EVALUATION OF A BIOCHAR ENHANCED CONSTRUCTED TREATMENT WETLAND FOR THE REMOVAL OF CONTAMINANTS FROM AGRICULTURAL WASTEWATER

by

STEFANIE GUGOLZ

(Under the Direction of Valentine Nzengung)

ABSTRACT

Nutrient-rich wastewater from concentrated animal feeding operations (CAFO's) is one of the largest sources of surface water contamination in the US. This study evaluated biochar as a media in vegetated constructed treatment wetlands (CTWs) for CAFO wastewater treatment. A wood biochar was found to adsorb up to 0.28 mg NH_4^+-N per g biochar but no NO_3^- or PO_4^{3-}. It was subsequently used in a greenhouse experiment, conducted with four simulated CTWs: R1 – biochar with aqueous plants; R2 - biochar, gravel and plants; R3 - biochar; and R4 - gravel and plants. The reactors were monitored for their removal of solids, nutrients and metals from swine wastewater as well as plant growth. Overall, there were no statistical differences between the mass of pollutants (TS, COD, NH_4^+-N, PO_4^{3-}-P, K, Na) removed by R1 and R2, but for almost all parameters (except PO_4^{3-}-P and Na) they outperformed R3 and R4. Plant growth was greatest in R1 and least in R4. These findings show that incorporation of biochar into CTW media can significantly improve treatment of wastewater. Additional studies using other types of biochar could yield even better results.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Background

Agricultural runoff is recognized as one of the largest sources of contamination of surface water in the US, particularly from concentrated animal feeding operations (CAFOs) (Puckett, 1994). CAFO is a legal designation for farms housing over a certain amount of animals on site. Large CAFOs, which can have thousands to hundreds of thousands of animals, can produce enough manure in a year to rival the sewage production of major US cities (GAO, 2008) and while all human waste is treated, there are no regulated treatment facilities for CAFOs.

CAFO waste management follows "best management practices" which includes the storage of manure wastewater in treatment lagoons, holding ponds and underground pits. It is typically disposed of via land application – sprayed onto cropland as a fertilizer (GAO, 2008). Rain and flooding events can, and often do, result in overflow from the holding ponds as well as wash out of the land-applied manure from fields. Leaks in holding ponds and storage tanks, as well as over-application onto fields, can cause nutrients and other contaminants to leach into the groundwater as well.

CAFO manure wastewater is high in nutrients like nitrogen and phosphorous, heavy metals, salts, hormones, antibiotics and pathogens like e-coli. When this runoff reaches surface water, it can cause algal blooms leading to eutrophication and the death of aquatic

species; and the other chemicals can also have devastating ecological and human health effects.

The EPA currently has limited regulatory power over CAFO waste and CAFO owners are not required under Resource Conservation and Recovery Act (RCRA) rules to treat their wastewater (Bradford et al., 2008; GAO, 2008) despite its harmful effects on the environment. Currently, CAFOs must develop nutrient management plans (NMP) for the land applied wastewater by accounting for the nutrient uptake capacity of the crops as well as the nutrient and water holding characteristics of the soil. If all of these variables are correctly taken into account the NMPs can theoretically prevent over-application and surface and groundwater impacts (Bradford et al., 2008; GAO, 2008). In Georgia, as well as many other states, the number of CAFOs is growing and the density of their operations with it (GAO, 2008). They tend to cluster in rural areas and the amount of waste they produce often exceeds that which can be used locally, which can make them the single largest source of non-point source pollution for a watershed.

Due to the ongoing negative environmental and human impacts that result despite NMPs, there is an immediate need for CAFO treatment technologies. If the wastewater could undergo pretreatment to significantly lower contaminant concentrations, land application systems may be much easier to manage successfully. Further, if the wastewater could be treated within acceptable levels then it could even be reused at the farm or discharged to local surface water without harm to the environment. In order to avoid significantly impacting CAFO operating costs and thus driving up the price of meat, there is a need for low-cost, low-maintenance treatment technologies for their wastewater.

Biochar: Nutrient sorption, water filtration, and plant growth

Biochar is a type a of charcoal produced by the pyrolysis of biomass (heating to at or above 350°C in the absence of oxygen). Biochar has very recently been found to be effective for the filtration of multiple contaminants from wastewater. The pyrolysis process decomposes the biomass' organic structures—like cellulose, hemicellulose and lignin in plant biomass—and can also change the structures of its inorganic constituents. A number of chemical reactions can occur during pyrolysis, including depolymerisation, aromatization and decarboxylation which lead to the formation of negatively charged functional groups on the surface of the biochar and the production of crystalline molecules and ash. These reactions also tend to change the physical structure of the biomass, increasing porosity and surface area.

The resulting amount of cation exchange capacity (CEC) and surface area of the biochar is directly related to the type of biomass feedstock and the pyrolysis temperature. There are different types of pyrolysis, including slow, fast, flash and hydrothermal pyrolysis. Typically slow pyrolysis produces the most biochar and highest surface area and charge and is therefore the preferred method to make char for use in soil amelioration and water filtration. Higher temperatures have also been found to increase the porosity of the biochar over those pyrolyzed at lower temperatures (Khalil, 2009; Zheng et al., 2013), but there is also some evidence that temperatures over a certain threshold may decrease surface area through the destruction of micropore structures within the char (Zheng et al., 2013). Higher temperature pyrolysis also decreases CEC over that of lower temperature for some feedstocks (Nguyen and Lehamn, 2009; Zheng et al., 2013; Hollister et al., 2013)

potentially due to the loss of carboxyl functional groups (Cheng et al., 2006; Liang et al., 2006).

