N were log transformed prior to analysis. All main effects and interactions were very significant, ($P < 0.0001$) (Table 5). According to the maximum likelihood ratio test, the whole model was extremely significant ($p = 9.19e-105$), and the Cox and Snell pseudo R^2 value was 0.809726.

Table 5

Results of repeated measures ANOVA for normalized nitrate N remaining.

Treatment	*Chisq*	*df*	*P*
Species	305.73	6	< 0.001 ***
Water level	419.84	2	< 0.001 ***
Week	218.23	1	< 0.001 ***
Species:Water level	295.89	12	< 0.001 ***
Species:Week	107.75	6	< 0.001 ***
Water Level:Week	34.19	2	< 0.001 ***
Species:Water level:Week	100.23	12	< 0.001 ***

Note. *Chisq* indicates the chi-squared test statistic values. *Df* indicates the degrees of freedom. *P* indicates the probability that random values would generate a test statistic greater than those observed.
* indicates $0.01 < p < 0.05$. ** indicates $0.001 < p < 0.01$. *** indicates $p < 0.001$.

When analyzing the percent of nitrate N remaining in the effluent there were significant differences between species (Figure 5, Table 6). Cattail had significantly higher amounts of nitrate N remaining than all other species. Sorghum and grass were significantly different than all other species in that they had the lowest amount of nitrate N remaining in their effluent. The remaining species – rice, taro, lotus and the control – were not significantly different from each other.

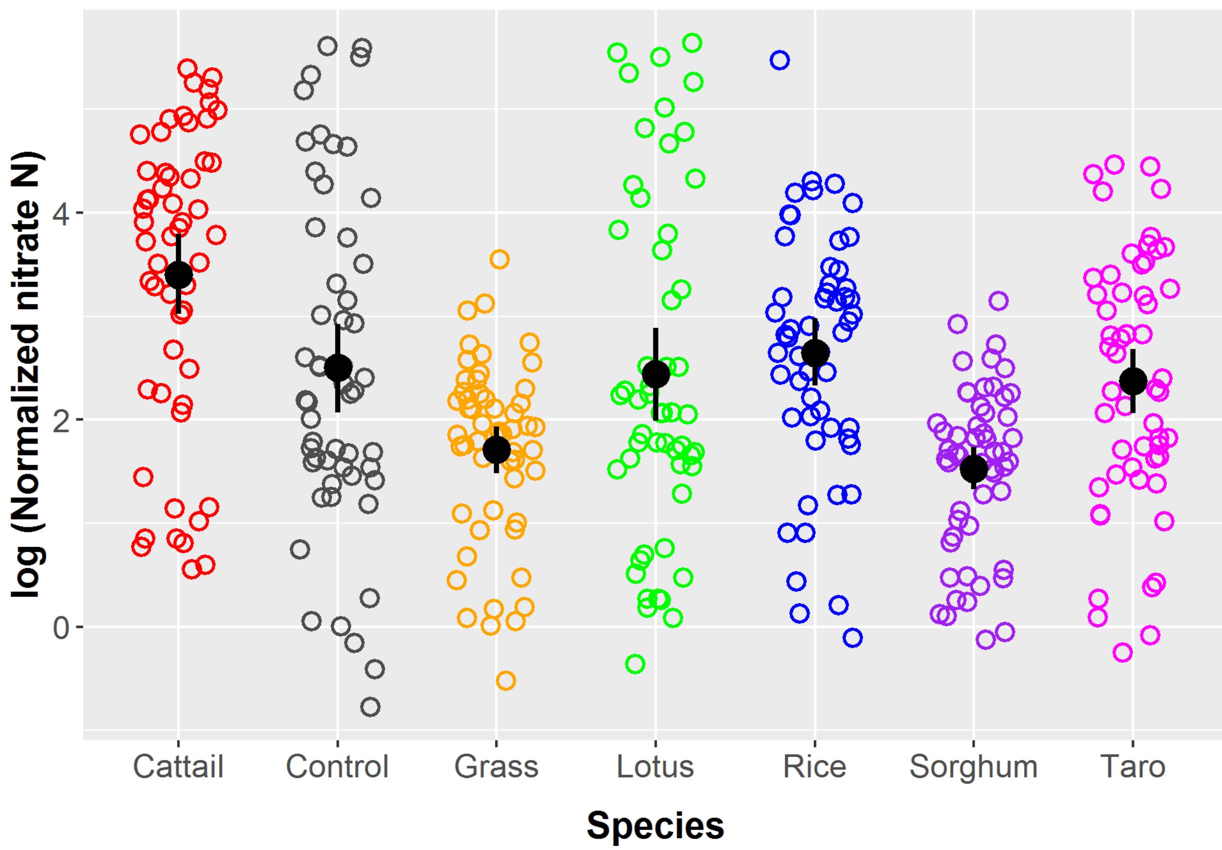

Figure 5. Normalized nitrate N remaining by species. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 6

Results of a least squared means post-hoc comparison test using a Tukey adjustment for normalized nitrate N remaining by species.

Species	*lsmean*	*SE*	*Df*	*lower.CL*	*upper.CL*	*group*
Sorghum	1.53	0.0866	42	1.28	1.77	a
Grass	1.72	0.0866	42	1.47	1.96	a
Taro	2.38	0.0866	42	2.14	2.62	b
Lotus	2.45	0.0866	42	2.20	2.69	b
Control	2.51	0.0866	42	2.26	2.75	b

Rice	2.65	0.0866	42	2.41	2.89	b
Cattail	3.41	0.0866	42	3.17	3.66	c

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

Variance in percent of nitrate N was also explained by the moisture regime they were assigned (Table 5). There were significant differences between each treatment (Figure 6, Table 7). Across the three treatments the flood mesocosms had the lowest percent of nitrate N remaining, with saturated having the second most remaining, and below surface mesocosms had the highest percent of nitrate N remaining.

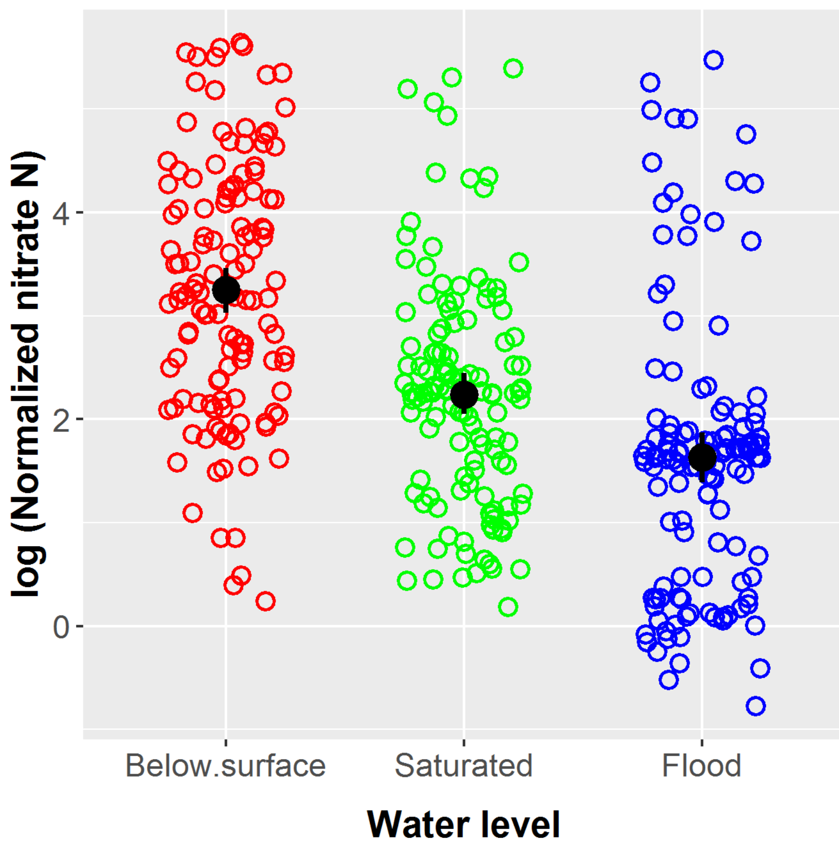

Figure 6. Normalized nitrate N remaining by water level. Colors denote moisture regime. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 7

Results of a least squared means post-hoc comparison test using a Tukey adjustment for normalized nitrate N remaining by water level.

Water level	*lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Flood	1.63	0.0567	42	1.49	1.77	a
Saturated	1.96	0.0567	42	2.10	2.38	b
Below surface	2.44	0.0567	42	3.11	3.40	c

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test.

Lower.CL and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different (α = 0.05).

The interaction between species and water level was significant within the model as well (Table 5). There were significant differences in the percent of nitrate N remaining in the effluent between the interaction of species and water level (Figure 7, Table 8).

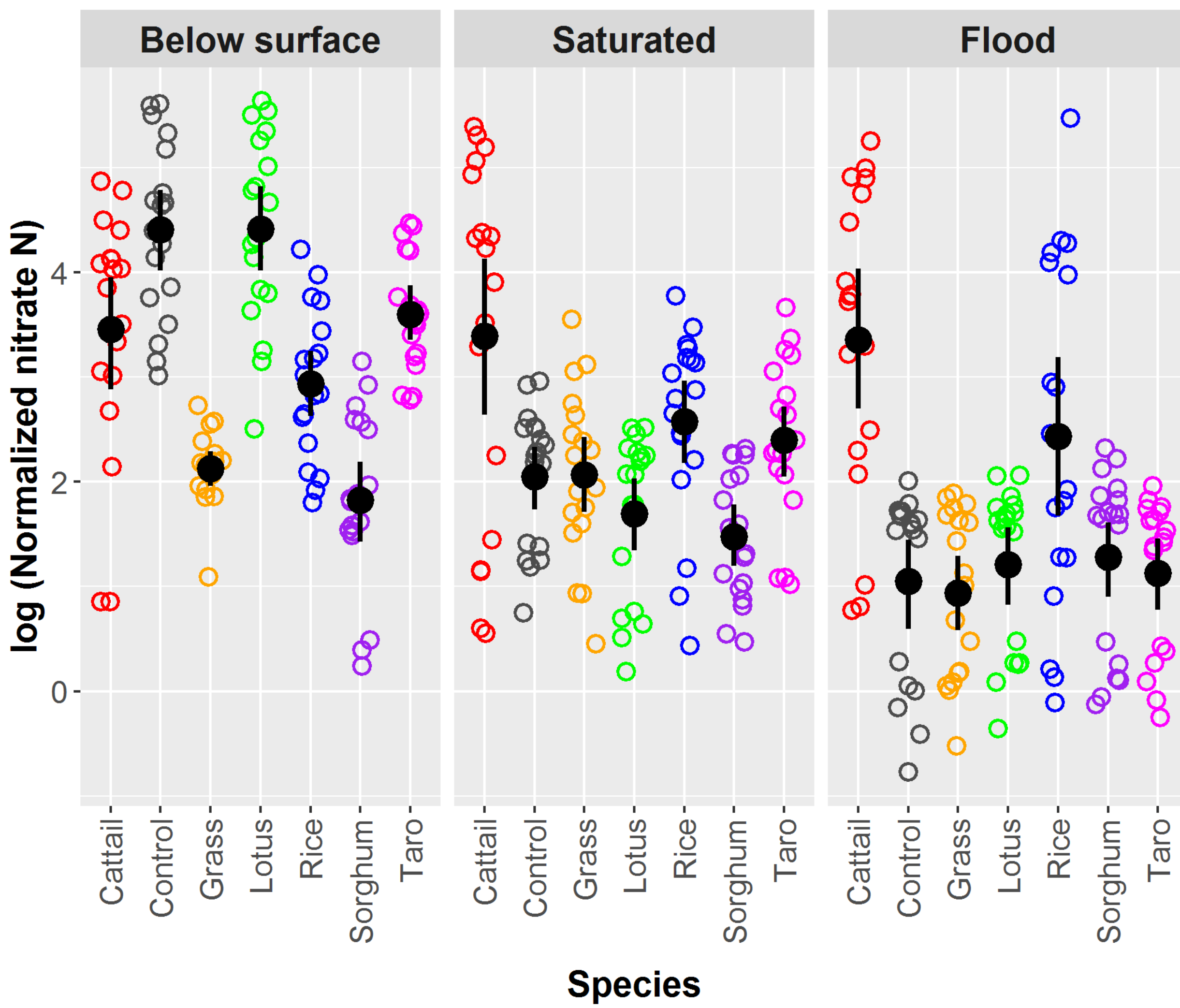

Figure 7. Normalized nitrate N remaining by species separated by water level. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 8

Results of a least squared means post-hoc comparison test using a Tukey adjustment for normalized nitrate N remaining by species and water level.

Species	Water level	*lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Grass	Flood	0.949	0.15	42	0.465	1.43	a
Control	Flood	1.056	0.15	42	0.572	1.54	ab
Taro	Below surface	1.129	0.15	42	0.2201	1.54	ab
Lotus	Below surface	1.217	0.15	42	0.3270	1.65	ab
Sorghum	Below surface	1.285	0.15	42	0.4447	1.77	abc
Sorghum	Flood	1.482	0.15	42	0.8295	2.15	abcd
Lotus	Saturated	1.702	0.15	42	0.8721	2.19	abcde
Sorghum	Below surface	1.819	0.15	42	0.9409	2.26	bcdef
Control	Saturated	2.052	0.15	42	0.9622	2.28	cdef
Grass	Saturated	2.075	0.15	42	1.3406	2.66	cdef
Grass	Saturated	2.127	0.15	42	1.3883	2.71	def
Taro	Flood	2.404	0.15	42	1.3935	2.72	efg
Rice	Flood	2.435	0.15	42	1.4863	2.81	efg
Rice	Flood	2.578	0.15	42	1.8847	3.21	fgh
Rice	Below surface	2.939	0.15	42	1.8941	3.22	ghi
Cattail	Below surface	3.364	0.15	42	1.9429	3.27	hi
Cattail	Saturated	3.402	0.15	42	2.1269	3.45	i
Cattail	Flood	3.470	0.15	42	2.2076	3.53	i
Taro	Saturated	3.606	0.15	42	2.3182	3.64	ij
Control	Flood	4.408	0.15	42	2.3346	3.66	jk
Lotus	Flood	4.418	0.15	42	2.3451	3.67	k

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

The interaction between species and week was significant and explained some of the variance within the model (Table 5). Between weeks 3 and 4 there was a clear increase in the amount of nitrate N remaining across all species, however that trend did not last in weeks 6 through 10 (Figure 8). In week 6, a trend started where cattail had greater amounts of nitrate N remaining than the other species and continued in the subsequent weeks. Also, there was a slight increase in the nitrate N remaining for rice, lotus and the control from week 6 through 10. Sorghum had its lowest percent nitrate N remaining in week 6, but it increased in week 7 and stayed at that level throughout the rest of the experiment.

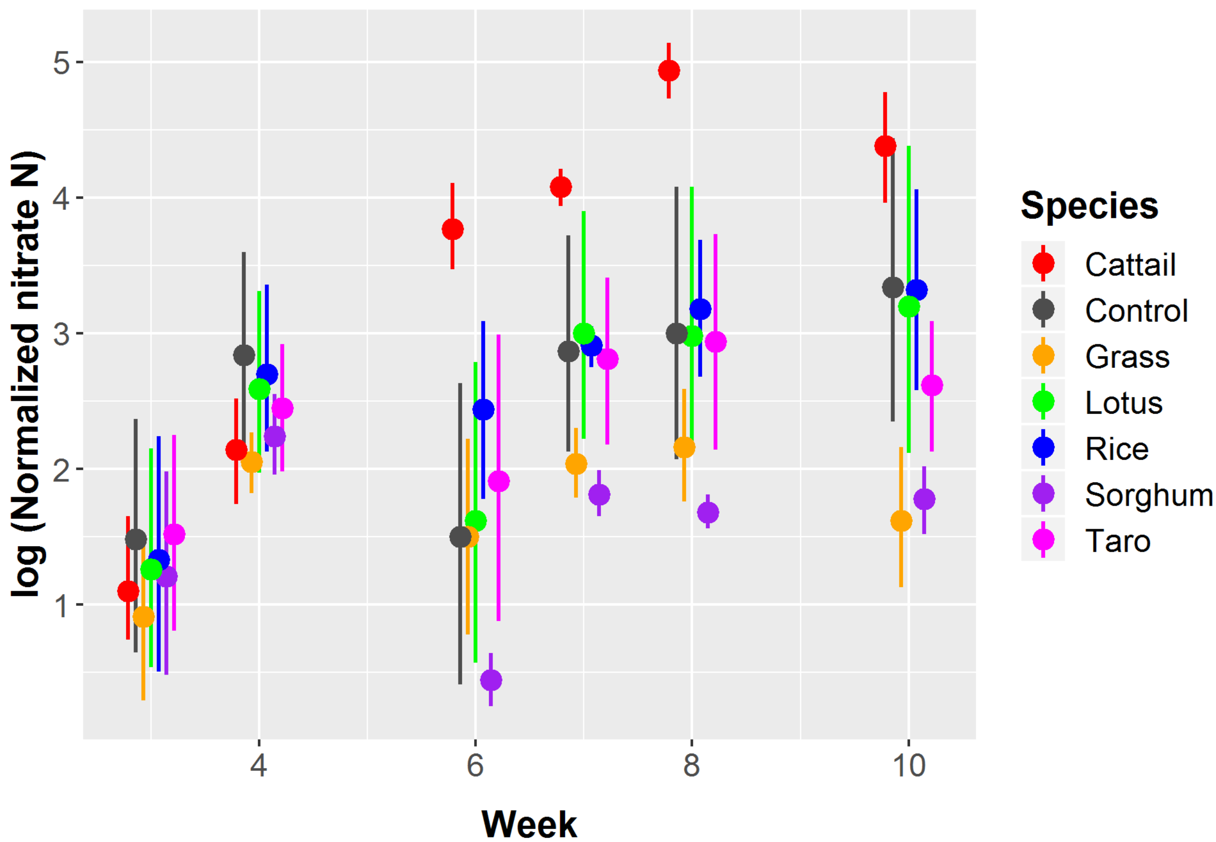

Figure 8. Normalized nitrate N remaining by species across weeks. Color denotes species. Colored points represent the arithmetic means of all values for a species in a given week. Error bars represent bootstrapped 95% confidence intervals. Week values are integers, points are jittered to improve clarity.

Normalized Total N remaining in effluent

The values of normalized total N were log transformed prior to analysis. Excluding water level by week, all main effects and interactions were very significant ($P < 0.0001$) (Table 9). According to the likelihood ratio test, the whole model is extremely significant ($p = 1.2761e-94$) and the Cox and Snell pseudo R^2 value was 0.782753.

Table 9

Results of repeated measures ANOVA for normalized total N remaining.

Treatment	*Chisq*	*df*	*P*
Species	187.045	6	< 0.001 ***
Water level	22.890	2	< 0.001 ***
Week	26.423	1	< 0.001 ***
Species:Water level	173.688	12	< 0.001 ***
Species:Week	215.974	6	< 0.001 ***
Water Level:Week	2.104	2	0.3492
Species:Water level:Week	110.478	12	< 0.001 ***

Note. *Chisq* indicates the chi-squared test statistic values. *Df* indicates the degrees of freedom. *P* indicates the probability that random values would generate a test statistic greater than those observed.
* indicates 0.01 < p < 0.05. ** indicates 0.001 < p < 0.01. *** indicates p < 0.001.

When analyzing the percent of total N remaining in the effluent, we saw significant differences between species (Figure 9, Table 10). Grass and sorghum were significantly different from all other species, having the lowest amount of total N remaining. Taro had significantly less total N remaining than lotus and the control. Rice had significantly less total N remaining than the control. All other species – cattail and lotus – were not significantly different than the control, which had the highest percent of total N remaining in the effluent.