Due to their high CEC and surface area, biochars are effective as adsorbents for a variety of contaminants from water. Thus, biochars are useful for filtration of nutrients and contaminants from water and contaminant immobilization in soils. There have been many studies characterizing the sorption of nutrients (ammonium – NH_4^+, nitrate – NO_3^-, and phosphate – PO_4^{3-}) onto biochars of various types. Most studies comparing feedstock materials agree that biochar surface functional groups and CEC are responsible for NH_4^+ adsorption differences between biochars. These studies found that the greater the CEC, the greater the NH_4^+ adsorption (Cui et al., 2016; Hollister et al., 2013). However, biochar surface area has also been found to influence NH_4^+ adsorption differences: Zheng et al. (2013) compared the effect of pyrolysis temperature on a variety of feedstocks and found that a higher NH_4^+ adsorption was observed using biochar produced at higher temperature (600-700°F vs. 500°C). This happened despite the consequent decrease in CEC (e.g., From 312 to 196 mm kg-1) and was attributed to an observed increase in the surface area (e.g., from 114 to 228 m^2 g^{-1} CO_2) of the higher temperature biochars. NH_4^+ adsorption to biochar is controlled both chemically by CEC and physically by surface area, but their order of importance may depend on the feedstock used.

Sarkhot et al., (2014) studied NH_4^+ adsorption to mixed hardwood biochar from both pure NH_4^+ solutions and dairy manure and found that NH_4^+ removal with KCl was higher from the manure than from the pure solution. This indicated that NH_4^+ adsorption may be aided by interactions with other ions in solution. In contrast, a study by Kizito et al. (2015) found the opposite was true for mixed wood biochar indicating the complex

relationship between biochar characteristics and adsorption. Sarkhot also found that the highest percentage of NH_4^+ was desorbable from biochar that had been in the highest NH_4^+ concentration solution. In addition, the longer the biochar was in contact with the NH_4^+ solution, the lower the desorbable fraction. These findings suggest multiple sorption mechanisms which are influenced by both NH_4^+ solution concentration and residence time in the biochar and ultimately affect the reversibility of adsorption. This would include weaker electrostatic interactions, cation and ligand exchange and chemisorption mechanisms.

NH_4^+ adsorption onto the biochar surface has been found to be at reversible to a certain degree (Sarkhot et al., 2014; Wang et al., 2015), implying that in a planted system the NH_4^+ would still be bioavailable. A study by Taghizadeh-Toosi et al., (2012) confirmed the bioavailability of biochar-adsorbed ammonia through the use of ^{15}N-tagged ammonia. They found elevated ^{15}N levels in plants grown in their biochar-amended soil.

Most fresh biochars are primarily negatively charged with a low anion exchange capacity (AEC). This apparently explains the findings of many studies that have found biochar to have a low capacity for removing NO_3^- from water (Sarkhot et al., 2014, Kameyama et al, 2016). This is potentially related to the biochar's pyrolysis temperature, with higher temperatures resulting in some NO_3^- adsorption (Mizuta et al., 2004; Kameyama et al., 2012). Some biochars have also been found to remove PO_4^{3-} even with a low AEC, probably due to PO_4^{3-} co-precipitation with other elements on the biochar surface, for example Mg^{2+} (Zheng et al., 2013; Yao, 2011). It has also been observed that in a manure solution, other anions can compete with PO_4^{3-} for co-precipitates or adsorption sites, decreasing the amount of PO_4^{3-} adsorbed compared to removal from pure solutions

(Sarkhot et al., 2014). This study also found that the higher the manure PO_4^{3-} concentration, the more PO_4^{3-} was adsorbed potentially due to a higher concentration of co-precipitates.

Biochars have also been found to adsorb heavy metals, salts, organics, other chemicals and suspended solids from wastewater (Mohan et al., 2014; Niazi et al., 2016), making biochar an important potential media for the filtration of various mixed contaminants in wastewaters. Several studies have used biochar to successfully filter multiple contaminants from storm (Reddy et al., 2014), dairy (Sarkhot et al., 2013), industrial—brewery (Huggins et al., 2016), and municipal (Kätzl et al., 2014) wastewaters.

In practice, using biochar only as an adsorbent means that once all the adsorption sites are filled, the biochar is spent and is no longer useful to treat the wastewater. It then has to be removed and replaced with fresh media. The useful life of biochar as a filtration media is dependent on the biochar's sorption capacity and the concentration of the contaminants of concern in the wastewater. Depending on the type of adsorbed contaminants, the biochar can be used for plant nutrients recovery and mixed into soil as a "slow-release fertilizer" (Taghizadeh-Toosi et al, 2012; Gezzehei et al., 2014). For use as a soil fertilizer, only biochar that has filtered only nutrients and no toxic chemicals may be used, otherwise it may contain metals that may be too toxic for plants to grow in, or could introduce toxic chemicals into food-crops (Chaukura et al., 2016). Spent biochar can be disposed of by burning, however if there are toxic volatile compounds within either the biochar structure or adsorbed by the biochar, combustion is not a desirable method of disposal (Lehman et al., 2011).

Biochar can also be used as a media for microbial filtration from wastewater. In a study by Kätzl et al. (2014), biochar was found to be equally or more effective than sand for

the treatment of municipal wastewater through the growth of biofilms. Biochar has been found to increase microbial activity in soils (Gregory et al., 2014; Lehman et al., 2011), but the reasons for this are not well understood. It is likely that a combination of interconnected factors are responsible, potentially including increased energy supply due to the labile carbon content of biochar (Hamer et al., 2004), microbial sheltering in biochar pore structures (Lehman et al., 2011; Thies and Rilling, 2009), pH increases and redox conditions brought on by biochar additions (Joseph et al., 2010), the removal of toxins from soil pore water through adsorption, and an increase in plant root exudates due to an increase in plant growth associated with biochar addition to soils (Gregory et al., 2014).

Microbial activity stimulation in biochar amended soils has also been suggested as a potential mechanism for the increased plant growth found in many studies (Lehman et al., 2011). Many studies which found increased microbial activity after biochar addition also noted that microbial community structure biodiversity increased (Khodadad et al., 2011), often resulting in an increase in rhizome associated microbes and fungi which form symbiotic relationships with plant roots (Jin, 2010). The increased microbial activity can lead to an increase in plant resistance to stressors like diseases (Matsubara et al., 2002) and drought (Herrmann et al., 2014).

Many studies have found biochar addition to soil to increase both roots and above ground plant biomass, plant germination and growth rates. These effects have been attributed to a combination of microbial activity and direct effects from biochar physicochemical properties that lead to water and nutrient retention, toxin immobilization, changes in pH, and soil aeration (Lehman et al., 2011).

Because of its contaminant adsorption ability and its positive impact on plant growth, biochar has been studied as a soil amendment for the phytostabilization and phytoextraction of heavy metal contaminated soils with some success (Paz-Ferreiro et al., 2014). Houben et al. (2013) observed that biochar reduced the bioavailability of cadmium (Cd) and zinc (Zn) and increased plant biomass. Fellet (2014) used biochar to increase plant uptake of lead (Pb) by phytoextractors, although they saw no change in biomass, whereas Chirakkara and Reddy (2015) found increased Cd and Pb uptake along with increased plant biomass. These studies make use of both the direct effect of biochar on contaminants, and the indirect effect through the environmental and ecological effects of plants.

Biochar as a phytoremediation enhancer has been studied more for soil than for water treatment and very few studies have so far used biochar in a wetland environment. Rozari et al. (2015) found biochar-amended sand to be more effective for removing chemical oxygen demand (COD), biological oxygen demand (BOD), suspended solids (TSS), and coliforms from septic tank sludge than sand alone in planted wetland mesocosms. There is great potential for a synergistic effect of biochar and plants to enhance the removal of contaminants by wetlands.

Constructed wetlands for the treatment of wastewater

Constructed treatment wetlands (CTWs) is a mature technology applied to treat wastewater from many sources including agriculture. One disadvantage of CTWs is that they tend to take up large land areas and may require two or more stages in order to effectively remove all the contaminants of concern in a waste stream. For instance, vertical

flow wetlands successfully target ammonia, typically through microbial oxidation, but are not effective denitrifiers. In contrast, horizontal flow wetlands tend to promote nitrification (Vymazal, 2008). Phosphate removal is similarly complex, requiring specialized steps that rely on chemical removal processes like sorption (Kadlec and Wallace, 2009). Gravel and coarse sands are used in the design of most CTWs in order to increase porosity and permeability.

Some other drawbacks of using CTWs include poor performance in winter months and the need for pretreatments or dilutions of wastewater in order to reduce any toxicity effects. In northern latitudes during winter months reduced plant uptake is caused by dormancy or plant death. The plants typically used in CTWs are hardy and strong, chosen for their longevity, nutrient uptake capacities and ability to withstand sudden changes in nutrient concentrations.

There has been increased interest in using more reactive media in CTWs to provide additional treatment mechanisms. The reactive media include natural minerals (like bauxite and dolomite), industrial by-products (slags), and man-made products like filtralite (Vohla et al., 2011). There is growing interest in the application of biochar to increase the specific surface area of media in CTWs and the physical and chemical removal of contaminants (de Rozari et al., 2015; Gezzehei et al., 2014)

Objectives

The goal of this study was to evaluate how biochar may enhance a CTW system used to passively or semi-passively remove nutrients from CAFO wastewater. The use of CTWs is a low-cost, low-maintenance, passive, solar-driven, and aesthetically pleasing technology.

By combining plants and biochar in a wastewater treatment system, the biochar with its large specific surface area and CEC should function as a sort of sponge that slows the transport of contaminants through the wetland. This retardation provides additional residence time for the plant roots to take up nutrients bound to the biochar and the residual dissolved in the wastewater. The removal of the sorbed nutrients by plants should keep the biochar sorption sites from becoming saturated. Additional benefits include the biochar enhancement of plant growth and microbial activity which should further enhance treatment in the system.

There is a growing focus on the development and application of green and sustainable remediation technologies for the management of CAFO wastes. This study will add to the growing body of research focused on developing and improving environmentally green and sustainable remediation practices for CAFOs. If a low-cost treatment system that provides added income gains to farmers is developed, then farmers are more likely to implement the solution. Biochar may offer farmers carbon sequestration credits, moreso if it is made on-site, with their own waste products like crop residues, solid manure or chicken litter. In addition, if the developed biochar enhanced CTW for CAFO wastewater is effective and adopted, the treated wastewater could be reused or recycled on the farm.

CHAPTER 2

MATERIALS AND METHODS

Biochar Characterization

Physical Properties

The biochar used in this study was acquired from Biochar Now, a biochar producer out of Colorado. The biochar was produced from soft wood pyrolyzed at 550°C. Soft wood biochar was selected for two reasons: 1) Its availability in large quanitities for large-scale applications, and 2) the fast growth rate of soft wood trees means it is an easily renewable resource.