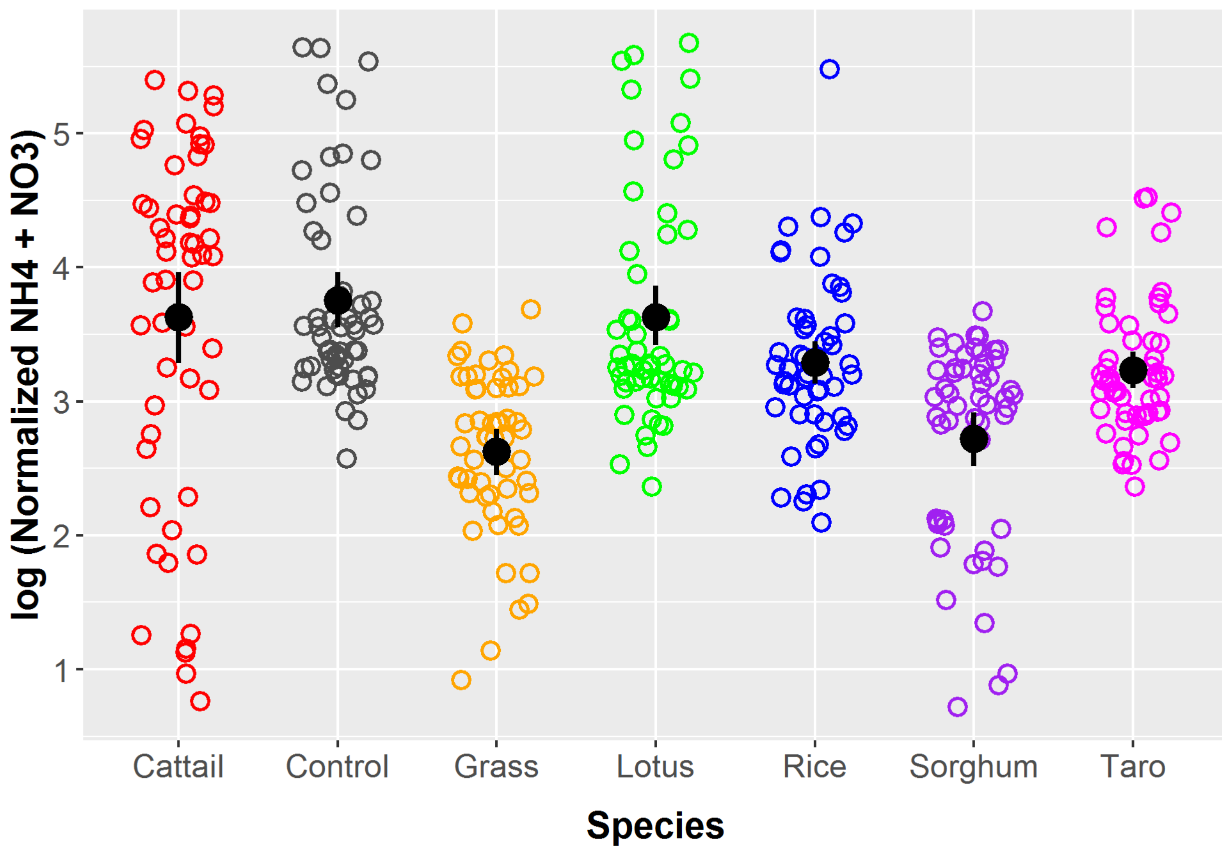

Figure 9. Normalized total N remaining by species. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 10

Results of a least squared means post-hoc comparison test using a Tukey adjustment for normalized total N remaining by species.

Species	*lsmean*	*SE*	*Df*	*lower.CL*	*upper.CL*	*group*
Grass	2.58	0.0826	42	2.35	2.82	a
Sorghum	2.72	0.0826	42	2.48	2.95	a
Taro	3.19	0.0826	42	2.96	3.43	b
Rice	3.29	0.0826	42	3.06	3.52	bc
Cattail	3.53	0.0826	42	3.30	3.76	bcd

Lotus	3.61	0.0826	42	3.38	3.84	cd
Control	3.73	0.0826	42	3.50	3.97	d

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

Water level explained a significant amount of variance within the model (Table 9). There were significant differences between some of the water levels (Figure 10, Table 11). Below surface had the highest percent of total N remaining in the effluent, being significantly different than the other water levels. Flood and saturated were not significantly different from each other.

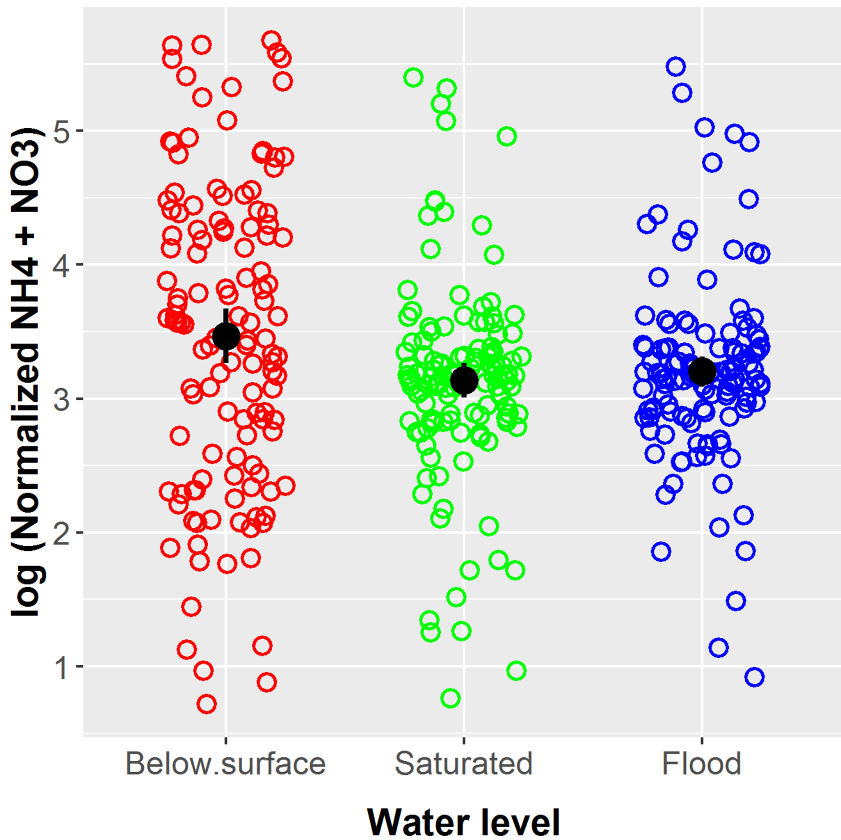

Figure 10. Normalized total N remaining by water level. Colors denote moisture regime. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 11

Results of a least squared means post-hoc comparison test using a Tukey adjustment for normalized total N remaining by water level.

Water level	*lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Saturated	3.09	0.0541	42	2.96	3.23	a
Flood	3.17	0.0541	42	3.04	3.31	a
Below surface	3.44	0.0541	42	3.31	3.58	b

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test.

Lower.CL and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

The interaction between species and water level was significant within the model as well (Table 9). There were significant differences in the percent of total N remaining in the effluent between the interaction of species and water level (Figure 11, Table 12).

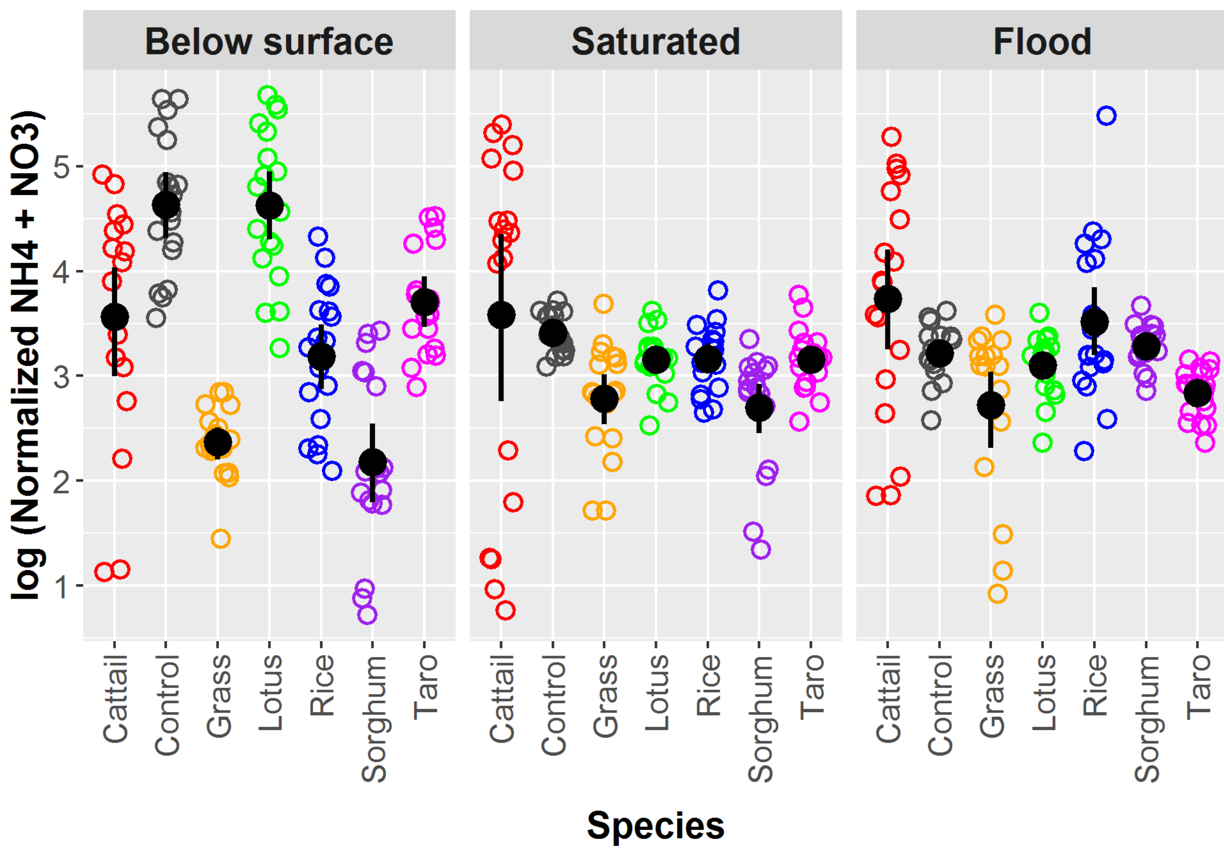

Figure 11. Normalized total N remaining by species separated by water level. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 12

Results of a least squared means post-hoc comparison test using a Tukey adjustment for normalized total N remaining by species and water level.

Species	Water level	*lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Sorghum	Below surface	2.24	0.143	42	1.78	2.70	a
Grass	Below surface	2.37	0.143	42	1.91	2.84	ab
Grass	Flood	2.62	0.143	42	2.16	3.08	abc
Sorghum	Saturated	2.66	0.143	42	2.19	3.12	abc
Grass	Saturated	2.75	0.143	42	2.29	3.21	abcd
Taro	Flood	2.82	0.143	42	2.36	3.28	abcde
Lotus	Flood	3.09	0.143	42	2.63	3.55	bcdef
Taro	Saturated	3.11	0.143	42	2.65	3.57	bcdef
Lotus	Saturated	3.12	0.143	42	2.66	3.58	dcdef
Rice	Below surface	3.16	0.143	42	2.69	3.62	cdef
Rice	Saturated	3.16	0.143	42	2.70	3.62	cdef
Control	Flood	3.18	0.143	42	2.72	3.64	cdef
Sorghum	Flood	3.25	0.143	42	2.79	3.71	cdef
Control	Saturated	3.39	0.143	42	2.93	3.85	cdef
Cattail	Below surface	3.44	0.143	42	2.98	3.90	def
Cattail	Saturated	3.48	0.143	42	3.01	3.94	def
Rice	Flood	3.56	0.143	42	3.10	4.02	ef
Taro	Below surface	3.65	0.143	42	3.19	4.11	f
Cattail	Flood	3.68	0.143	42	3.22	4.14	f
Lotus	Below surface	4.61	0.143	42	4.15	5.07	g
Control	Below surface	4.63	0.143	42	4.17	5.09	g

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

Within the full model, the interaction between species and week was seen to be significant (Table 9). Prior to week 6, cattail had the smallest percent of total N in the effluent across all other species; however, from week 6 onward, it had the largest of all species (Figure 12). Rice, lotus, and the control were consistent across all weeks, with a slight gradual increase as the experiment went on. Grass was also consistent except for the final week where it had a lower amount of total N in the effluent than all other weeks. Taro showed a similar trend to that of grass, however across weeks it was gradually increasing until the final week in which it decreased. All species were relatively clumped together in the first week, but by the end of the experiment the species were clearly stratified in the percent of total N remaining in the effluent.

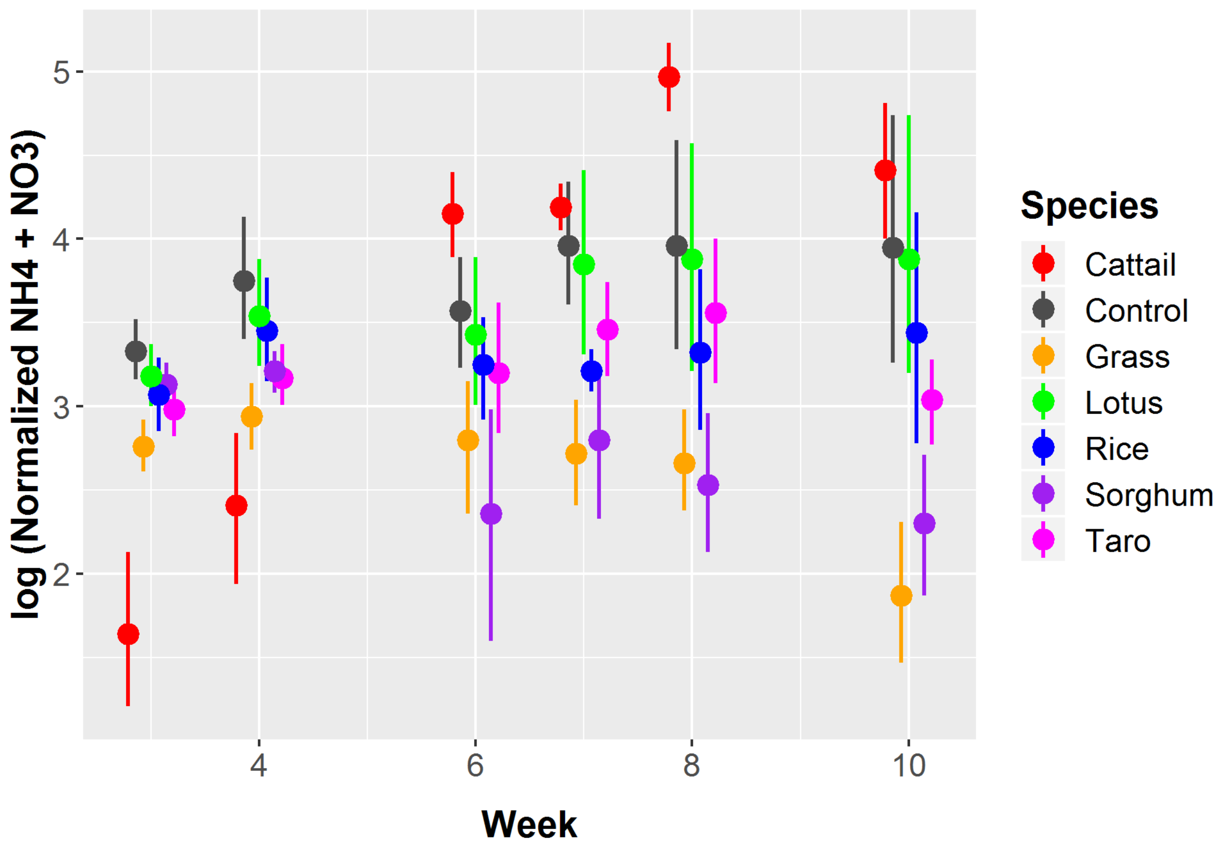

Figure 12. Normalized total N remaining by species across weeks. Color denotes species. Colored points represent the arithmetic means of all values for a species in a given week. Error bars represent bootstrapped 95% confidence intervals. Week values are integers, points are jittered to improve clarity.

Total N Removed

The values of total N removed were square root transformed prior to analysis but were not normalized, unlike most of our other response variables. Excluding the water level by week interaction, all main effects and interactions were very significant ($P < 0.0001$) (Table 13). According to the likelihood ratio test, the whole model was extremely significant ($p = 1.06e-129$), and the Cox and Snell pseudo R^2 value was 0.863699.

Table 13

Results of repeated measures ANOVA for total N removed.

Treatment	*Chisq*	*df*	*P*
Species	221.6625	6	< 0.001 ***
Water level	1313.1558	2	< 0.001 ***
Week	86.0738	1	< 0.001 ***
Species:Water level	179.1653	12	< 0.001 ***
Species:Week	212.5461	6	< 0.001 ***
Water Level:Week	0.5707	2	0.7518
Species:Water level:Week	175.6230	12	< 0.001 ***

Note. *Chisq* indicates the chi-squared test statistic values. *Df* indicates the degrees of freedom. *P* indicates the probability that random values would generate a test statistic greater than that observed.
* indicates 0.01 < p < 0.05. ** indicates 0.001 < p < 0.01. *** indicates p < 0.001.

Species was shown to explain a significant amount of variance within the full model (Table 13). There were significant differences between species in terms of total N removed (Figure 13, Table 14). Cattail was significantly different from all other species, and it had the lowest total N removed. Lotus and the control removed significantly less total N than taro, sorghum, or grass, but was not significantly different from rice. Rice was also not significantly different than taro or sorghum but removed significantly less total N than did grass. Grass removed the greatest amount of total N, being significantly greater than all other species except taro and sorghum.

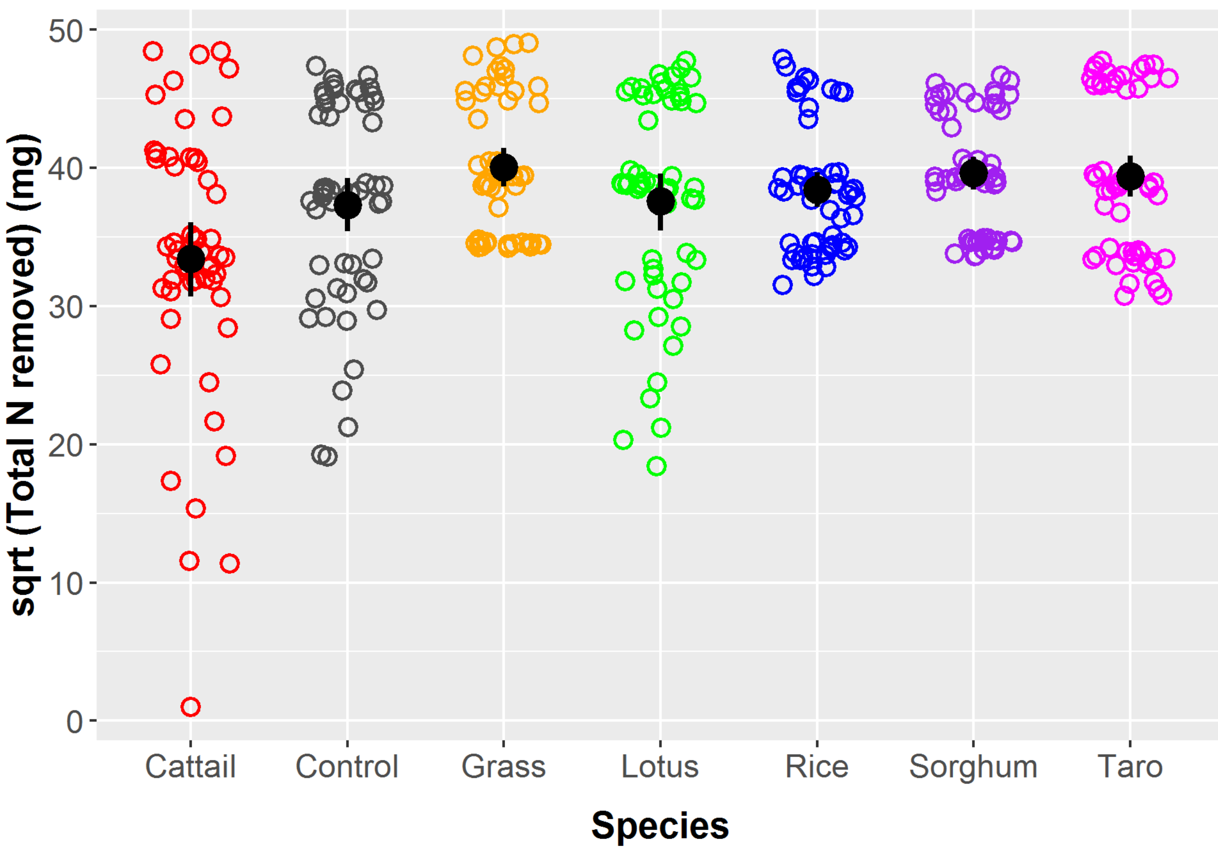

Figure 13. Total N removed by species. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 14

Results of a least squared means post-hoc comparison test using a Tukey adjustment for total N removed by species.

Species	*lsmean*	*SE*	*Df*	*lower.CL*	*upper.CL*	*group*
Cattail	33.4	0.363	42	32.4	34.4	a
Control	37.3	0.363	42	36.2	38.3	b
Lotus	37.6	0.363	42	36.6	38.6	b
Rice	38.5	0.363	42	37.4	39.5	bc
Taro	39.4	0.363	42	38.4	40.4	cd

Sorghum	39.6	0.363	42	38.6	40.6	cd
Grass	40.0	0.363	42	39.0	41.1	d

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

Within the model, water level did explain a significant amount of variance (Table 13). There were significant differences in the amount of total N removed between water levels (Figure 14, Table 15). Below surface exhibited the least amount of total N removed, saturated the second most N removed, and flood the greatest amount of total N removed. Each difference was significant.

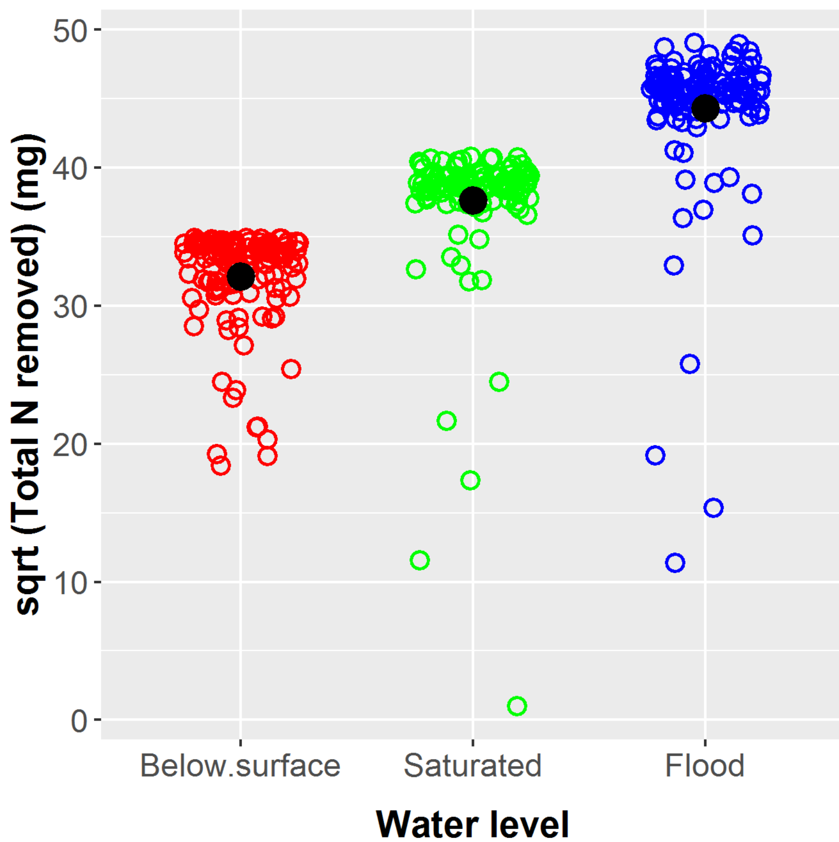

Figure 14. Total N removed by water level. Colors denote moisture regime. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 15

Results of a least squared means post-hoc comparison test using a Tukey adjustment for total N removed by water level.

Water level	*lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Below surface	32.2	0.0235	42	31.6	32.7	a
Surface	37.6	0.0235	42	37.0	38.2	b
Flood	44.1	0.0235	42	43.6	44.7	c

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test.

Lower.CL and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

When considering the full model, the interaction of species and water level explained a significant amount of the variance (Table 13). There were significant differences between the interactions (Figure 15, Table 16). The greatest difference between the species and water level interactions occurred between water level, as most species within each water level were not significantly different than each other, while the overall trend showed the most N was removed in flood treatments, saturated the second most, and below surface showing the least removed. However, within water levels there were some species that stood out among the others and were significantly different. In the flood category cattail removed significantly less than all other species. Also, in flood, taro and rice were significantly different, with taro removing more total N than rice. Cattail in flood was not significantly different from all saturated species except cattail, which removed significantly less total N than all other species in saturated. All species in below surface were not significantly different from each other except for lotus and the control which removed significantly less than the others. Total N removal from cattail in saturated was not significantly different from lotus and the control from below surface.

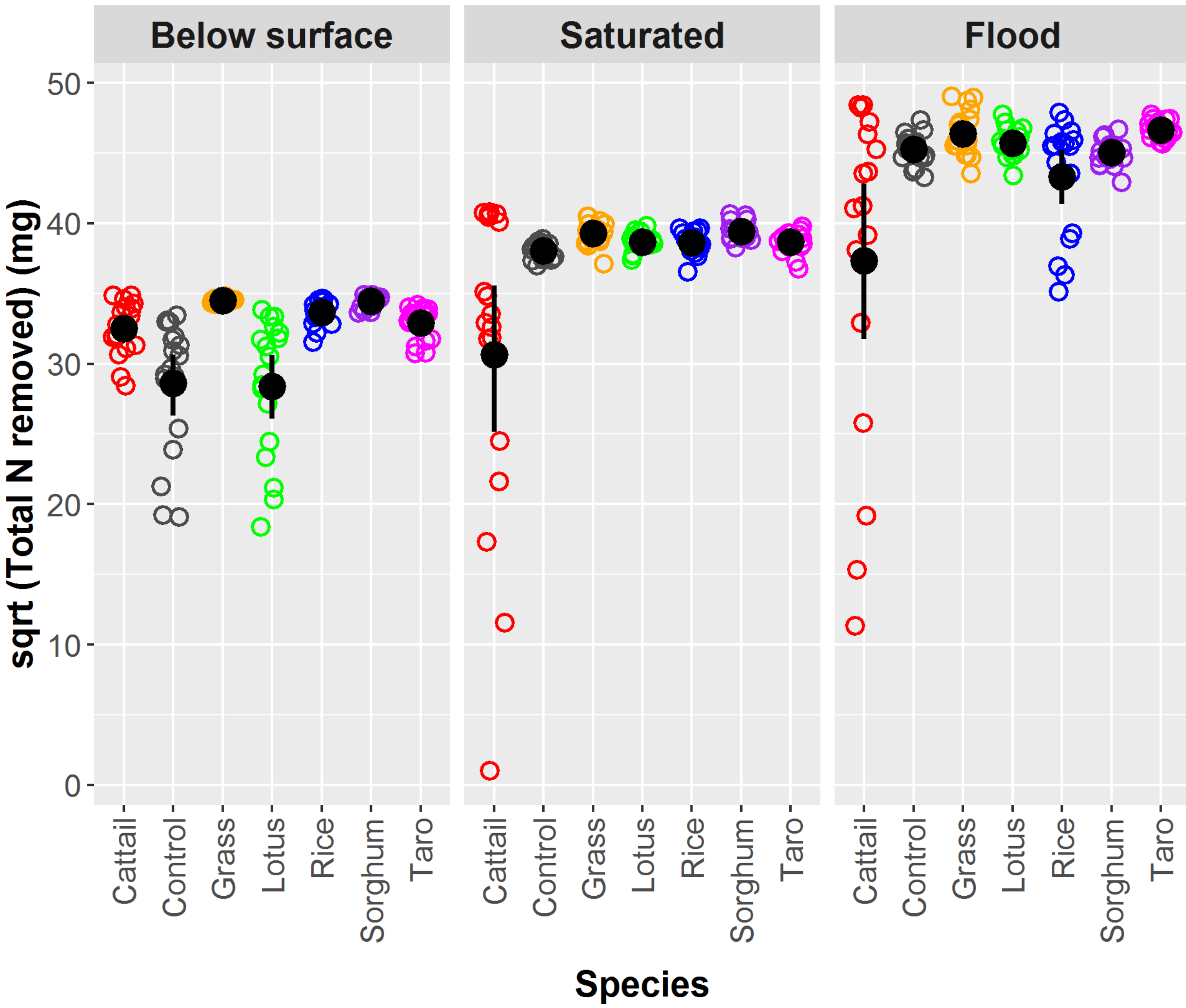

Figure 15. Total N removed by species separated by water level. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 16

Results of a least squared means post-hoc comparison test using a Tukey adjustment for total N removed by species and water level.

Species	Water level	*lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Lotus	Below surface	28.5	0.622	42	26.5	30.5	a
Control	Below surface	28.7	0.622	42	26.6	30.7	a
Cattail	Saturated	30.7	0.622	42	28.7	32.7	ab

Cattail	Below surface	32.5	0.622	42	30.5	34.5	bc
Taro	Below surface	32.9	0.622	42	30.9	34.9	bc
Rice	Below surface	33.7	0.622	42	31.7	35.7	bcd
Sorghum	Below surface	34.5	0.622	42	32.4	36.5	cd
Grass	Below surface	34.5	0.622	42	32.5	36.5	cd
Cattail	Flood	37.0	0.622	42	35.0	39.1	de
Control	Saturated	38.0	0.622	42	36.0	40.0	e
Rice	Saturated	38.6	0.622	42	36.6	40.6	e
Taro	Saturated	38.6	0.622	42	36.6	40.7	e
Lotus	Saturated	38.7	0.622	42	36.7	40.7	e
Grass	Saturated	39.3	0.622	42	37.3	41.3	e
Sorghum	Saturated	39.4	0.622	42	37.4	41.4	e
Rice	Flood	43.1	0.622	42	41.0	45.1	f
Sorghum	Flood	45.0	0.622	42	43.0	47.0	fg
Control	Flood	45.2	0.622	42	43.2	47.2	fg
Lotus	Flood	45.7	0.622	42	43.7	47.7	fg
Grass	Flood	46.4	0.622	42	44.4	48.4	fg
Taro	Flood	46.6	0.622	42	44.6	48.6	g

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

Normalized Percent of Total N Removed

The values of total N removed were not transformed prior to analyses but were normalized, like other response variables, as a percentage of the total N added. All main effects and interactions were very significant ($P < 0.0001$) (Table 17). According to the likelihood ratio test, the whole model was extremely significant ($p = 6.0653e-108$), and the Cox and Snell pseudo R^2 value was 0.817445.

Table 17

Results of repeated measures ANOVA for normalized percent total N removed.

Treatment	*Chisq*	*df*	*P*
Species	220.540	6	<0.0001 ***
Water level	88.198	2	<0.0001 ***
Week	111.871	1	<0.0001 ***
Species:Water level	341.135	12	<0.0001 ***
Species:Week	147.109	6	<0.0001 ***
Water Level:Week	20.939	2	<0.0001 ***
Species:Water level:Week	296.560	12	<0.0001 ***

Note. *Chisq* indicates the chi-squared test statistic values. *Df* indicates the degrees of freedom. *P* indicates the probability that random values would generate a test statistic greater than those observed.
* indicates $0.01 < p < 0.05$. ** indicates $0.001 < p < 0.01$. *** indicates $p < 0.001$.

Species explained a significant amount of variance within the model (Table 17). We saw significant differences between species in terms of the proportion of total N removed (Figure 16, Table 18). Grass and sorghum removed a significantly higher proportion of total N than all other species except taro. Rice, while not different than taro, removed a lower proportion than grass and sorghum, but more than cattail, lotus, and the control, which removed the lowest proportion of total N out of all the focal species.

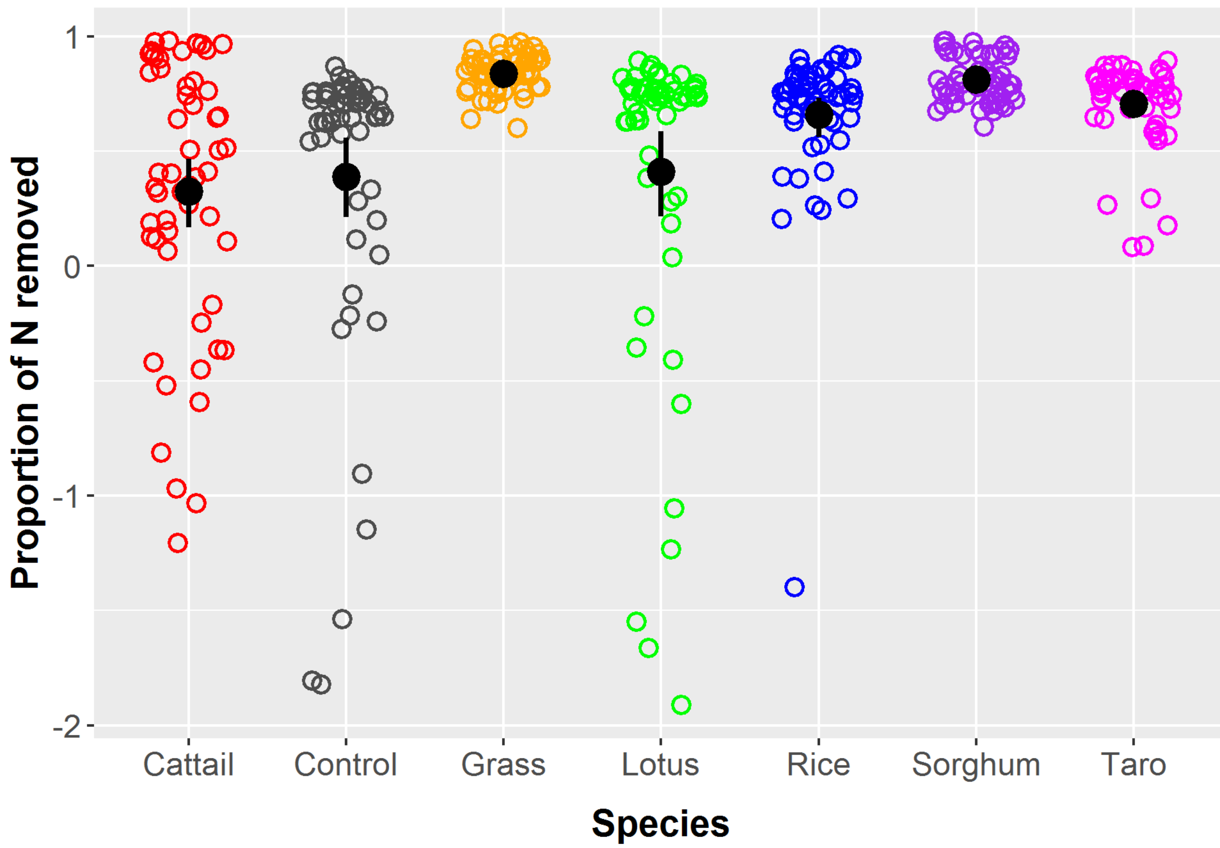

Figure 16. Normalized percent of total N removed by species. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 18

Results of a least squared means post-hoc comparison test using a Tukey adjustment for normalized percent total N removed by species.

Species	*lsmean*	*SE*	*Df*	*lower.CL*	*upper.CL*	*group*
Cattail	0.338	0.0363	42	0.235	0.440	a
Control	0.386	0.0363	42	0.284	0.489	a
Lotus	0.407	0.0363	42	0.305	0.510	a

Rice	0.652	0.0363	42	0.550	0.755	b
Taro	0.715	0.0363	42	0.612	0.817	bc
Sorghum	0.815	0.0363	42	0.712	0.917	c
Grass	0.842	0.0363	42	0.740	0.945	c

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

The water level treatments explain a significant amount of variance in the full model (Table 17). There was a significant difference between water levels (Figure 17, Table 19). Surface and flood were not significantly different from each other but had a significantly greater proportion of total N removed than below surface.

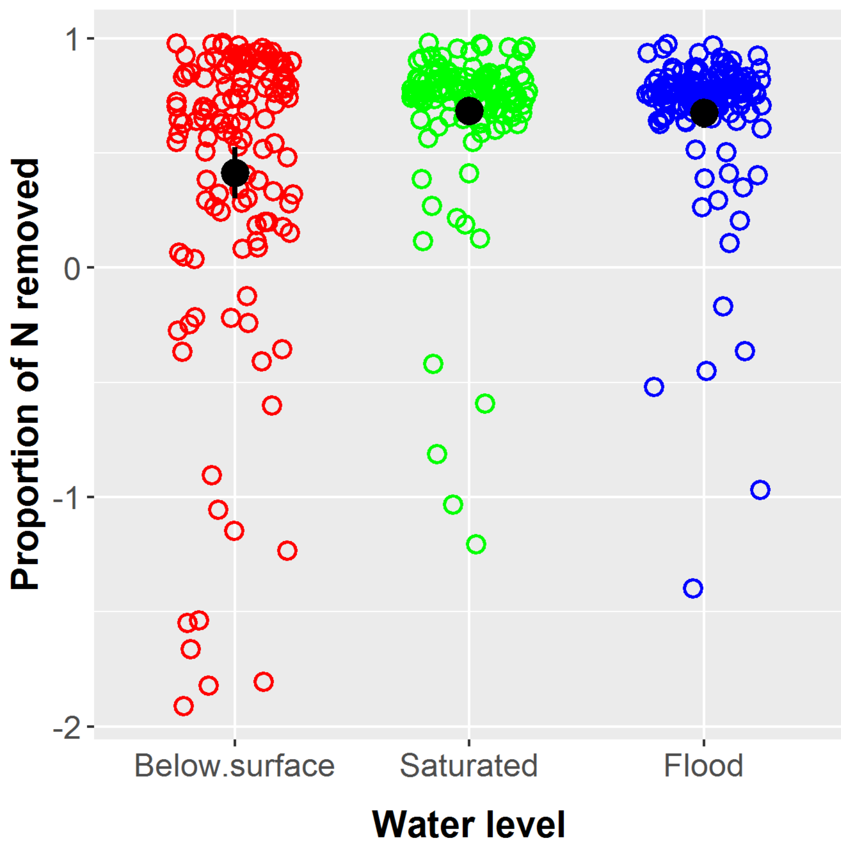

Figure 17. Normalized percent of total N removed by water level. Colors denote moisture regime. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 19

Results of a least squared means post-hoc comparison test using a Tukey adjustment for normalized percent total N removed by water level.

Water level	*lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Below surface	0.415	0.0238	42	0.356	0.474	a
Flood	0.676	0.0238	42	0.617	0.735	b
Surface	0.690	0.0238	42	0.631	0.749	b

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test.

Lower.CL and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

The interaction between species and water level explained a significant amount of variance within the full model (Table 17). We saw significant differences within individual interactions between species and water level in terms of the proportion of total N removed (Figure 18, Table 20). Lotus and the control in below surface removed a significantly lower proportion of total N than all other species and water level interactions. Cattail was not significantly different across each treatment, but those in saturated and flood removed significantly less than all other interactions except for rice in flood and taro in below surface. The majority of interactions were not significantly different from one another.