The grain size distribution of the biochar was determined by sieving using sieves of sizes #5, 10, 20 and 40 (method ASTM D422-63(2007)). The biochar was sieved to remove particles finer than 0.425 mm (#40 mesh) and the >0.425 mm biochar fraction was used in the batch sorption and constructed treatment wetland tests.

Nutrient Sorption and Desorption

Batch sorption isotherms were measured and used to characterize the equilibrium sorption of ammonium, nitrate and phosphate by the biochar. Stock solutions of the three nutrients of interest (NH_4^+-N, NO_3^--N and PO_4^{3-}-P) and were prepared by dissolving KNO_3, $(NH_4)_2SO_4$ and K_2HPO_4, separately in distilled water. Each 50 mL nominal volume crimp top vial received a 5g sample of the biochar and 45 mL of the prepared nutrient solution. The batch sorption of each nutrient was measured in a separate test. The control samples

consisted of nutrient solutions alone and biochar with distilled water, respectively, handled in parallel with the treatments.

To determine the amount of time needed for the approach to sorption equilibrium, a preliminary sorption kinetics experiment was performed using solutions of ~100 mg L^{-1} NH_3-N, ~5 mg L^{-1} NO_3—N or ~100 mg L^{-1} PO_4^{3-} and mixing times of 5 min, 10 min, 20 min, 2 hrs, 10 hrs and 24 hrs. Sorption equilibrium isotherms were produced using 5 grams of biochar mixed for 24 hours in solutions of ~20, 40, 60, 80, 100, 150 and 200 mg L^{-1} NH_3-N, respectively. The tests were done in duplicate and then in triplicate if the variance in the results were >5%. Sorption isotherms were not produced for NO_3^- or PO_4^{3-} because the kinetics experiment showed insignificant sorption of either. The prepared samples and control vials were mixed continuously on a rotary mixer at a speed of 3.3 revolutions per minute.

To determine if the nutrients were reversibly sorbed, the biochar samples from the sorption isotherm experiment were dried in an oven for 24 h. at 40°C. Duplicate samples of the biochar (each consisting of ~5 g) that had adsorbed the most ammonium were each mixed with 45 mL of distilled water and equilibrated on the mixer for 48 h., filtered and the solution was analyzed as described below.

At the end of each sorption or desorption test, the solution and solid mixture was filtered through a Fisher Brand P8 qualitative filter to remove the biochar particles. The remaining solution was analyzed using a HACH DR3900 spectrophotometer (Hach Method 10031 for high range ammonia nitrogen, Hach Method 10206 for low range nitrate nitrogen, and Hach Method 8048 for low range reactive phosphorous).

Data Analysis

The Freundlich and Langmuir sorption isotherm equations were fitted to the experimental data. The linearized form of the Freundlich model, linearized by taking the log, is shown in equation 1.

$$\log S = \log K_f + n\log C \quad (1)$$

where n = constant, and K_f = Freundlich coefficient (mg L^{-1}). A plot of log$S$ vs. log$C$ gives a straight line with a slope of n and intersect of logK_f.

The linearized form of the Langmuir model (equation 2) was applied to the data,

$$\frac{1}{S} = \frac{1}{K_L C S_m} + \frac{1}{S_m} \quad (2)$$

where S = mg g^{-1} sorbed, C = solution concentration (mg L^{-1}), K_L = Langmuir coefficient, S_m = sorption maximum (mg g^{-1}). A plot of $\frac{1}{S}$ vs. $\frac{1}{C}$ gives a straight line with a slope of $\frac{1}{K_L S_m}$ and y-intercept of $\frac{1}{S_m}$. The goodness of the Langmuir and Freundlich model fits were confirmed by linear regression.

Greenhouse Experiments

Greenhouse setup

The role of biochar in the enhancement of nutrient removal from wastewater was investigated using four simulated constructed treatment wetland (CTW) reactors in a greenhouse (Figure 1). These reactors were 140L rectangular planter tanks. At the bottom of each reactor was a drainage channel topped with a perforated (0.5cm holes) false bottom to support the media in the reactor and allow effluent drainage into the channel. This channel spanned the entire length of the reactors and ended in a drainage port for

effluent removal. A 2.5 cm layer of pea gravel was added to the bottom of each of the four reactors, then landscape fabric and a 2.5 cm layer of sand on top to keep any biochar from washing out of the reactors. Reactor 1 (Figure 1, R1) was then filled with 20 cm of biochar (approx. 15.7 kg), sieved first over a #40 mesh sieve to remove fines and prevent clogging. This was topped by another 2.5 cm layer of sand to keep the biochar from floating while simultaneously functioning as a pre-filter for suspended solids. This reactor was planted.

Reactor 2 (Figure 1, R2) was similarly designed, except that it contained half the amount of biochar in CTW R1, so that the treatment media consisted of a 10 cm layer of pea gravel and a 10 cm layer of biochar, and was planted like R1. Reactor 3 (Figure 1, R3) was packed with the same quantity of biochar as in R1 except that it was unplanted. Reactor 4 (Figure 1, R4) contained only pea gravel with no biochar and was planted. R1, R2 and R4 provide a gradient from R1 “full biochar”, R2 “half biochar” and R4 “no biochar”. R3 provided the biochar control, representing the contribution of biochar only to the nutrient removal while R4 served as the plant control by providing the contribution of the plants only. Each reactor had one inch of wastewater above the packed treatment media.