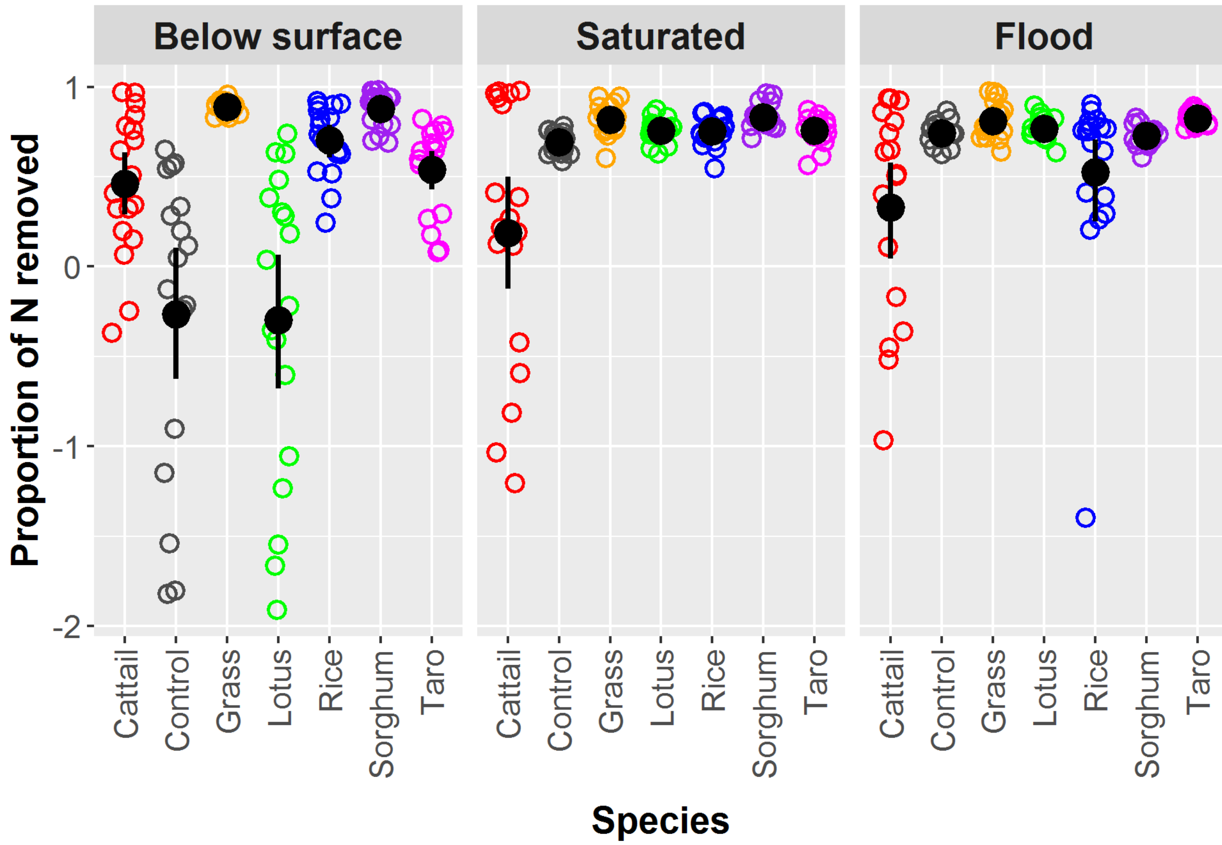

Figure 18. Normalized percent total N removed by species separated by water level. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 20

Results of a least squared means post-hoc comparison test using a Tukey adjustment for normalized percent total N removed by species and water level.

Species	Water level	*lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Lotus	Below surface	-0.314	0.0629	42	-0.51677	-0.1107	a
Control	Below surface	-0.286	0.0629	42	-0.48895	-0.0829	a
Cattail	Saturated	0.198	0.0629	42	-0.00521	0.4008	b
Cattail	Flood	0.329	0.0629	42	0.12643	0.5325	bc
Cattail	Below surface	0.485	0.0629	42	0.28230	0.6883	bcd

Rice	Flood	0.501	0.0629	42	0.29772	0.7038	bcde
Taro	Below surface	0.555	0.0629	42	0.35218	0.7582	cdef
Control	Saturated	0.696	0.0629	42	0.49320	0.8992	def
Rice	Below surface	0.704	0.0629	42	0.50138	0.9074	def
Sorghum	Flood	0.734	0.0629	42	0.53138	0.9374	def
Control	Flood	0.749	0.0629	42	0.54615	0.9522	def
Rice	Saturated	0.752	0.0629	42	0.54879	0.9548	def
Taro	Saturated	0.762	0.0629	42	0.55899	0.9650	def
Lotus	Saturated	0.764	0.0629	42	0.56071	0.9667	def
Lotus	Flood	0.772	0.0629	42	0.56882	0.9749	def
Grass	Flood	0.819	0.0629	42	0.61551	1.0215	def
Grass	Saturated	0.821	0.0629	42	0.61750	1.0235	def
Taro	Flood	0.827	0.0629	42	0.62361	1.0296	ef
Sorghum	Saturated	0.836	0.0629	42	0.63283	1.0389	ef
Sorghum	Below surface	0.873	0.0629	42	0.67037	1.0764	f
Grass	Below surface	0.887	0.0629	42	0.68434	1.0904	f

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different (α = 0.05).

When considering the interaction of species and time within the model, it explained a significant amount of variance (Table 17). There were differences in the proportion of total N removed by species between weeks (Figure 19). In weeks 3 and 4 there were differences between species, but they were more similar than different with the control and lotus removing the smallest proportion of total N. However, in the following weeks, excluding the final week, cattail removed the smallest proportion of all species, especially in week 8 were its proportion of total N removed was negative, showing a greater amount of total N in the effluent than was added at the beginning of that week. Throughout all weeks, grass and sorghum removed the greatest proportion of all other species and the amount they removed was consistent from beginning to end. For taro, there was a trend of a slight gradual decline in the proportion of total N removed except in week 10 where it rose to roughly the same proportion as week 3. Rice displayed an oscillating trend where it dropped between weeks 3 and 4, increased between weeks 4, 6, and 7, and from week 7 through the final week showed a gradual decrease in the proportion of total N removed.

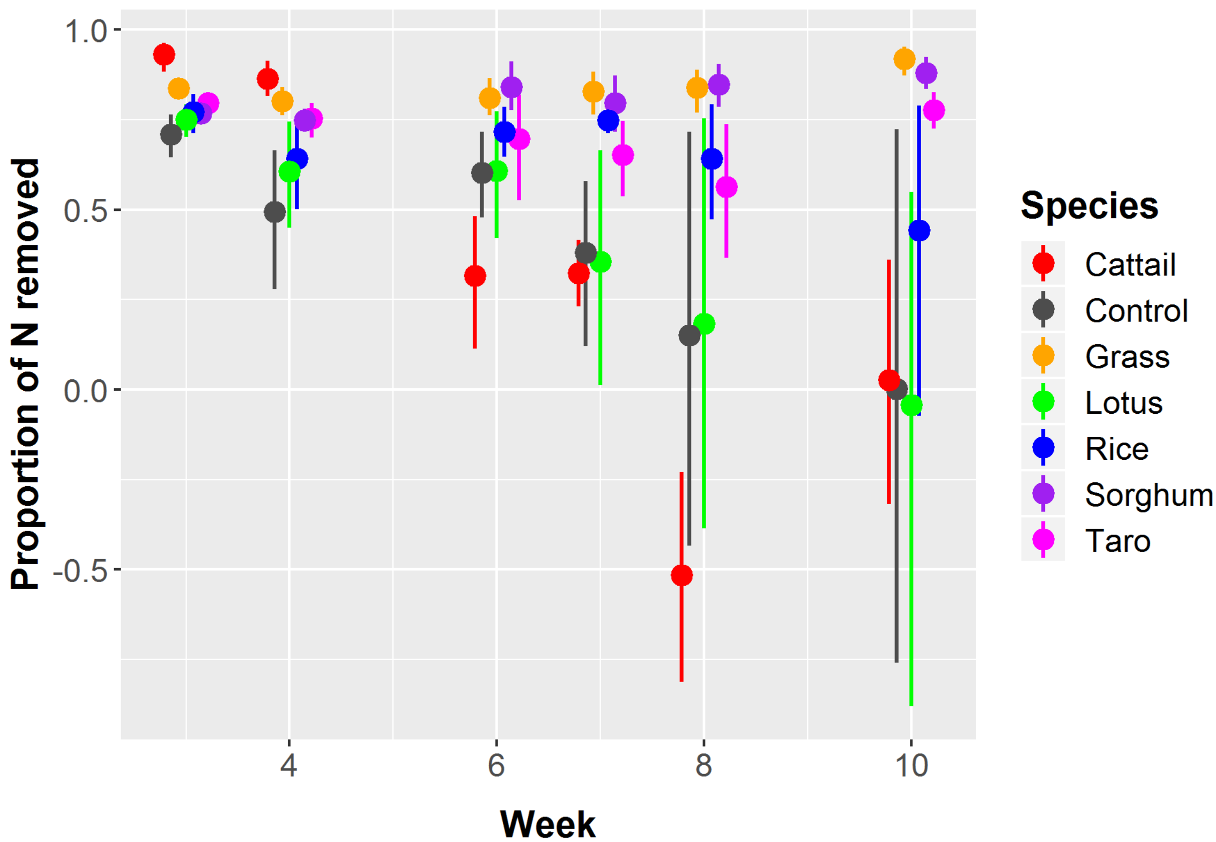

Figure 19. Normalized percent total N removed by species across weeks. Color denotes species. Colored points represent the arithmetic means of all values for a species in a given week. Error bars represent bootstrapped 95% confidence intervals.

A significant amount of variance within the model was also explained by the interaction of water levels and time (Table 17). We saw distinct temporal trends in the proportion of total N removed within different water level treatments throughout the experiment (Figure 20). Below surface exhibited the smallest proportion removed across all weeks and displayed a trend of decreasing removal week by week. Flood and saturated were both very similar in their proportion of total N removed, and, much like below surface, the trends they show are that of decrease as the weeks go by except for the final week where the proportion of total N removed increases.

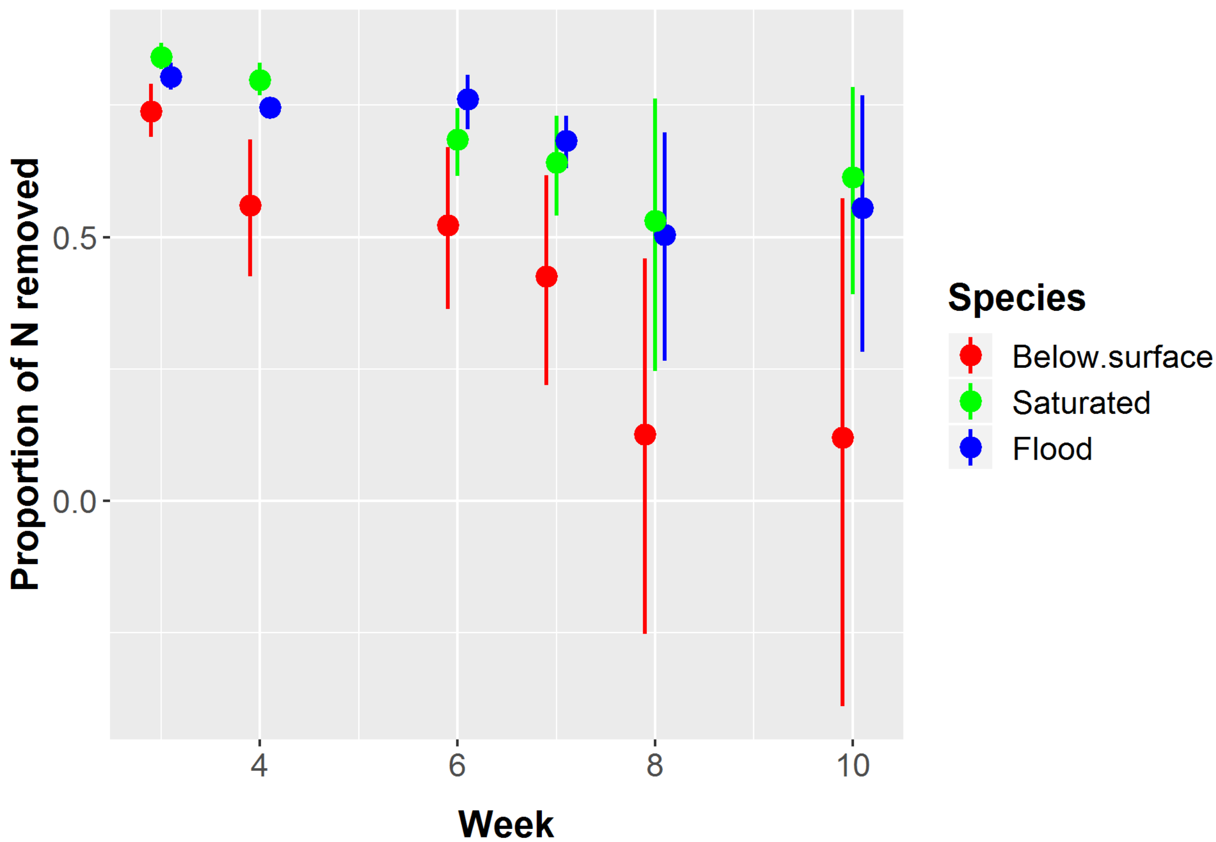

Figure 20. Normalized percent total N removed by water level across weeks. Color denotes species. Colored points represent the arithmetic means of all values for a species in a given week. Error bars represent bootstrapped 95% confidence intervals.

Water Balance

The values of rinse volumes recovered were square root transformed prior to analysis and were normalized as a percent of the water added to the mesocosm every week. All main effects and interactions were very significant ($P < 0.0001$), except for the 3-way interaction of species, water level, and week which was significant with a p-value of 0.0085 (Table 21). According to the likelihood ratio test, the whole model was extremely significant ($p = 2.4638e-98$), and the Cox and Snell pseudo R^2 value was 0.793068.

Table 21

Results of repeated measures ANOVA for percent of rinse volume recovered from effluent.

Treatment	*Chisq*	*df*	*P*
Species	377.497	6	<0.0001 ***
Water level	563.838	2	<0.0001 ***
Week	360.129	1	<0.0001 ***
Species:Water level	88.088	12	<0.0001 ***
Species:Week	121.077	6	<0.0001 ***
Water Level:Week	55.715	2	<0.0001 ***
Species:Water level:Week	26.720	12	0.0085 **

Note. *Chisq* indicates the chi-squared test statistic values. *Df* indicates the degrees of freedom. *P* indicates the probability that random values would generate a test statistic greater than those observed.
* indicates 0.01 < p < 0.05. ** indicates 0.001 < p < 0.01. *** indicates p < 0.001.

Species explained a significant amount of variance within the model (Table 21). There were significant differences between species in terms of the percent of rinse volume recovered (Figure 21, Table 22). Of all species, cattail had the significantly smallest percent of water recovered in the effluent. The percent recovered from rice was significantly greater than cattail, but significantly lower than the other species except for grass. Grass was not significantly different from sorghum but significantly less rinse water was recovered than from the remaining species. Sorghum and taro were not significantly different from each other, but the amount of water recovered was significantly less than from lotus and the control. Lotus and the control had significantly greater percentages of water recovered than all other species.

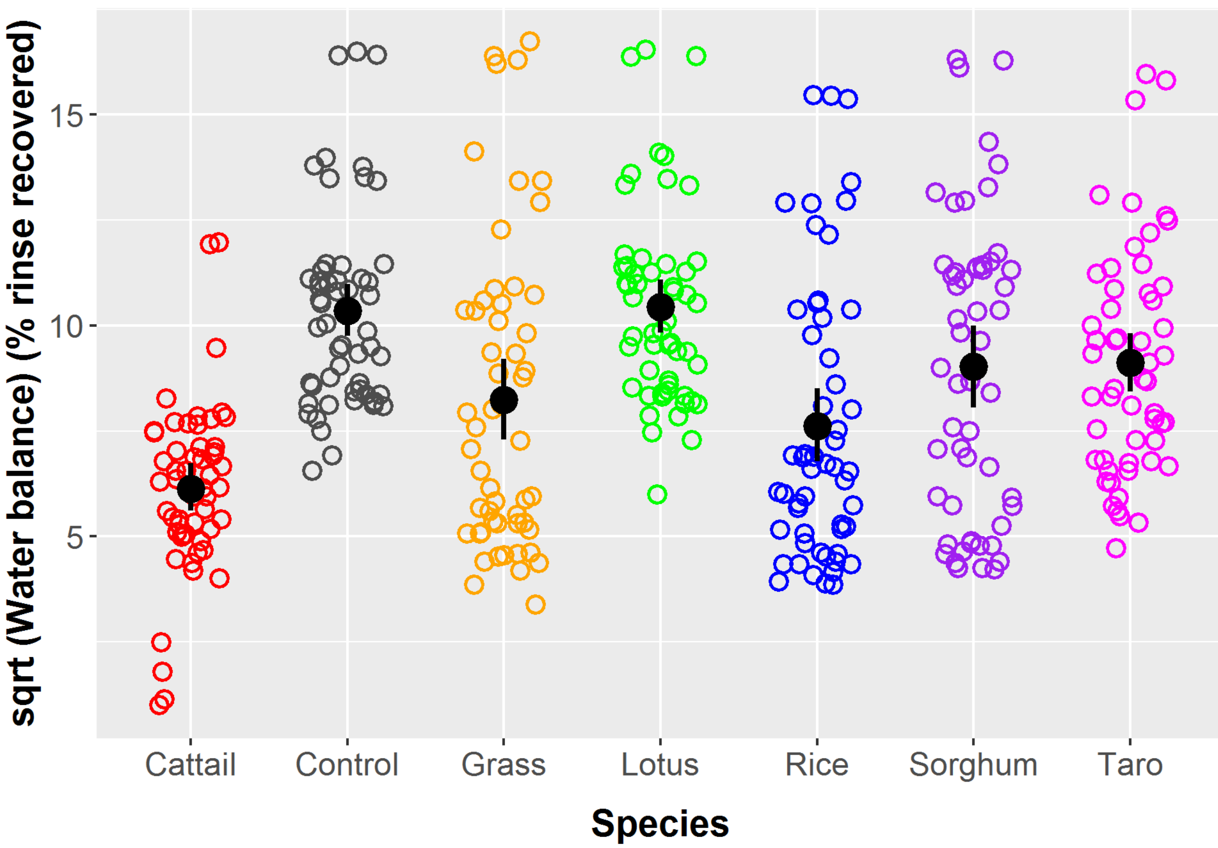

Figure 21. Percent of rinse volume recovered from effluent by species. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 22

Results of a least squared means post-hoc comparison test using a Tukey adjustment for percent of rinse volume recovered from effluent by species.

Species	*lsmean*	*SE*	*Df*	*lower.CL*	*upper.CL*	*group*
Cattail	6.15	0.19	42	5.62	6.69	a
Rice	7.51	0.19	42	6.98	8.05	b
Grass	8.21	0.19	42	7.67	8.74	bc
Sorghum	8.93	0.19	42	8.40	9.47	cd
Taro	9.07	0.19	42	8.53	9.60	d

Control	10.28	0.19	42	9.74	10.81	e
Lotus	10.37	0.19	42	9.83	10.90	e

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

When considering the full model, the water level assigned also explained a significant amount of variance (Table 21). The percent of water recovered from water level treatments were all significantly different from each other (Figure 22, Table 23). The greatest percentage of water was recovered from flood treatments, the second most was recovered from saturated, and the smallest percentage was recovered from below surface treatments.