An effluent hose was attached to the drainage port on each reactor. The hose rose vertically and then bent at the same level as the top of the planter tank so it matched up with the water level inside the tank (Figure 1). As wastewater was added to the reactors, the water level would rise and the pressure would cause the downward movement of the wastewater through the tanks into the drainage channel, out the port and up the hose where it was allowed to drip out and equalize the water level on each side. This kept the effluent flow rate consistent with the level of water in the reactor so that the effluent flow rate depended both on the influent flow rate and the evapotranspiration rate from each

reactor. Another hose was connected to a spigot at the bottom to allow for full, quick and complete draining of the reactors. Each reactor had an influent hose on the opposite side of the reactor effluent drainage port through which water and wastewater were pumped into the reactor from a 200 L drum.

In August reactors R1, R2 and R4 were initially planted each with 6 common cattails (*Typha latifolia*), 3 soft rush (*Juncus effusus*), 2 willow (*Salix*) branch cuttings and a handfull of duckweed (Figure 2). The plants were collected from ponds and lakes around Athens, GA. Cattails and rush are both commonly used in constructed treatment wetlands and cattails especially are known for their hardiness and ability to withstand stressors like sudden spikes in nutrient concentration as well as seasonal temperature changes. The reactors were filled with an equal mixture of clean water and dairy wastewater collected from the UGA dairy farm lagoon. The reactors were kept full for three months to allow the plants to acclimate, establish large roots and spread.

Three tests lasting several weeks each were conducted by applying wastewater to the reactors during different seasons: An initial test was conducted in November 2015, a second in April-June 2016, and a third in October 2016. For the first test the influent flow rate was $\sim$ 2 L hr^{-1} for a residence time in each reactor of 33.5 hrs. Diluted swine wastewater was used for these tests instead of dairy wastewater as it was easier to collect from the UGA swine farm anaerobic digester. The swine wastewater, however, had a much higher N concentration (>1200 mg L^{-1} NH_4^+-N, undiluted) than the dairy wastewater ($\sim$200 mg L^{-1} NH_4^+-N, undiluted) . The initial November test resulted in the death of plants in R4 so they were removed and healthy plants from R1 and R2 were transplanted, and all the reactors were allowed time to regrow for the subsequent tests. None of the planted

reactors had any rush, willow or duckweed left, however there were some other aquatic plants, parrot's feather (*Myrophyllum aquaticum*) and knotweed (*Persicaria*) that had volunteered along with the original plants when they were collected. These were divided and planted equally within all three planted reactors. Photographs of the reactors were taken at the start of the test and subsequently every few days for the duration in order to document the growth and health of the plants.

For the second test, the flow rate was lowered to 1 L hr^{-1} for a residence time of 67 hrs. The test ran for 53 days and each reactor treated a total of 1272 L of the wastewater. For the first two sampling sets (over the course of 2 weeks), the wastewater was diluted >10x to obtain an initial NH_3-N concentration of ~50 mg L^{-1}. For the subsequent 4 sampling events this concentration was doubled. One set of samples had to be discarded due to pump and reactor problems. Photographs were taken of the reactors at the start of the test as well as every other day for the duration of the test. These, along with yardsticks were used to measure the growth of the cattails in each tank over the course of the test.

The plants were cut down to ~2.5 cm above the water in all the reactors after the second test. The biomass was dried in an oven at 44°C and 24% humidity and weighed. The reactors were allowed to regrow until the third test when they were all cut to the same height of 3ft. For the third test run during October, the wastewater was diluted to the nutrient concentration level of the wastewater used in the second part of the second test. The test's duration was 11 days at a flow rate of 1L hr^{-1} for a total of 264 L treated by each reactor. A total of three sample sets were taken during the third test and photographs were also taken to document the relative plant growth in each of the three planted CTW reactors.

The diluted CAFO wastewater was supplied to the reactors from a 200 L CAFO reservoir that contained enough wastewater to last for 2 days. The reservoir was refilled with a new "batch" of diluted wastewater every 48 hours. The baseline concentration consisted of 1 L influent samples taken from the reservoir prior to the treatment of each fresh 200L batch of the wastewater. Effluent samples were taken 45.5 hrs (for the 2 L hr^{-1} influent rate) or 91 hrs (1 L hr^{-1}) after treatment began, corresponding to half of the inflow volume. For each test, the effluent flow rate (F_e) of each reactor was measured at 6 am and 6 pm during a 24hr day. The effluent flow rates were generally less than the influent flow rate (F_i), with the difference being attributed to the evapotranspiration rate (E) from each reactor (L hr^{-1}):

$$E = F_i - F_e \quad (3)$$

The samples from the second test were analyzed for total solids (TS), PO_4^{3-}, chemical oxygen demand (COD), total Kjedahl nitrogen (TKN), NH_4^+-N, NO_3^--N and total minerals (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, Zn). The TS was estimated gravimetrically by drying 20 mL of the wastewater sample in an oven at 104°C. PO_4^{3-} was measured using the Hach spectrophotometer method. The other parameters were analyzed by the UGA Extension Soil, Plant and Water Analysis Laboratory. During the third test, the samples were analyzed only for NH_4^+-N and PO_4^{3-} using the Hach spectrophotometer methods.