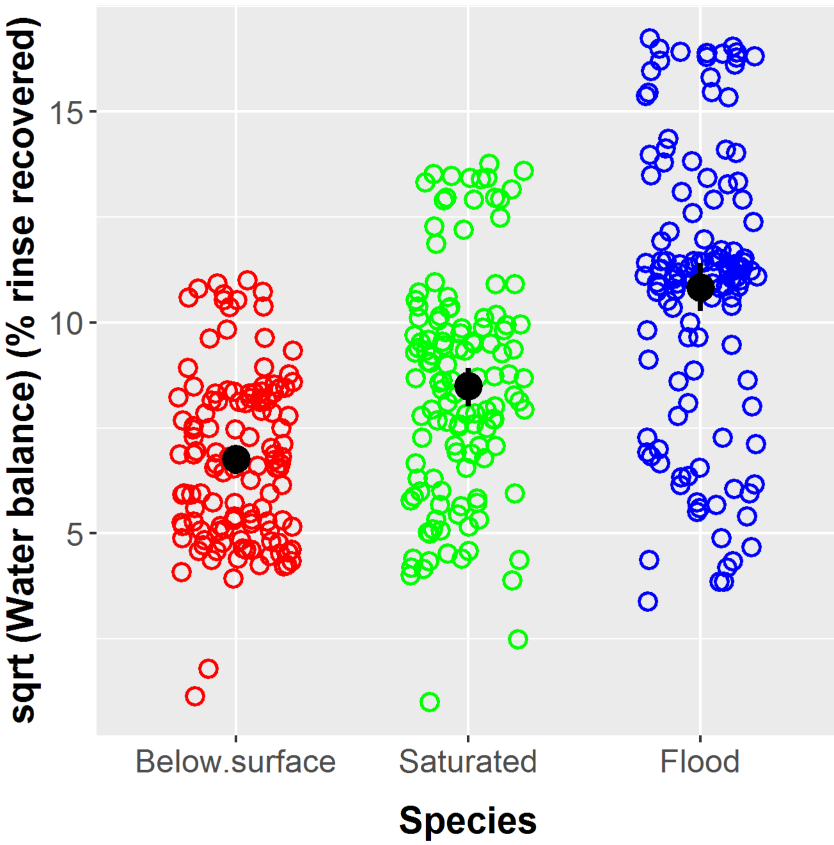

Figure 22. Percent of rinse volume recovered from effluent by water level. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 23

Results of a least squared means post-hoc comparison test using a Tukey adjustment for percent of rinse volume recovered from effluent by water level.

Water level	*lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Below surface	6.72	0.124	42	6.41	7.03	a
Surface	8.44	0.124	42	8.13	8.75	b
Flood	10.78	0.124	42	10.47	11.09	c

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test.

Lower.CL and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

The interaction between species and water level explained a significant amount of variance within the model (Table 21). We saw significant differences in the percent of water recovered from the effluent between interactions (Figure 23, Table 24). Across all water levels cattails were not significantly different from one another and recovered the smallest percent of all other species. Across all treatments, lotus, sorghum, and control under flooded conditions demonstrated the greatest percentages of rinse water recovered and were significantly higher than all other treatment combinations except for grass and taro in flood. In both saturated and below surface, lotus and the control recovered the greatest percent of water. In flood, rice recovered a significantly smaller percent in the effluent than all other species except for cattail.

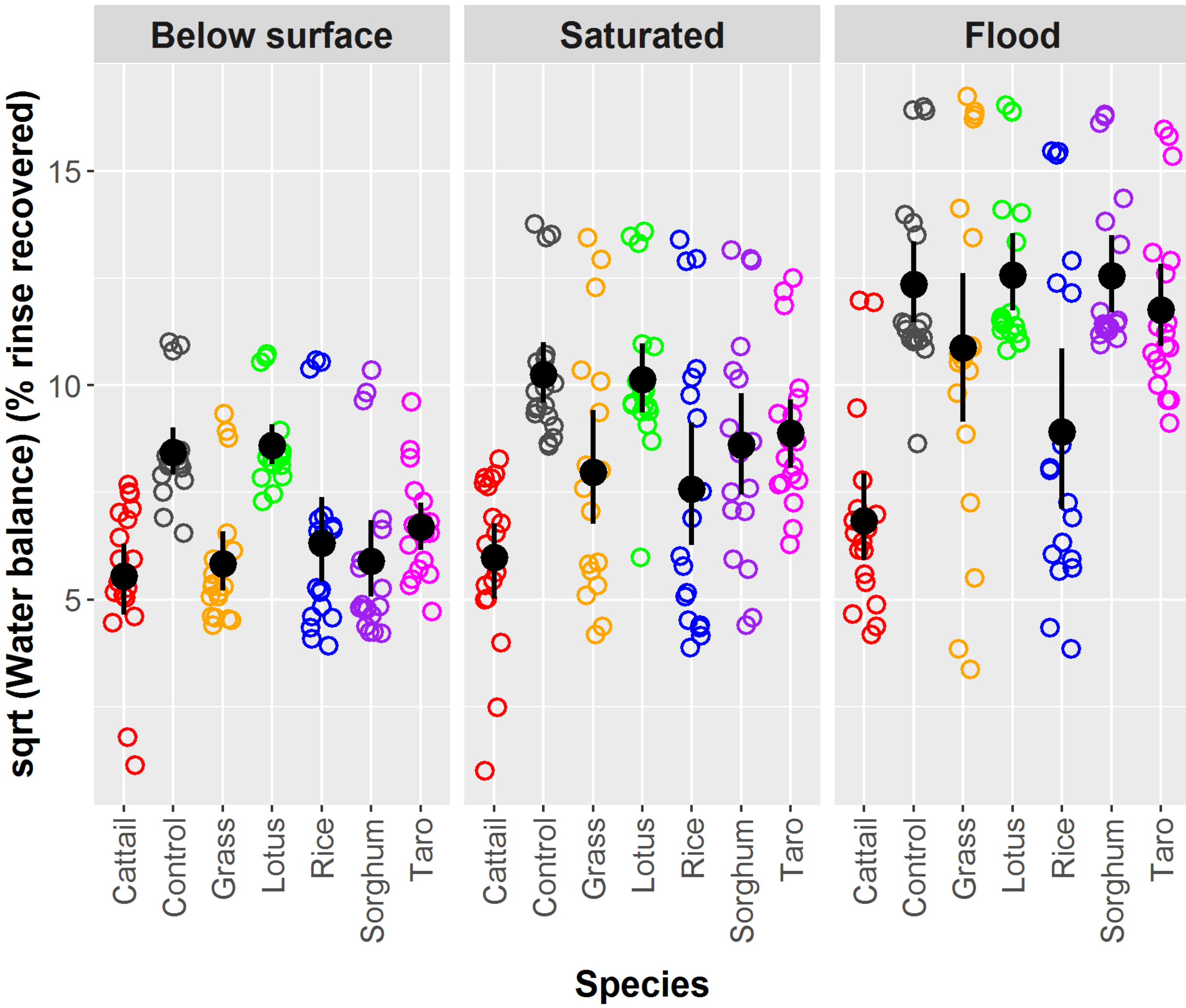

Figure 23. Percent of rinse volume recovered from effluent by species separated by water level. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 24

Results of a least squared means post-hoc comparison test using a Tukey adjustment for percent of rinse volume recovered from effluent by species and water level.

Species	Water level	*lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Cattail	Below surface	5.56	0.329	42	4.50	6.62	a
Grass	Below surface	5.78	0.329	42	4.72	6.84	ab
Sorghum	Below surface	5.79	0.329	42	4.73	6.85	ab

Cattail	Saturated	6.06	0.329	42	5.00	7.12	ab
Rice	Below surface	6.25	0.329	42	5.19	7.31	abc
Taro	Below surface	6.69	0.329	42	5.63	7.75	abcd
Cattail	Flood	6.84	0.329	42	5.78	7.90	abcde
Rice	Saturated	7.48	0.329	42	6.42	8.54	bcdef
Grass	Saturated	7.92	0.329	42	6.86	8.98	cdef
Control	Below surface	8.41	0.329	42	7.35	9.47	defg
Sorghum	Saturated	8.54	0.329	42	7.48	9.60	efg
Lotus	Below surface	8.56	0.329	42	7.50	9.62	efg
Rice	Flood	8.81	0.329	42	7.75	9.87	fg
Taro	Saturated	8.84	0.329	42	7.78	9.91	fg
Lotus	Saturated	10.07	0.329	42	9.01	11.13	gh
Control	Saturated	10.16	0.329	42	9.10	11.22	gh
Grass	Flood	10.93	0.329	42	9.87	11.99	hi
Taro	Flood	11.67	0.329	42	10.61	12.73	hi
Control	Flood	12.27	0.329	42	11.21	13.33	i
Sorghum	Flood	12.47	0.329	42	11.41	13.53	i
Lotus	Flood	12.48	0.329	42	11.42	13.54	i

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

The species by time (week) interaction explained a significant amount of variance within the model (Table 21). Throughout the weeks we saw trends within and differences between species (Figure 24). In weeks 3 and 4 the greatest things to note are that cattail recovered the smallest percentage of water of all species, the other species in those weeks are all very similar, and that between week 3 and 4 there was an increase in the percent of water recovered for all species. From week 6 and beyond we saw a greater stratification of species as time progressed. In week 6, all species are much more similar to one another, but with less still being recovering from cattail than the others, although not as drastically as in previous weeks. From week 6 onward, the greatest percentages of water were recovered from lotus and the control, and these percentages did not vary much. For taro and sorghum, although less water was recovered than from lotus and the control, they behaved similarly in that the percent recovered was consistent. For the last three species – cattail, rice and grass – they displayed a decreasing trend from week 6 until week 10. Starting in week 7, as little water began to be recovered from rice as from cattail, but from week 8 to 10 less was recovered than from cattail. A greater amount of water was consistently recovered from grass than both cattail and rice, but in week 10, the water recovered from grass was slightly less than cattail.

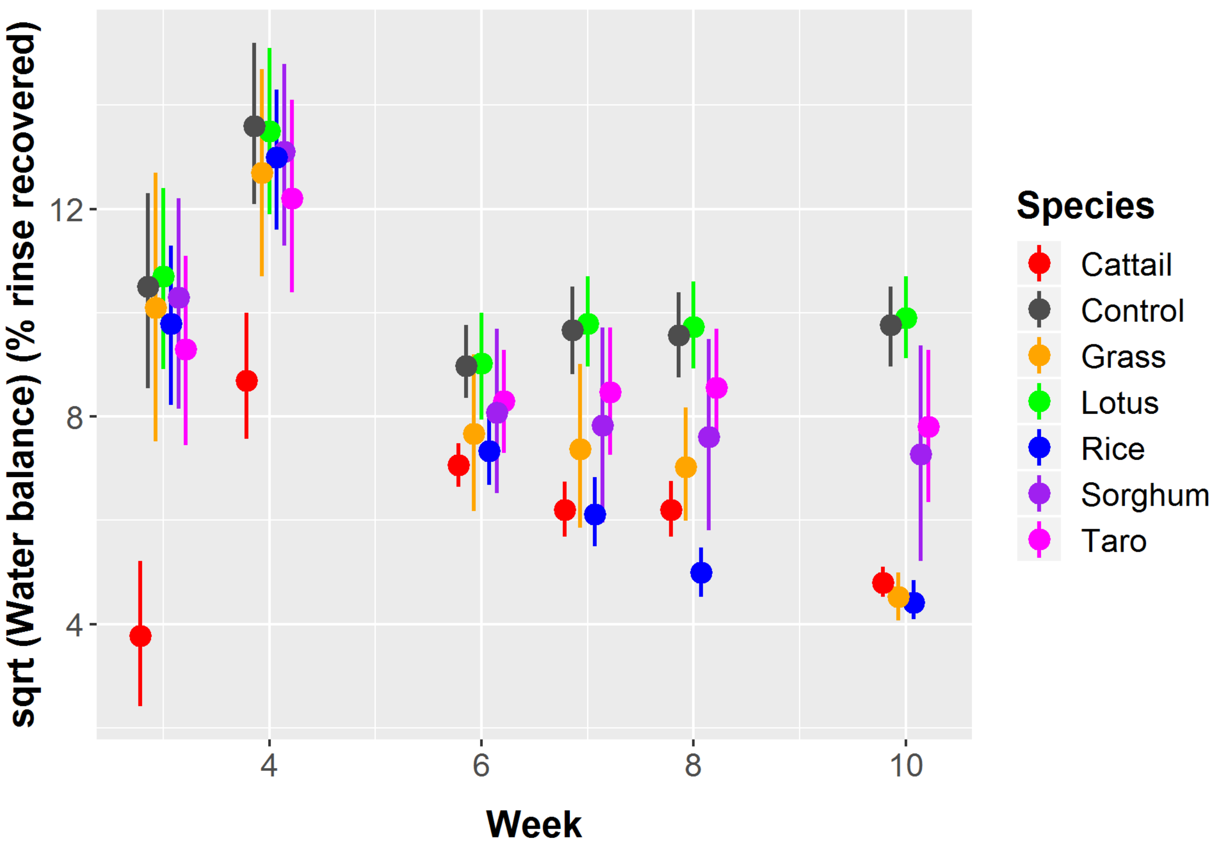

Figure 24. Percent of rinse volume recovered from effluent by species across weeks. Color denotes species. Colored points represent the arithmetic means of all values for a species in a given week. Error bars represent bootstrapped 95% confidence intervals.

Total Biomass Harvested

Given that there was no time dimension for total biomass harvested, we could not run a repeated measures analyses, and total biomass violated the assumptions of a traditional ANOVA, so we performed a permutational ANOVA (bootstrapping) to evaluate the effects of our treatments on total biomass. We ran 10,000 permutations using values sampled with replacement from our observed biomass values. No transformations or normalizations were necessary, thus none were performed prior to analysis. Species and the interaction of species and water level were very significant ($P < 0.0001$), but water level was not significant (Table 25). Alongside the permutational ANOVA we also ran a traditional ANOVA to compare and cross-validate the results of each approach (Table 26), and the results were very similar. According to the permutational F-test, the whole model was highly significant ($p < 2.2e-16$), and the adjusted R^2 value from the traditional ANOVA was 0.8902.

Table 25

Results of permutational ANOVA for total biomass harvested.

Treatment	*df*	*F obs*	*F sim*	*P*
Species	6	70.352	1.063	<0.0001 ***
Water level	2	0.125	0.707	0.8816
Species:Water level	12	8.372	1.051	<0.0001 ***
Residuals	42			

Note. *Df* indicates the degrees of freedom. *F obs* indicates the F statistic generated from observed values. *F sim* indicates the average of all F statistics generated from the bootstrap procedure. *P* indicates the proportion of trials where the simulated F statistic was greater than or equal to the observed F statistic.
* indicates $0.01 < p < 0.05$. ** indicates $0.001 < p < 0.01$. *** indicates $p < 0.001$.

Table 26

Results of traditional Analysis of Variance for total biomass harvested.

Treatment	*SS*	*df*	*F value*	*P*
Species	218490	6	70.3521	<0.0001 ***
Water level	130	2	0.1253	0.8826
Species:Water level	52003	12	8.3723	<0.0001 ***
Residuals	21740	42		

Note. *SS* indicates the sum of squares values. *Df* indicates the degrees of freedom. *P* indicates the confidence level, based on the probability that random values would generate a test statistic greater than those observed.

* indicates 0.01 < p < 0.05. ** indicates 0.001 < p < 0.01. *** indicates p < 0.001.

Species explained a significant amount of variance within the model (Table 25, Table 26). We saw significant differences in terms of total biomass harvested between species (Figure 25, Table 27). Lotus and the control were not significantly different from each other, but from them significantly less total biomass was harvested than from all other species. The greatest amount of total biomass harvested was from cattail and grass, the two of which were not significantly different from each other. The remaining species – taro, sorghum, and rice – were not significantly different from one another, but had significantly more biomass harvested than lotus and the control and significantly less than cattail and grass.

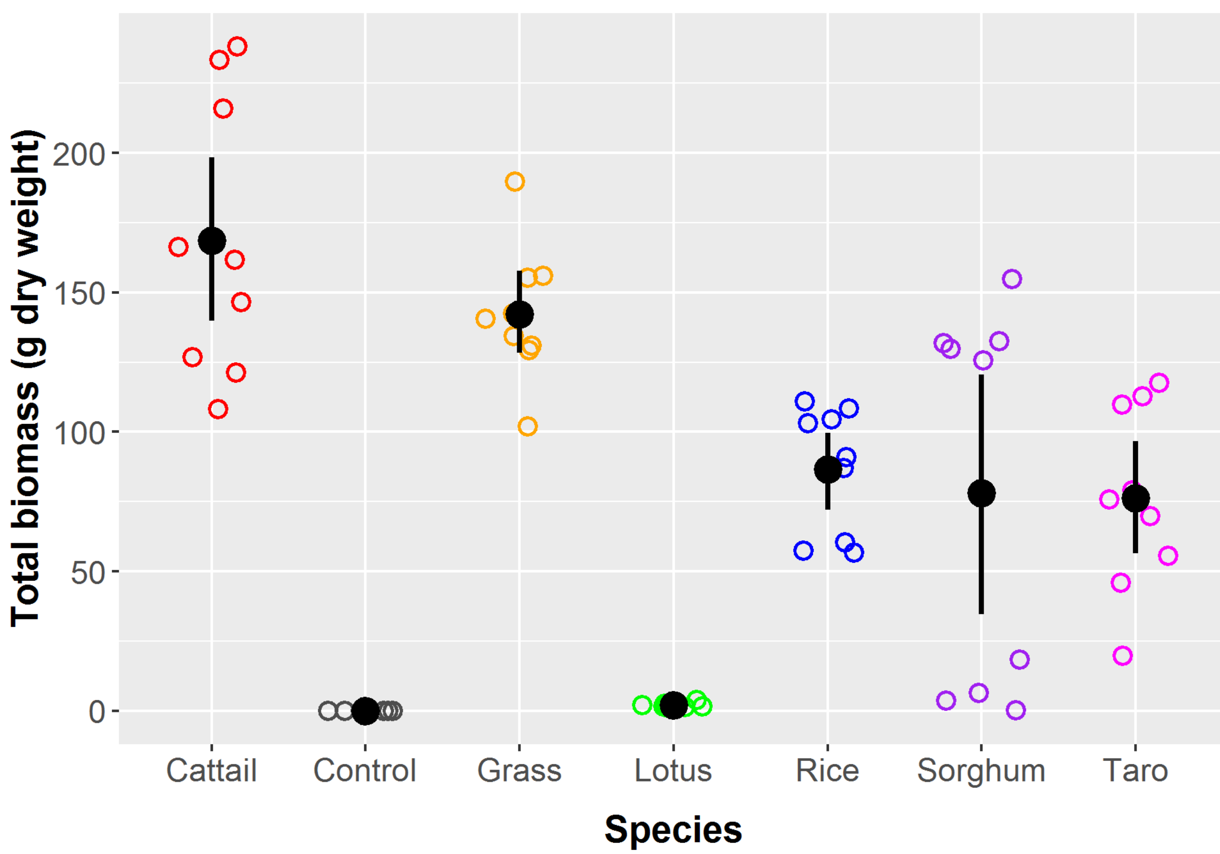

Figure 25. Total biomass harvested by species. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 27

Results of a least squared means post-hoc comparison test using a Tukey adjustment for total biomass harvested by species.

Species	*lsmean*	*SE*	*Df*	*lower.CL*	*upper.CL*	*group*
Control	0.00	7.58	42	-21.4	21.4	a
Lotus	1.94	7.58	42	-19.4	23.3	a
Taro	76.16	7.58	42	54.8	97.5	b
Sorghum	78.11	7.58	42	56.7	99.5	b
Rice	86.56	7.58	42	65.2	107.9	b
Grass	142.21	7.58	42	120.8	163.6	c
Cattail	168.64	7.58	42	147.3	190.0	c

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

The interaction of species and water level explained a significant amount of variance within the model (Table 25, Table 26). There were significant differences between the interactions of species and water level (Figure 26, Table 28). We saw certain trends within species across treatments. Cattail in both flood and saturated had the greatest amount of total biomass, and as the amount of water available increased the greater the harvestable biomass. Grass had the second most total harvested biomass in flood and saturated, and across all three treatments total biomass was homogenous. Rice showed a positive relationship to the increase in water level in terms of total biomass. Taro, much like grass, was not significantly different across treatments. Lotus and the control were not significantly different from one another and produced significantly less biomass than all other species and water level combinations except for sorghum in flood. Sorghum had the greatest amount of total biomass of all species in below surface, and in saturated we harvested similar amounts, however in flood the amount harvested was not significantly different from the control.

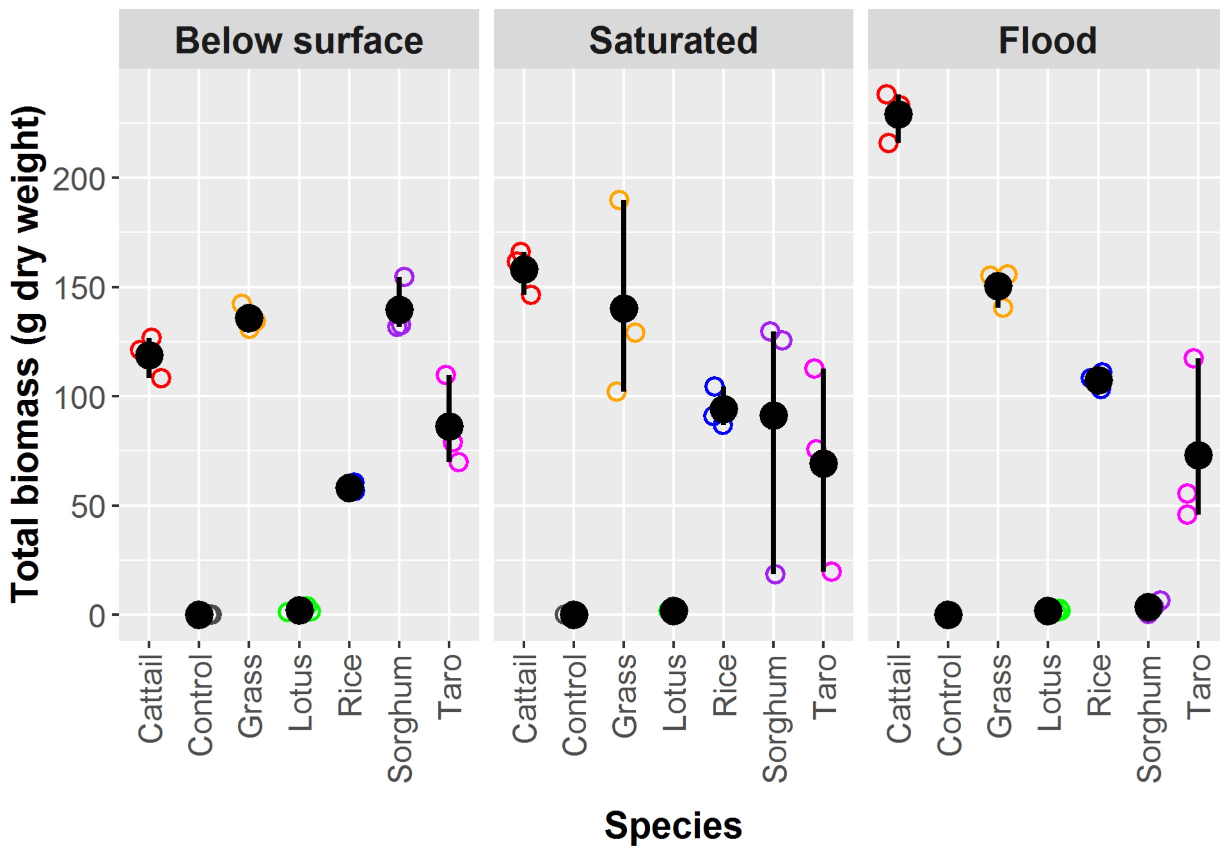

Figure 26. Total biomass harvested by species separated by water level. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 28

Results of a least squared means post-hoc comparison test using a Tukey adjustment for total biomass harvested by species and water level.

Species	Water level	*Lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Control	Flood	0.00	13.1	42	-42.4	42.4	a
Control	Saturated	0.00	13.1	42	-42.4	42.4	a
Control	Below surface	0.00	13.1	42	-42.4	42.4	a
Lotus	Flood	1.80	13.1	42	-40.6	44.2	a
Lotus	Saturated	1.84	13.1	42	-40.5	44.2	a
Lotus	Below surface	2.17	13.1	42	-40.6	44.5	a
Sorghum	Flood	3.40	13.1	42	-39.0	45.8	ab
Rice	Below surface	58.12	13.1	42	15.8	100.5	abc
Taro	Saturated	69.38	13.1	42	27.0	111.7	abcd
Taro	Flood	72.93	13.1	42	30.6	115.3	bcde
Taro	Below surface	86.18	13.1	42	43.8	128.5	cdef
Sorghum	Saturated	91.25	13.1	42	48.9	133.6	cdefg
Rice	Saturated	94.08	13.1	42	51.7	136.4	cdefg
Rice	Flood	107.48	13.1	42	65.1	149.8	cdefg
Cattail	Below surface	118.69	13.1	42	76.3	161.1	cdefg
Grass	Below surface	135.85	13.1	42	93.5	178.2	defg
Sorghum	Below surface	139.69	13.1	42	97.3	182.1	defg
Grass	Saturated	140.30	13.1	42	97.9	182.7	efg
Grass	Flood	150.49	13.1	42	108.1	192.9	fg
Cattail	Saturated	158.13	13.1	42	115.8	200.5	g
Cattail	Flood	229.09	13.1	42	186.7	271.5	h

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

Edible Biomass Harvested

As with total biomass, there was no time dimension for edible biomass harvested and edible biomass also violated the assumptions of ANOVA, so we performed a permutational ANOVA to evaluate the effects of our treatments on edible biomass. We ran 10,000 permutations using values sampled with replacement from our observed values. No transformations or normalizations were necessary or performed prior to analysis. Only species was significant ($P < 0.0001$), while the other treatments were not significant (Table 29). Alongside the permutational ANOVA we also ran a traditional ANOVA to compare and cross-validate the results of each approach (Table 26), and the results were very similar. According to the permutational F-test, the whole model was significant ($p = 0.0002627$), and the adjusted R^2 value from the traditional ANOVA was 0.4519.

Table 29

Results of permutational ANOVA for edible biomass harvested.

Treatment	*Df*	*F obs*	*F sim*	*P*
Species	6	10.407	1.046	<0.0001 ***
water.level	2	0.268	1.063	0.7824
species:water.level	12	0.679	1.049	0.7865

Residuals	42

Note. *Df* indicates the degrees of freedom. *F obs* indicates the F statistic generated from observed values. *F sim* indicates the average of all F statistics generated from the bootstrap procedure. *P* indicates the proportion of trials where the simulated F statistic was greater than or equal to the observed F statistic.
* indicates 0.01 < p < 0.05. ** indicates 0.001 < p < 0.01. *** indicates p < 0.001.

Table 30

Results of traditional Analysis of Variance for edible biomass harvested.

Treatment	*SS*	*df*	*F value*	*Pr(>F)*
Intercept	1747.0	1	20.9918	<0.0001 ***
Species	5196.6	6	10.4072	<0.0001 ***
Water level	44.6	2	0.2678	0.7663
Species:Water level	678.1	12	0.6791	0.7614
Residuals	3495.3	42		

Note. *SS* indicates the sum of squares values. *Df* indicates the degrees of freedom. *Pr(>F)* indicates the probability that random values would generate a test statistic greater than those observed.
* indicates 0.01 < p < 0.05. ** indicates 0.001 < p < 0.01. *** indicates p < 0.001.

Species explained a significant amount of variance within the model (Table 29, Table 30). We saw significant differences between species in terms of edible biomass harvested (Figure 27, Table 31). Only two species produced edible biomass, namely sorghum and taro, and taro was significantly different from all other species.

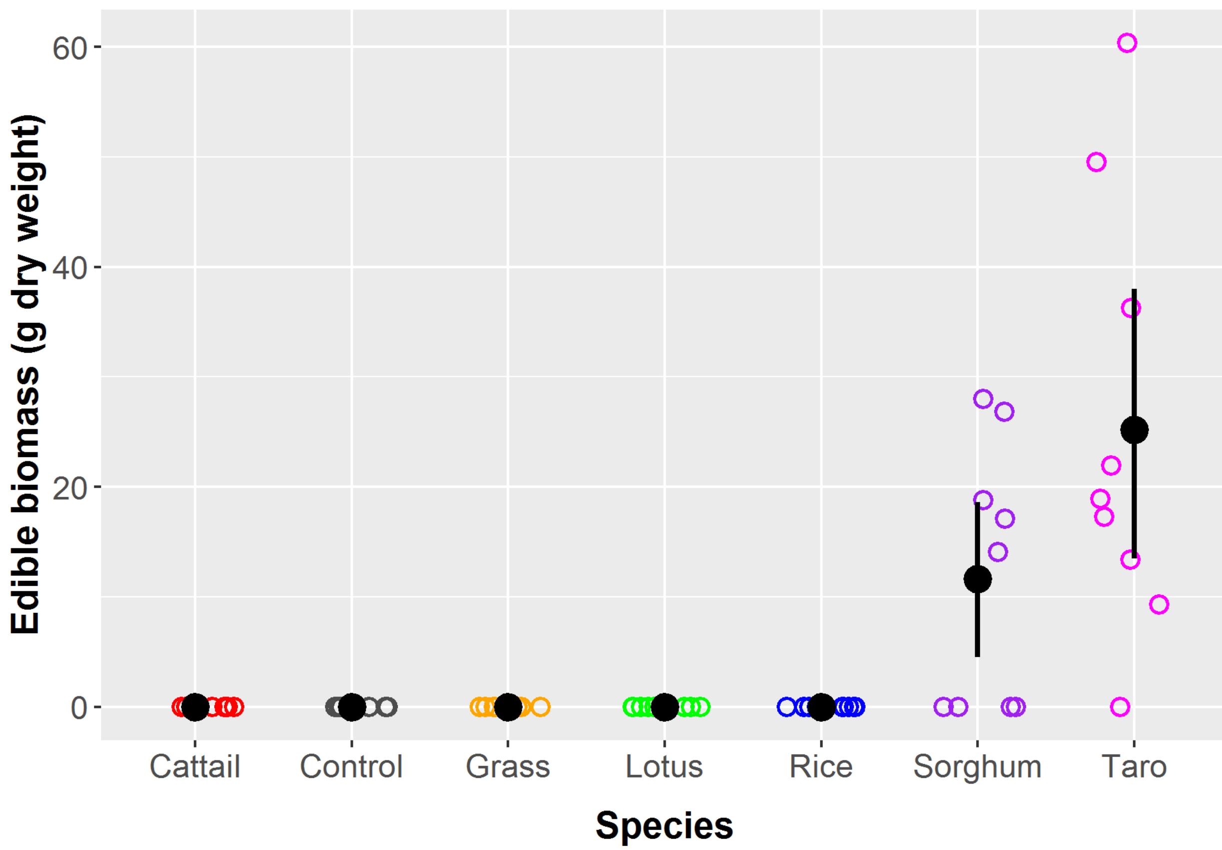

Figure 27. Edible biomass harvested by species. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 31

Results of a least squared means post-hoc comparison test using a Tukey adjustment for edible biomass harvested by species.

Species	*lsmean*	*SE*	*Df*	*lower.CL*	*upper.CL*	*group*
Control	0.00	3.04	42	-8.57	8.57	a
Lotus	0.00	3.04	42	-8.57	8.57	a
Cattail	0.00	3.04	42	-8.57	8.57	a
Rice	0.00	3.04	42	-8.57	8.57	a
Grass	0.00	3.04	42	-8.57	8.57	a

Sorghum	11.6	3.04	42	3.07	20.22	a
Taro	25.2	3.04	42	16.64	33.79	b

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different (α = 0.05).

While the interaction of species and water level was not significant within the model, we did see some differences within species across separate treatments (Figure 28, Table 32). Taro was not significantly different across treatments, but sorghum in flood produced no edible biomass whereas edible biomass was produced in both below surface and saturated treatments.

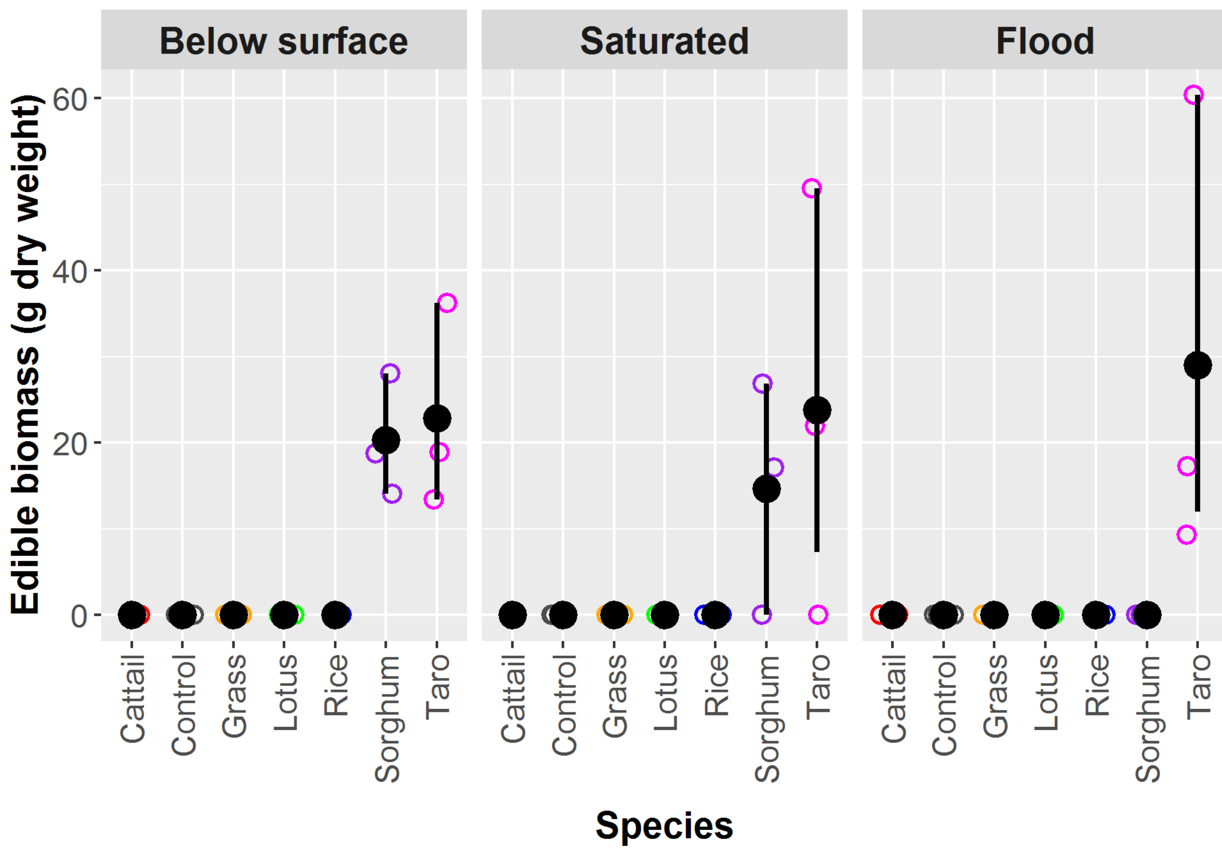

Figure 28. Edible biomass harvested by species separated by water level. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 32

Results of a least squared means post-hoc comparison test using a Tukey adjustment for edible biomass harvested by species and water level.

Species	Water level	*Lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Control	Flood	0.00	5.27	42	-16.99	17.0	a
Control	Below surface	0.00	5.27	42	-16.99	17.0	a
Control	Saturated	0.00	5.27	42	-16.99	17.0	a
Sorghum	Flood	0.00	5.27	42	-16.99	17.0	a
Rice	Below surface	0.00	5.27	42	-16.99	17.0	a

Lotus	Below surface	0.00	5.27	42	-16.99	17.0	a
Cattail	Below surface	0.00	5.27	42	-16.99	17.0	a
Lotus	Flood	0.00	5.27	42	-16.99	17.0	a
Lotus	Saturated	0.00	5.27	42	-16.99	17.0	a
Rice	Saturated	0.00	5.27	42	-16.99	17.0	a
Cattail	Saturated	0.00	5.27	42	-16.99	17.0	a
Cattail	Flood	0.00	5.27	42	-16.99	17.0	a
Rice	Flood	0.00	5.27	42	-16.99	17.0	a
Grass	Flood	0.00	5.27	42	-16.99	17.0	a
Grass	Below surface	0.00	5.27	42	-16.99	17.0	a
Grass	Saturated	0.00	5.27	42	-16.99	17.0	a
Sorghum	Saturated	14.6	5.27	42	-2.34	31.6	ab
Sorghum	Below surface	20.3	5.27	42	3.29	37.3	ab
Taro	Below surface	22.9	5.27	42	5.89	39.8	ab
Taro	Saturated	23.8	5.27	42	6.84	40.8	ab
Taro	Flood	29.0	5.27	42	11.99	46.0	b

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

Aboveground Biomass Harvested

As with prior biomass response variables, we performed a permutational ANOVA to evaluate the effects of our treatments on aboveground biomass and used 10,000 permutations sampled with replacement from our observed data. No transformations or normalizations were necessary or performed prior to analysis. Species and the interaction of species and water level were highly significant ($P < 0.0001$) (Table 33). There was strong agreement with the permutational ANOVA and a traditional type III ANOVA that was also run for aboveground biomass using the same model terms (Table 34).

Table 33

Results of permutational ANOVA for aboveground biomass harvested.

Treatment	*Df*	*F obs*	*F sim*	*P*
Species	6	106.951	1.050	<0.0001 ***
water.level	2	1.654	0.716	0.2081
species:water.level	12	10.145	1.049	<0.0001 ***
Residuals	42			

Note. *Df* indicates the degrees of freedom. *F obs* indicates the F statistic generated from observed values. *F sim* indicates the average of all F statistics generated from the bootstrap procedure. *P* indicates the proportion of trials where the simulated F statistic was greater than or equal to the observed F statistic.
* indicates $0.01 < p < 0.05$. ** indicates $0.001 < p < 0.01$. *** indicates $p < 0.001$.

Table 34

Results for traditional Analysis of Variance for aboveground biomass harvested.

Treatment	*SS*	*df*	*F value*	*Pr(>F)*
(Intercept)	172513	1	919.866	<0.0001 ***
Species	120346	6	106.951	<0.0001 ***
water.level	621	2	1.6544	0.2034
species:water.level	22831	12	10.1447	<0.0001 ***
Residuals	7877	42		

Note. *SS* indicates the sum of squares values. *Df* indicates the degrees of freedom. *Pr(>F)* indicates the probability that random values would generate a test statistic greater than those observed.
* indicates 0.01 < p < 0.05. ** indicates 0.001 < p < 0.01. *** indicates p < 0.001.

Within the model, species explained a significant amount of variance (Table 33). We saw significant differences between species in terms of aboveground biomass harvested (Figure 29, Table 35). Grass and cattail were not significantly different from one another, but significantly more aboveground biomass was harvested from these than all other species. Rice produced a significantly greater amount of aboveground biomass than the remaining species except for sorghum. Sorghum and taro were not significantly different from one another, but they had more aboveground biomass than lotus or the control. Lotus and the control had significantly less aboveground biomass than all other species.