Data analysis

The amount of each measured constituent removed by each reactor was calculated using the evapotransipration rates (E) and influent and effluent concentrations (C_i and C_e) by applying the following equations:

The mass loading (M) of each pollutant into each reactor over a given period of time (T) in mg,

$$M = C_i * F_i * T \quad (4)$$

where C_i = influent concentration (mg L^{-1}) and F_i = influent flow rate (L hr^{-1}); the total volume (V_E) of wastewater removed from the reactor by evapotranspiration (E) over a given period of time (T) in L,

$$V_E = T * E \quad (5)$$

the total volume (V_R) of wastewater remaining in the reactor after evapotranspiration in L,

$$V_R = (F_i * T) - V_E \quad (6)$$

where F_i = influent flow rate (L hr^{-1}), T = time (hr), and V_E = volume of water removed by evapotranspiration as defined in equation 5; the total mass (M_R) of pollutant removed from the wastewater in the reactor (mg),

$$M_R = M - (C_e * V_R) \quad (7)$$

where M = total mass of pollutant loaded into each reactor (mg, equation 4), C_e = effluent concentration (mg L^{-1}), and V_R = volume of water remaining after evapotranspiration (L, equation 6); and the concentration (C_R) removed by each reactor (mg L^{-1}),

$$C_R = \frac{M_R}{T * F_i} \quad (8)$$

where M_R = mass of pollutant removed (mg, equation 7), T = time (hr), and F_i = influent flow rate (L hr^{-1}).

Percent removal was calculated using equation 9:

$$R(\%) = (M - M_R) * 100 \quad (9)$$

For the second test, two-way (2x3) type 3 ANOVA was performed to understand the effect of influent concentration and amount of char on the contaminant removal capacity of

the planted reactors. The low initial concentration loading had only two observations per reactor, while the high initial concentration loading had three, making the ANOVA unbalanced, so a type 3 was applied. R statistical software was used to do the ANOVA, using the "Anova" function from the package "car" which can be easily set to do a type 3 ANOVA. For each parameter that the reactors actually removed from the wastewater, the effects of influent concentration ("low" or "high"), amount of biochar ("0", "50" or "100") and the interaction between them was tested for significance. If there was no significant ($p>0.1$) interaction, this was removed from the ANOVA so that only each factor's individual effect was evaluated. If there was a significant ($p<0.1$) effect, Tukey's Honestly Significant Difference (TukeyHSD) test was performed to see which levels were different from one another. For some of the parameters the ANOVA had to be altered to account for influent concentrations not matching the wastewater dilution or effluent parameter concentrations that were higher than the influent. For the third greenhouse test, the CTW performance was compared using one-way ANOVA and TukeyHSD.