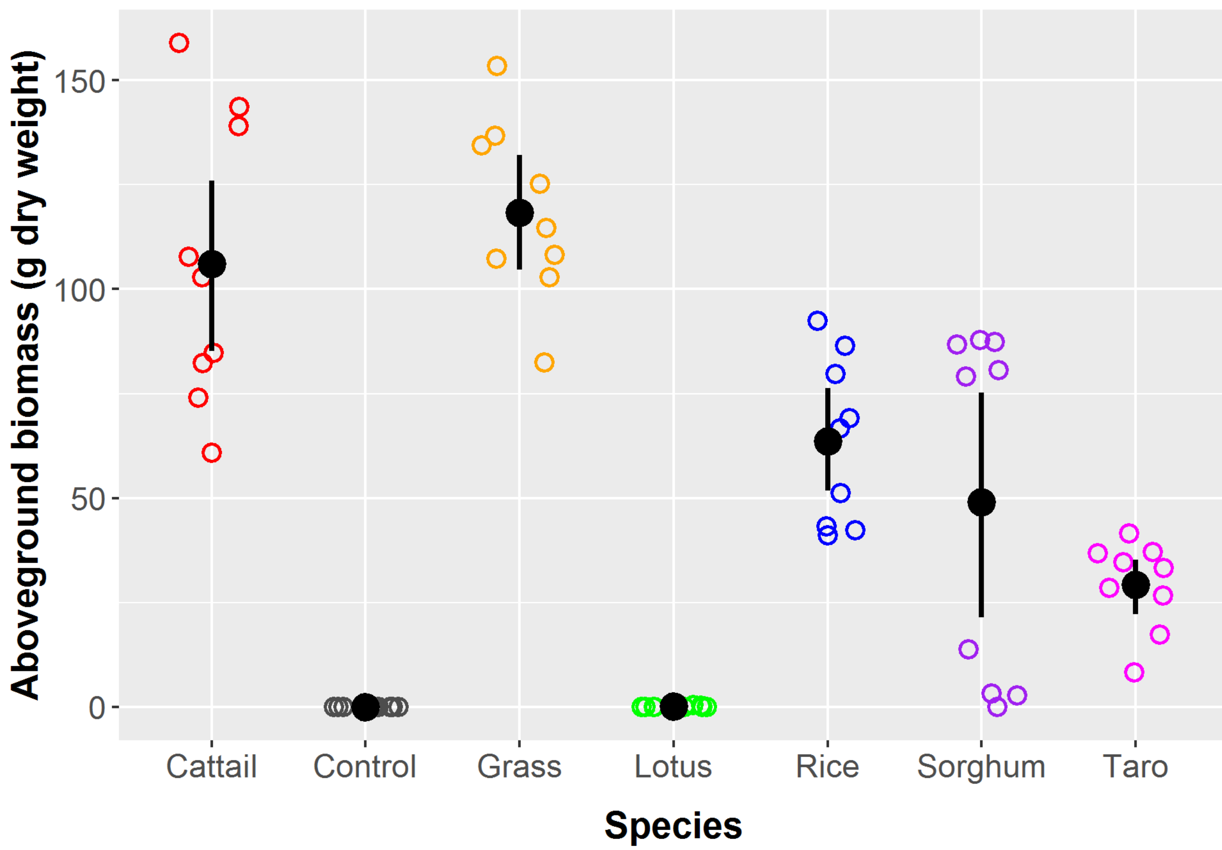

Figure 29. Aboveground biomass harvested by species. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 35

Results of a least squared means post-hoc comparison test using a Tukey adjustment for aboveground biomass harvested by species.

Species	*lsmean*	*SE*	*Df*	*lower.CL*	*upper.CL*	*group*
Control	0	5	42	-12.9	12.9	a
Lotus	0.0778	5	42	-12.8	13	a
Taro	29.32	5	42	16.4	42.2	b
Sorghum	49.006	5	42	36.1	61.9	bc
Rice	63.57	5	42	50.7	76.4	c

Cattail	106	5	42	93.1	118.9	d
Grass	118.33	5	42	105.5	131.2	d

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different (α = 0.05).

The interaction of species and water level also explained a significant amount of variance within the model (Table 33). Significant differences and clear trends in aboveground biomass were seen among treatment combinations (Figure 30, Table 36). Across all treatments, lotus and the control produced the least aboveground biomass of all species, also sorghum in flood was not significantly different from them. The aboveground biomass of sorghum showed a negative relationship to the increase in water added. Grass, cattail, and rice, by contrast, displayed a positive relationship to the increase in water added in terms of aboveground biomass harvested. Taro was consistent across water levels.

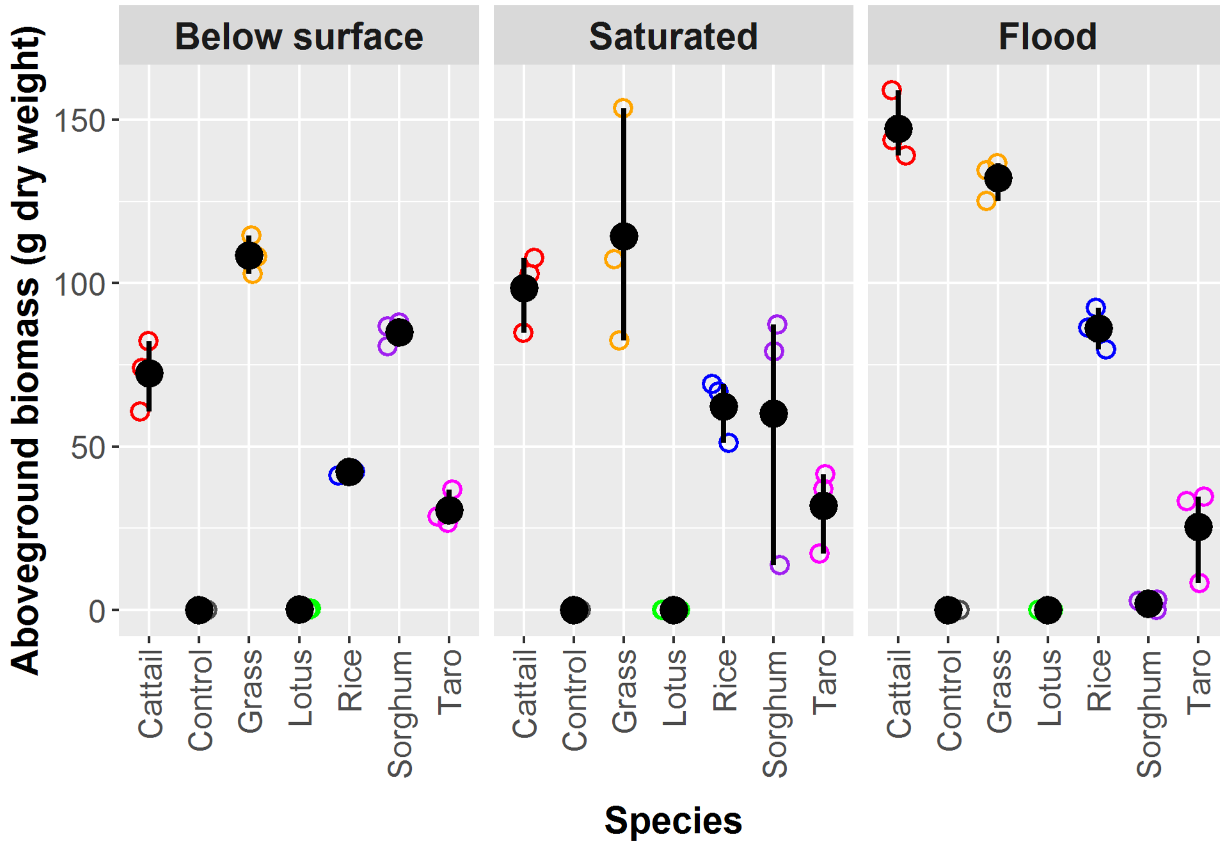

Figure 30. Aboveground biomass harvested by species separated by water level. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 36

Results of a least squared means post-hoc comparison test using a Tukey adjustment for aboveground biomass harvested by species and water level.

Species	Water level	*Lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Control	Below surface	0	7.91	42	-25.5	25.5	a
Control	Saturated	0	7.91	42	-25.5	25.5	a
Control	Flood	0	7.91	42	-25.5	25.5	a
Lotus	Saturated	0	7.91	42	-25.5	25.5	a
Lotus	Flood	0	7.91	42	-25.5	25.5	a

Lotus	Below surface	0.233	7.91	42	-25.267	25.7	a
Sorghum	Flood	1.933	7.91	42	-23.567	27.4	a
Taro	Flood	25.373	7.91	42	-0.127	50.9	ab
Taro	Below surface	30.643	7.91	42	5.143	56.1	abc
Taro	Saturated	31.943	7.91	42	6.443	57.4	abc
Rice	Below surface	42.22	7.91	42	16.72	67.7	abc
Sorghum	Saturated	60.047	7.91	42	34.547	85.5	bcd
Rice	Saturated	62.34	7.91	42	36.84	87.8	bcd
Cattail	Below surface	72.35	7.91	42	46.85	97.8	cde
Sorghum	Below surface	85.037	7.91	42	59.537	110.5	de
Rice	Flood	86.15	7.91	42	60.65	111.6	de
Cattail	Saturated	98.44	7.91	42	72.94	123.9	def
Grass	Below surface	108.5	7.91	42	82.997	134	efg
Grass	Saturated	114.38	7.91	42	88.88	139.9	efg
Grass	Flood	132.11	7.91	42	106.61	157.6	fg
Cattail	Flood	147.21	7.91	42	121.707	172.7	g

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

Belowground Biomass Harvested

As with prior biomass variables, we performed a permutational ANOVA with 10,000 permutations sampled with replacement to evaluate the effects of our treatments on belowground biomass. No transformations or normalizations were necessary or performed. Species and the interaction of species and water level were highly significant within the model (P < 0.0001 (Table 37). There was strong agreement between the permutational ANOVA and the traditional type III ANOVA that was also run for this variable (Table 38).

Table 37

Results of permutational ANOVA for belowground biomass harvested.

Treatment	*Df*	*F obs*	*F sim*	*P*
Species	6	102.953	1.053	<0.0001 ***
water.level	2	0.669	0.693	0.5127
species:water.level	12	10.320	1.039	<0.0001 ***
Residuals	42			

Note. *Df* indicates the degrees of freedom. *F obs* indicates the F statistic generated from observed values. *F sim* indicates the average of all F statistics generated from the bootstrap procedure. *P* indicates the proportion of trials where the simulated F statistic was greater than or equal to the observed F statistic.
* indicates 0.01 < p < 0.05. ** indicates 0.001 < p < 0.01. *** indicates p < 0.001.

Table 38

Results of traditional Analysis of Variance for belowground biomass harvested.

Treatment	*SS*	*df*	*F value*	*Pr(>F)*
Species	23082.5	6	102.953	<0.0001 ***
water.level	50	2	0.6689	0.5176

species:water.level	4627.4	12	10.3195	<0.0001 ***
Residuals	1569.4	42		

Note. *SS* indicates the sum of squares values. *Df* indicates the degrees of freedom. *Pr(>F)* indicates the probability that random values would generate a test statistic greater than those observed.
* indicates $0.01 < p < 0.05$. ** indicates $0.001 < p < 0.01$. *** indicates $p < 0.001$.

Species explained a significant amount of variance within the model (Table 37). There were significant differences between species in belowground biomass (Figure 31, Table 39). Of all species the greatest amount of belowground biomass came from cattail, which was significantly different from all other species. Grass, rice, taro, and sorghum were not significantly different from each other, and, while producing less biomass than cattail, they had significantly more harvestable belowground biomass than lotus or the control.

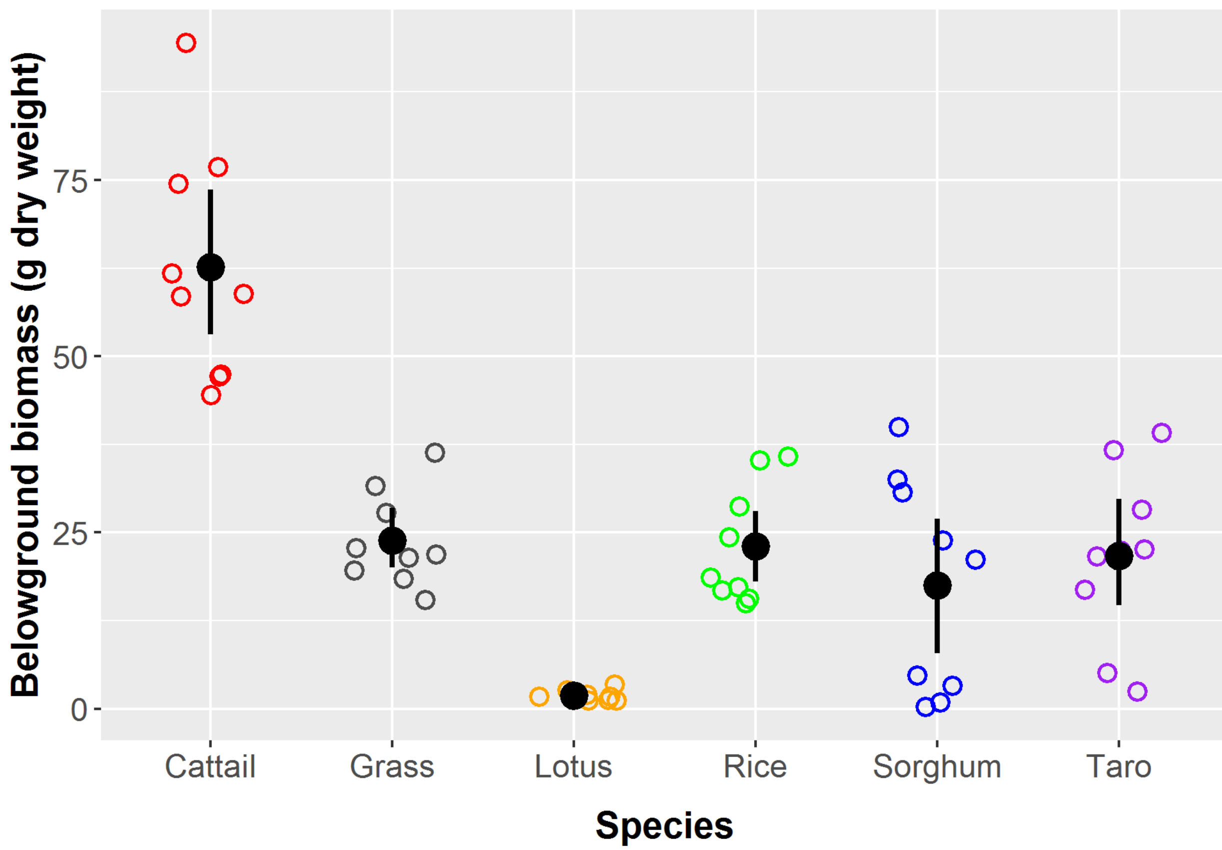

Figure 31. Belowground biomass harvested by species. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 39

Results of a least squared means post-hoc comparison test using a Tukey adjustment for belowground biomass harvested by species.

Species	*lsmean*	*SE*	*Df*	*lower.CL*	*upper.CL*	*group*
Control	0	2.04	42	-5.75	5.75	a
Lotus	1.86	2.04	42	-3.89	7.6	a
Sorghum	17.46	2.04	42	11.72	23.21	b
Taro	21.63	2.04	42	15.88	27.37	b
Rice	22.99	2.04	42	17.24	28.73	b

Grass	23.88	2.04	42	18.14	29.63	b
Cattail	62.64	2.04	42	56.89	68.39	c

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different ($\alpha = 0.05$).

The interaction of species and water level explained a significant amount of variance within the model (Table 37). There were significant differences between the interactions in terms of belowground biomass (Figure 32, Table 40). Lotus and the control across all water levels and sorghum in flood had essentially no belowground biomass harvested and, as such, produced less than all other species in all water levels. Cattail was had the greatest amount of belowground biomass harvested of all species in each of the water levels, and its harvested belowground biomass showed a positive relationship to the amount of water added. Sorghum showed a negative relationship to the water added. Taro in below surface produced a greater amount of belowground biomass than in other water level treatments. Grass and rice were fairly consistent across all treatments, and they were not significantly different from one another.

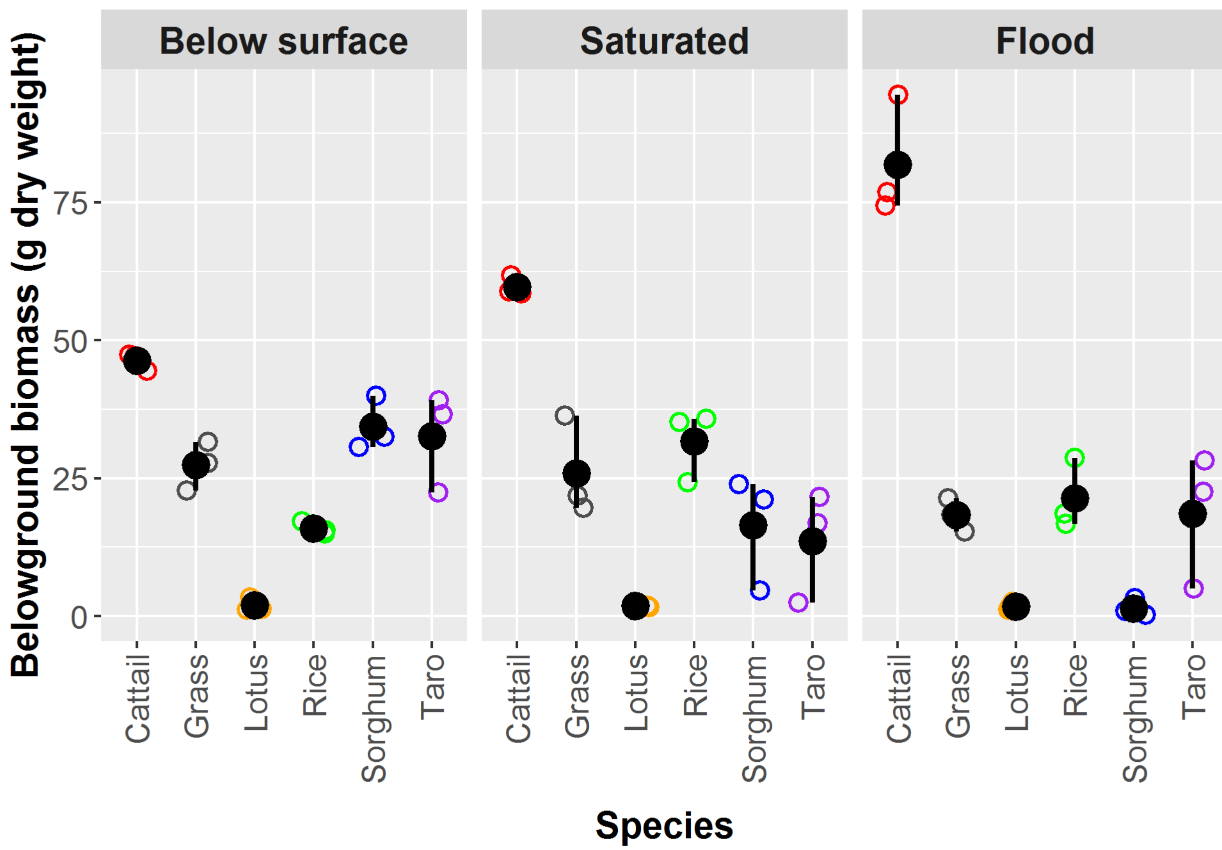

Figure 32. Belowground biomass by species separated by water level. Colors denote species. The black dot represents the arithmetic mean for each species and the black error bars represent a bootstrapped 95% confidence interval.

Table 40

Results of a least squared means post-hoc comparison test using a Tukey adjustment for belowground biomass by species and water level.