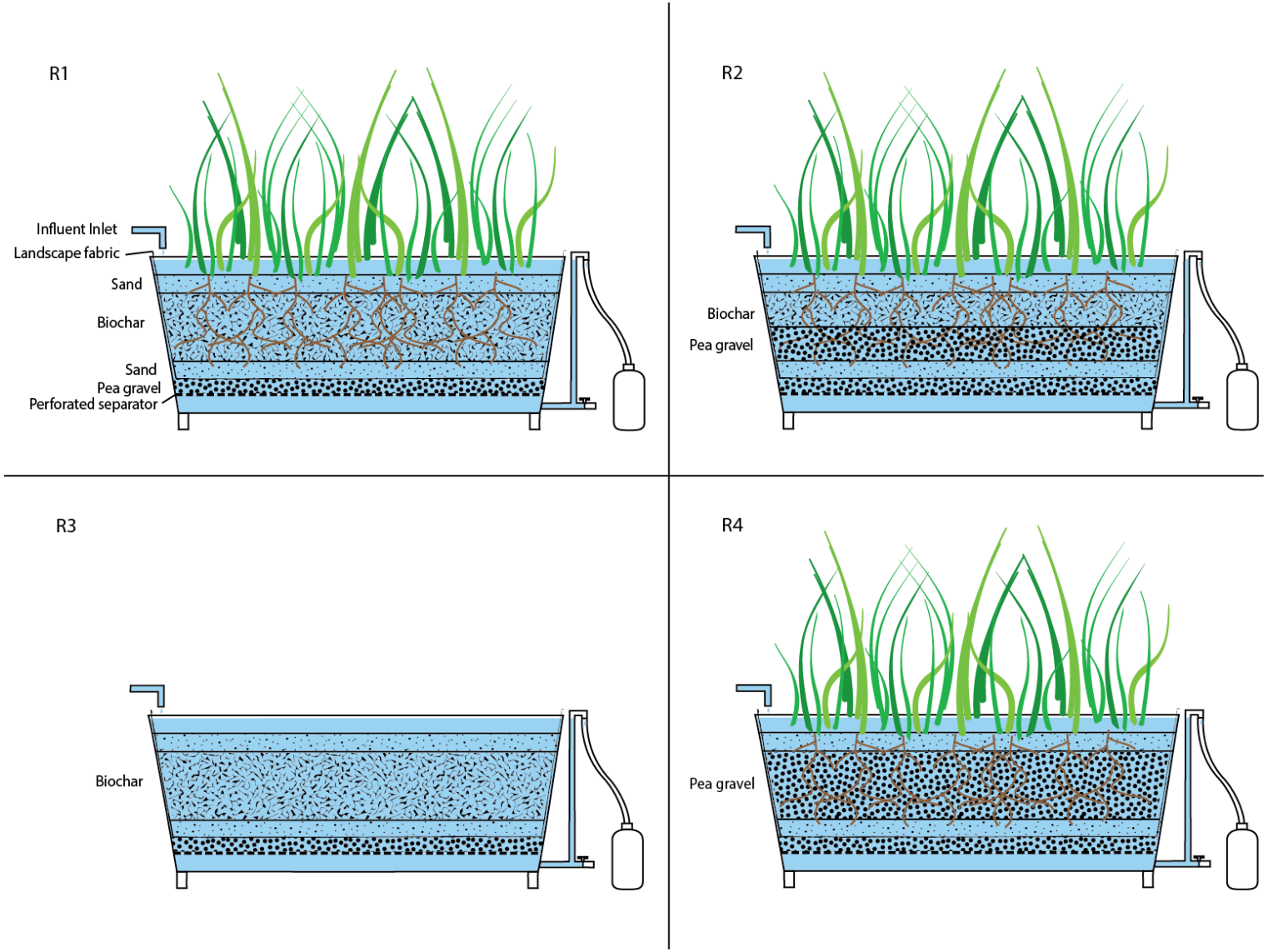

Figure 1. Layout of experimental wetland reactors. R1 is the full biochar (20 cm) planted reactor, R2 is the half biochar (10 cm) planted, R3 is the full biochar unplanted and R4 is the no biochar planted reactor.

Figure 2. Photo of reactors after initial planting. August 7, 2015.

CHAPTER 3

RESULTS AND DISCUSSION

Biochar Characterization

The biochar obtained from the commercial supplier (Biochar Now) in a one ton bag consisted of elongated grains that were thin and flat, so that the grain size analysis provided is a measure of its second largest dimension. The had a grain size distribution of the biochar was 0% gravel, 18.2% coarse sand, 80% medium sand and 1.8% fine sand or smaller (Figure 3).

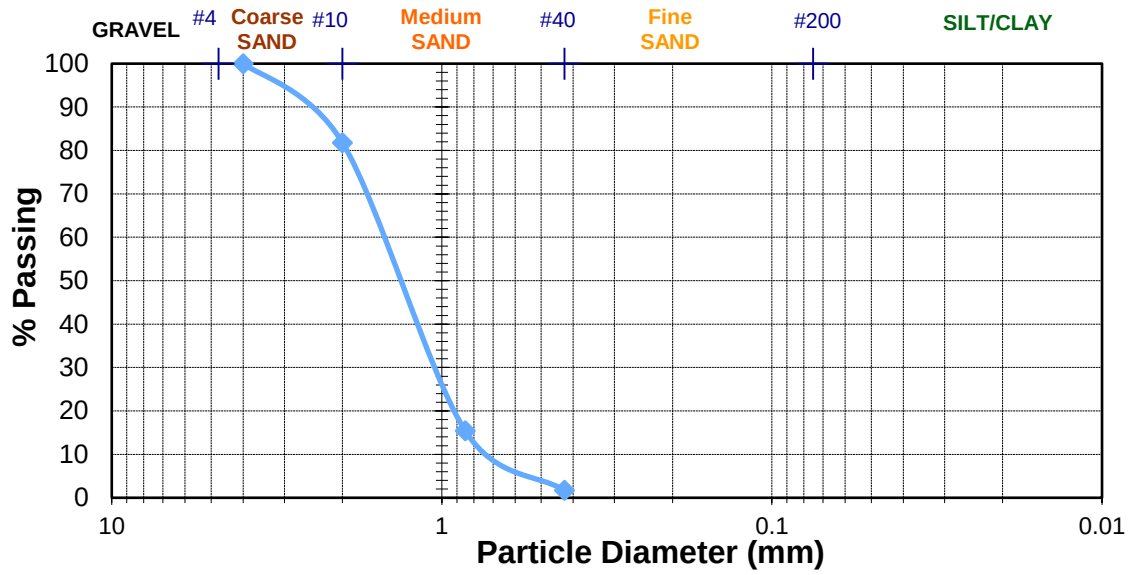

Figure 3. Biochar grain size distribution, chart provided by UM Lowell Geotechnical Engineering Research Laboratory

Biochar Sorption and Desorption

The batch sorption kinetics tests indicated that the approach to sorption equilibrium for NH_4^+-N was achieved in about 24 hours. There was no measureable adsorption of NO_3^--N or PO_4^{3-}-P, indicating that, like most un-activated, fresh biochar, this biochar's surface was primarily negatively charged. Therefore, only the NH_4^+-N equilibrium sorption isotherms were measured. The Langmuir isotherm (Figure 4a) model was a better fit to the data ($R^2 = 0.92$) than the Freundlich (Figure 4b, $R^2 = 0.74$). The Langmuir coefficient (K_L) was estimated as 0.026 and the sorption maximum (S_m) was 0.28 mg g^{-1}. This adsorption maximum for NH_4^+-N is quite low compared to many other wood biochars found in the literature whose S_m range from 0.73- 44.64 mg g^{-1} (Cui et al., 2016; Kizito et al., 2015; Sarkhot et al., 2013; Wang et al., 2015; Zheng et al., 2013). The low sorption capacity for this soft wood biochar has been attributed in the literature to the type of feedstock, pyrolysis temperatures and other pyrolysis characteristics. In the desorption test, 97% of the adsorbed ammonia was desorbed from the biochar into distilled water, indicating its potential bioavailability to plants in a wetland system.