Species	Water level	*Lsmean*	*SE*	*df*	*lower.CL*	*upper.CL*	*group*
Control	Below surface	0	3.53	42	-11.38	11.4	a
Control	Saturated	0	3.53	42	-11.38	11.4	a
Control	Flood	0	3.53	42	-11.38	11.4	a
Sorghum	Flood	1.47	3.53	42	-9.91	12.9	a
Lotus	Flood	1.8	3.53	42	-9.58	13.2	a

Lotus	Saturated	1.84	3.53	42	-9.55	13.2	a
Lotus	Below surface	1.94	3.53	42	-9.45	13.3	a
Taro	Saturated	13.62	3.53	42	2.23	25	ab
Rice	Below surface	15.9	3.53	42	4.51	27.3	abc
Sorghum	Saturated	16.55	3.53	42	5.17	27.9	abc
Grass	Flood	18.38	3.53	42	7	29.8	abc
Taro	Flood	18.58	3.53	42	7.19	30	abc
Rice	Flood	21.33	3.53	42	9.94	32.7	bc
Grass	Saturated	25.92	3.53	42	14.53	37.3	bc
Grass	Below surface	27.36	3.53	42	15.97	38.7	bcd
Rice	Saturated	31.74	3.53	42	20.36	43.1	bcd
Taro	Below surface	32.69	3.53	42	21.3	44.1	cd
Sorghum	Below surface	34.37	3.53	42	22.99	45.8	cd
Cattail	Below surface	46.34	3.53	42	34.96	57.7	de
Cattail	Saturated	59.69	3.53	42	48.31	71.1	e
Cattail	Flood	81.88	3.53	42	70.5	93.3	f

Note. Lsmean indicates the predicted marginal means for the specified factors, which are not the same as the arithmetic means shown in the associated figure that are based on observed values. *SE* indicates predicted marginal standard error for the factor tested, not the standard error for each factor level. *Df* indicates degrees of freedom for the least squared means post-hoc test. *Lower.CL* and *upper.CL* indicate the lower and upper limits of the 95% confidence interval for the post hoc test, which are not the same as the confidence levels shown in the associated figure that are based on observed values. *Group* indicates significant differences; if factor levels share letters, they are not significantly different (α = 0.05).

CHAPTER IV

DISCUSSION

In the first chapter we stressed the importance of discovering novel means of bolstering agricultural production while simultaneously mitigating nutrient pollution in waterways. While there are many, the solution we chose to examine is constructed wetlands. There are multiple aspects that go into the application of effective constructed wetlands, but within this study our focus was the plant species and the flood regimes therein. The plants were grown in a variety of ecological contexts to which, in practice, they may likely be subjected. The key goals of the study were twofold, to test how well each species removed nitrogen from nutrient rich water as well as how much biomass they generated, most importantly edible biomass. The results, as laid out in the previous chapter, will henceforth be explained by examining the performance of each species individually as it pertains to both the nitrogen and production components. Exploring the strengths of each species in these contexts will provide valuable insight into the development of constructed wetlands that are capable of food production or will otherwise maximize the value of ecosystem services that they provide. Our findings may encourage more producers to consider implementation of constructed wetlands as a means to reduce their impact on natural aquatic environments, while still maintaining their productive acreage and possibly even increasing their overall profit. However, before addressing individual performance it is best to discuss idiosyncrasies of the experiment and overarching trends that appeared over the course of the study.

To properly explain the presence of various forms of nitrogen in the effluent it should be reiterated that the fertilizer we applied weekly consisted primarily of urea and ammoniacal nitrogen and contained no nitrate. However, each mesocosm was inoculated with nitrifying bacteria which allowed for conversion of the nitrogen present in the fertilizer into nitrate. Therefore, abundance or lack of nitrate in the effluent speaks to the natures of the individual species, i.e. their preference for certain nitrogenous compounds over others. Due to the inoculation, the ubiquity of soil bacteria possessing urease (which catalyzes conversion of urea to ammonia/ammonium) (Mobley' & Hausinger, 1989), and the difficulties of directly measuring urea concentrations, it should be noted that we did not measure urea content in the water and focused instead on ammoniacal nitrogen and nitrate. Additionally, when we speak of nitrogen remaining in the effluent, it should be understood as a proportion of the total nitrogen added to the mesocosm at the beginning of each week. The findings of this study may be used to help elucidate to the planner of the constructed wetland which of the species we examined is most appropriate given their specific needs in terms of the form of nitrogen in their effluent.

During this study, water containing fertilizer was only added at the beginning of each week at one of three volumes – 5 L, 2.5 L, and 1 L – as such throughout the experiment water availability within each mesocosm progressively decreased. The suspected mechanisms being increased evaporation and transpiration rates caused by seasonal changes in temperature and daily total sunlight and the water requirements for the plants increasing as they grew. As a result, in the latter portions of the study, species in the below surface treatment had no water remaining within their mesocosms by the end of the week. This was also seen in those mesocosms containing cattail, rice, and grass across all treatments due to their high total biomasses and rates of transpiration. These facts presented a challenge to the study given our entire means of measuring nitrogen uptake was through water quality analysis. To combat this, as stated in the second chapter, at the end of each week prior to draining the mesocosms we would add municipal water hoping to both provide a sufficient volume of effluent to accurately measure, as well as “unlock” and flush nitrogenous compounds that may have been trapped within the pore spaces of the soil. Later in this chapter the implications of the water stress felt by each species will be examined.

A variety of species were examined in this study, each with particular environmental tolerances, life history characteristics, nitrogen uptake rates, and preferences for nitrogen sources (i.e., ammonium vs. nitrate), and, as such, significant differences were seen between the contents of the effluent between species. Henceforth, the response each individual species had to the different water level treatments will be examined as it pertains to the presence of various forms of nitrogen in their effluent as well as biomass accumulated.

Nitrogen in Effluent and Growth

Control

Prior to discussing the species, first the response of the control should be considered. Given that the control did not contain plants, it did not generate any biomass. Across all treatments, the control consistently had the greatest proportion of ammoniacal nitrogen in its effluent of all species. The same can be said about it for nitrate and total nitrogen in the below surface treatment, however it performed surprising well in the other treatments. When considering nitrate, in saturated treatments it had the third lowest proportion remaining in its effluent and second lowest in flood treatments. For total nitrogen, in saturated it had the second highest proportion of all species, but in flood the control outperformed half of the other species. Most of these findings were expected, but its low amount of nitrate in the effluent needs further explanation. The abundance of water in the control mesocosms are the most likely cause for the low levels of nitrate. Throughout the experiment, the controls in flood and saturated consistently had water leftover by the end of the week. We saw high levels of nitrate in mesocosms that had low amounts of water during the week. This is at least partially explained by the fact that nitrification is an aerobic process. This is seen in the fact that the control in below surface had the greatest amount of nitrate in its effluent of all other species. However, those in saturated and flood had a significant amount of water remaining, limiting the amount of oxygen in the mesocosm and therefore limiting the nitrification process, whereas other treatments with species that had higher transpiration resulted in less water during the week in the saturated and flood treatments and likely increased the rate of conversion of ammonium to nitrate.

Lotus

Lotus was the most disappointing of all species in that it failed to survive. Regarding nitrogen in effluent, its behavior was extremely similar to that of the control. Given that none of the mesocosms survived beyond the first week, it generated no biomass. There are two likely causes for its failure: the sensitivity of the young lotus to the sun and the size of the mesocosm. The most probable of these causes is its sensitivity to sun. Although the size of the mesocosms may be too small for a full-grown lotus, due to its complex root system, this was never a factor because none of the lotus developed beyond a juvenile. We do believe that if implemented into a large enough constructed wetland, this species could thrive. Lotus typically prefer deeper waters than the other species studied here. Future research should be done on this species to determine its viability as a nitrogen filter and crop in a constructed wetland context.

Cattail

The performance of cattail was one of the more interesting findings, as we expected its effluent to have the lowest proportion of both forms of nitrogen of all species. While it consistently had one of the smallest proportions of ammoniacal nitrogen remaining in the effluent across all treatments of all species, especially in flood and saturated, it however had the greatest proportion of nitrate in the effluent of all species in flood and saturated, and one of the highest in below surface. This agrees with previous research on *Typha* spp. preferences for various forms of nitrogen, which have shown that cattail perform better in the presence of ammoniacal N in comparison to nitrate (Brix et. al, 2002). This helps to explain our findings, namely the consistently low levels of ammoniacal N remaining across treatments, however further explanation is required to understand the abundance of nitrate remaining across the water level treatments.

Cattail was the first species to accumulate large amounts of biomass during the experiment and this fact had a large impact on the nitrate in the effluent mainly due to the water requirements of this species. As cattail grew, it quickly got to the point where it would uptake all the water within the mesocosm prior to the week's end. Given its preference for ammoniacal nitrogen and the excessive amount applied at the beginning of each week, cattail would be incapable of taking up all of it before running out of water. It is our understanding that these facts are the main contributing factors to the persistently high levels of nitrate across all treatments. Noting that the nitrification process is aerobic, leftover ammoniacal nitrogen within the pore space could be converted to nitrate in the increased presence of oxygen than in mesocosms that had more water throughout the week. This process is likely the main reason cattail had the highest amount of total nitrogen in the effluent of all species, including the control, in both the flood and saturated treatments. It performed better in below surface because it was able to consume the majority of the total nitrogen before much of it could be transformed into nitrate.

In terms of growth cattail excelled, producing the most total biomass in both flood and saturated as well as the third most in below surface. As it pertains to edible biomass, in this study we did not consider any parts of cattail as edible, primarily because it is not marketable. Its rhizomes and shoots are edible if harvested at the appropriate time, but it functions more as food in survival situations. For these reasons this species was selected for this study due to its ubiquity within constructed wetlands, and it functions as a good control against the crop species.

According to the findings of this study, if the constructed wetland in question is in an area that can support large amounts of water, the main fertilizer input is ammoniacal, and additional food production is not important, then cattail would be an appropriate choice. If, on the contrary, water availability is low, nitrate is the primary input, and marketable crop support is needed, then there are much better options.

Rice

The behavior of rice regarding nitrogen in effluent, in many ways, reflected that of cattail, primarily in showing a preference for ammoniacal nitrogen compared to nitrate. In flood, while not as successful as cattail, rice had the second lowest amount of ammoniacal nitrogen remaining in the effluent, and the third least by a narrow margin in the saturated treatment.

However, in below surface it did not perform as well as in the other treatments, this likely being due to insufficient water availability.

As it pertains to nitrate, the proportion remaining in the effluent was highly consistent across all treatments, and those proportions were relatively high. Rice had the highest proportion of nitrate in their effluent, second only to cattail, in both flood and saturated, and this was likely due to the same mechanisms as those operating in the cattail mesocosms. In those two treatments, rice had large amounts of biomass, which resulted in a high water demand. This high demand meant that, like cattail, before the end of the week, the mesocosm had little water remaining, which enabled greater amounts of nitrification owing to the increased presence of oxygen in the pore spaces. In below surface this did not occur; the water availability was stressed even sooner every week simply because less water was added at the beginning of the week, but this also meant that there was a smaller amount of fertilizer added. Again, noting its preference for ammoniacal nitrogen, rice was able to uptake most of it before the added fertilizer could transition into nitrate.

The proportion of total nitrogen remaining in the effluent of rice, while consistent across treatments, had varied success when compared to other species. In flood, it had the second highest proportion remaining of all species including the control, and this was heavily skewed due to the abundance of nitrate. It is probable that the total nitrogen in the flood treatment would have been lower had the water availability been greater throughout the week. In both saturated and below surface, the total nitrogen remaining ranked third of all species and was lower than most of the other species.

The total biomass harvested from rice was the greatest of all crop species in this study. In below surface, the accumulated biomass was not as high as in the other treatments owing to water requirements of rice. Regarding edible biomass, this species did not produce any in this experiment. The likely causes being that this study was beginning in early spring and concluding only ten weeks later in the same season. Had the experiment lasted longer we expect that at least some of the rice would have produced edible seed given that most of the plants grew to maturity.

Overall, in this study rice has shown that it can function successfully in many constructed wetland contexts. Much like cattail, if access to water is not an issue and the primary form of nitrogen entering the system is ammoniacal, then rice is a viable nitrogen filter. Despite not fruiting in this study, rice has demonstrated its abilities as a food source worldwide in similar contexts (FAO, 1995).

Sorghum

In many respects, sorghum was one the strongest performers of all species, especially in the below surface treatment. The proportion of ammoniacal nitrogen in the effluent in below surface had the second least remaining of all species and least of the crop species, and in this treatment, sorghum had the least of all species remaining in terms of both nitrate and total nitrogen. The same can be said about nitrate and total nitrogen in the saturated treatment, in that sorghum had the least proportion remaining of all species. However, the ammoniacal nitrogen remaining in the effluent of sorghum mesocosms within this treatment was less than lotus and the control but no other species. The most likely explanation for this being that sorghum has a preference for nitrate over ammoniacal nitrogen and that the water requirements for this species were relatively low. As we have discussed, when there is sufficient water present to limit oxygen levels in the soil and an abundance of nutrients, the overall nitrification process is slowed. With this process slowed, a small proportion of the ammoniacal nitrogen would be transformed, thereby leaving larger amounts of it in the water available to the plant. However, given its preference for nitrate, sorghum would readily uptake the newly transformed nitrate leaving little of that in the effluent, but not consume the ammoniacal nitrogen, resulting in a greater proportion remaining in the effluent.

For all its success in the below surface and saturated, in this study sorghum showed a complete inability to thrive in the flood treatment. All the individuals in flood failed to survive after the first few weeks of the experiment. Therefore, in this treatment the presence of nitrogen in the effluent were essentially the same as lotus and the control. The most plausible cause for their failure being that the juvenile plants were incapable of surviving total submersion. However, had the plants grown larger prior to transplant then they may have survived; future studies should be conducted with sorghum that is more developed.

The biomass generated by sorghum also varied greatly across treatments. Given that it did not survive in the flood treatment, essentially no biomass was harvested. On the other side of the spectrum, in below surface, of all species, sorghum generated the greatest amount of biomass, owing yet again to its ability to thrive in water limited environments. The total biomass produced by sorghum in saturated was ranked fourth among all species in treatment, showing that it can grow in situations where water availability is not an issue. While general biomass accumulation represents successful uptake of nutrients and therefore achieves one of the major goals of constructed wetlands, within this study we wanted to see harvestable edible biomass, and sorghum provided. It was one of only two species in this experiment to do so, and sorghum generated the second most edible biomass of all species.

Overall, despite struggling in a flooded in environment, sorghum showed that is a viable constructed wetland crop species within many of the contexts of this study. For those planning to construct a wetland in a relatively dry area with nitrate being the primary form of nitrogen in the runoff, this species may be ideal to further bolster agricultural production while also filtering the water. In the Lower Rio Grande Valley – the study site – sorghum is a major crop species and water availability is a common issue, so additionally growing this species in low lying areas on the edge of a farms in this region could reduce nutrient pollution into major waterways and growers would not necessarily have to sacrifice yield.

Bermuda Grass

Grass was the most consistently successful species in terms of proportion of nitrogen in effluent across all treatments. As mentioned previously, Bermuda grass is a particularly hardy species, and highly generalist in its ecological range, and it showed in the results of this study. When considering the driest treatment, below surface, grass had the least amount of ammoniacal nitrogen remaining in its effluent of all other species and the second least for both nitrate and total nitrogen remaining, all likely owing to its nature as a drought tolerant species. In the saturated treatment, while not as relatively successful, grass still managed to have the second smallest proportion of ammoniacal nitrogen and total nitrogen. As for nitrate, the proportion remaining in the effluent fell in the middle of all other species. In consideration of the flood treatment, once again grass performed well, leaving the third most ammoniacal nitrogen in the effluent and the least in terms of both nitrate and total nitrogen. The success in both of the wetter treatments show that grass is highly effective at taking up all forms of nitrogen as long as there is water present at some point throughout the week. In the latter half of the study grass rarely had water remaining by the end of the week, meaning that while high water availability is not necessary, if present, grass will consume it as well as the nutrients therein.

Drought tolerance and simultaneous flood tolerance paired with a lack of clear preference for form of nitrogen resulting in consistent, substantial growth in all treatments. In each of the three treatments, grass generated the second highest amount of total biomass of all species. The one major downside to this species is that it does not produce biomass that is edible to humans. This fact in the context of this study makes grass a weaker choice as a constructed wetland plant. However, if a grower also raises livestock then this can supplement that aspect of their operation, given that the wetland goes through cycles of wet and dry thereby allowing grazing opportunities.

Grass is a highly effective plant in terms of filtration no matter the water availability but does fail to meet the second goal of the study, providing the focus is on food that is edible to humans. Given its success at reducing all forms of nitrogen in the effluent measured in this study, Bermuda grass is a good check to most fertilizers. If farmers are willing to sacrifice a portion of their land with the aim of reducing the amount of nutrient pollution running off their farm, while not needing to supplement crop yield, then based on the findings of this study, Bermuda grass will fulfill this need.

Taro

While not being the most effective at reducing nitrogen from the effluent in all water levels, taro was the best performing crop species in the flood treatment. In this treatment, taro had the second lowest total nitrogen remaining of all species, the third least in terms of nitrate, and ranked fourth for ammoniacal nitrogen. However, taro showed a preference for ammoniacal nitrogen, despite consistently ranking fourth amongst all species across all treatments for this nitrogenous form, it seldom had significantly more ammoniacal nitrogen remaining than the higher ranked species. The reason for its strong performance in terms of nitrate and total nitrogen in the flood treatment, is likely because of its somewhat low transpiration rate. In every week throughout this experiment, taro had water remaining within the mesocosm by the end of the week; this fact speaks to a lower nitrification rate as previous discussed. In flood, taro would uptake the ammoniacal nitrogen it needed to grow – which, based on the total biomass results, was less than most other species, given that there is a direct link to nitrogen uptake and biomass accumulation – and would forego taking up whatever little nitrate that had formed. In both saturated and below surface, despite its small water requirements, it rarely had water remaining or at most very little, thereby leading to increased nitrification rates. Pairing this with its preference for ammoniacal nitrogen and minimal nitrogen needs generally, taro had higher nitrate levels as compared to other species in these treatments.

When considering its biomass accumulation, taro in this regard was also ranked in the middle of all species. In each mesocosm, we saw consistent growth across all treatments, but simply due to the morphology of this species, its mass was never very high. While its total biomass was not particularly large, taro did produce the greatest amount of edible biomass of all species. As stated in the first chapter, almost every part of this plant is edible, but for this study we only counted the belowground corm formation as edible biomass. Much like its total biomass the edible biomass was consistent across water level treatments, with only a slight increase in the flood treatment.

Bearing in mind the two foci of this study, taro was shown to be one of the most well-rounded of the species. Despite not taking up large amounts of nitrogen, in a place where there is a constant presence of water and comparatively small inputs of ammoniacal nitrogen, taro can successfully filter out excess fertilizer. So too, it does provide an additional food source, so if a grower were to construct an on-site wetland, they could supplement their regular yield with this crop. While it is perfectly edible, it is a niche food crop in the continental US, so that is both a benefit and a hindrance. By growing this crop, one may have a tough time finding a buyer, but once found, have a substantial portion of the market.

CHAPTER V

CONCLUSION

Through this study we have shown that not only are crop species capable of greatly reducing the amount of nitrogen in nutrient rich effluent across a variety of moisture regimes but also generate viable produce. Our hope is that the results of this study will encourage producers to take steps to limiting their impacts on the aquatic environment by constructing wetlands on their facilities that utilize crop species so that they may generate a profit from this decision.

While we have tested a variety of species these are but a few of the possible choices for wetland plants and as such further studies should be performed to test the viability of those species not included in our study. Also, there are many other elements to examine within the ecological contexts we have chosen such as other macronutrients like phosphorus and potassium, or heavy metals like arsenic and selenium; future studies of these will provide a fuller picture of how species might respond in a constructed treatment wetland. Another important area for future research would be the food safety aspect of producing crops in wetlands that are fed by wastewater runoff.

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BIOGRAPHICAL SKETCH

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