Although the loading of NH_4^+-N to this biochar was relatively low, it was still used for the greenhouse CTW tests because sorption was not the only hypothesized benefit of biochar in a CTW system for the treatment of wastewater. In addition, there is evidence that the sorption effectiveness of the biochar might change with age. For instance, while there have been no studies on biochar aged in a saturated system, biochars have been found to undergo biotic and abiotic oxidation and experience an increase in CEC when aged in soil (Cheng et al., 2006; Cheng et al., 2008). This, combined with the biochar's potential

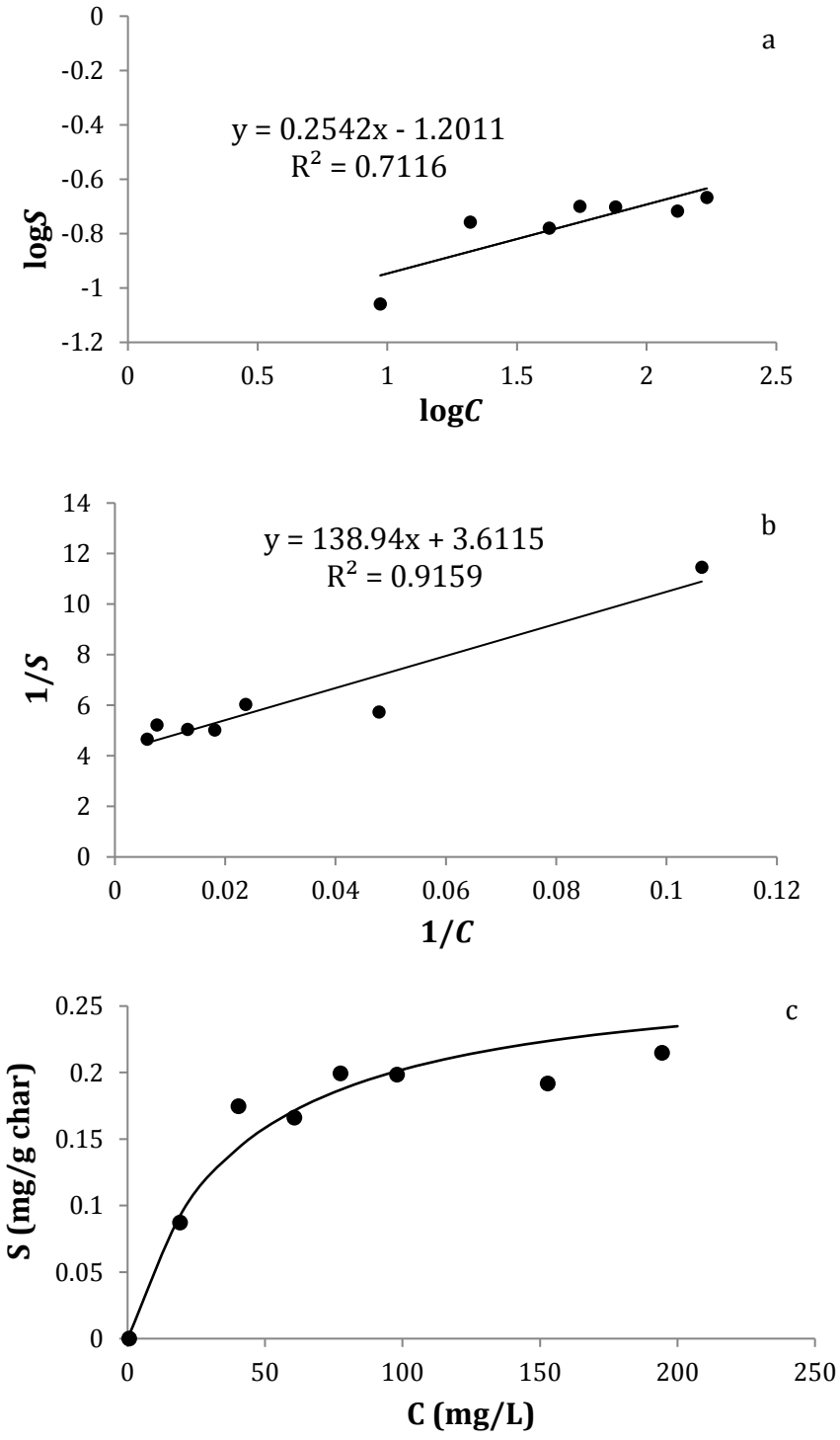

Figure 4. a: Linearized Freundlich isotherm where n = 0.33 and K_F = 0.063 mg L^{-1}. b: Linearized Langmuir isotherm where Sm = 0.28 mg g^{-1} and K_L = 0.026. c: Plot of the Langmuir isotherm curve and experimental data.

stimulation of the growth of microbial communities within the CTWs with time was expected to lead to enhanced pollutant removal over time.

Greenhouse Constructed Treatment Wetland Test #1

The initial treatment test carried out in November (Fall season) was performed with 2x diluted swine wastewater from the University of Georgia CAFO operation. The initial concentrations of nitrogen and phosphorous ions were 636.2 mg L^{-1} for NH_3-N, 9.45 mg L^{-1} for NO_3^--N, 30.8 mg L^{-1} for PO_4^{3-}-P and pH = 8.01. Within three days of this initial test, all the plants in R4 began yellowing and showed signs of stress, which was attributed to high influent nutrient concentrations. The reactors were drained and filled with the lower strength wastewater (diluted to NH_3-N ~ 200 mg L^{-1}) in order to attempt to keep the plants from dying and continue with the test. By the end of the month all of the plants in R4 (the no biochar reactor) had died. Although the plants in R1 (100% biochar) and R2 (50% biochar) also wilted, not nearly as many of the plants died (Figure 5). It was observed that half of the cattails and all the rush, willow and duckweed in R2 died while the remaining cattails showed some yellowing. In R1 only a few (~4 of 30 cattails, 2 rush, all willow) died and many were yellowing. The latter observations confirmed the decrease in plant mortality in a CTW bioreactor with an increase in biochar content in the growth media. Biochar appears to improve cattails' resistance to stressors like sudden nutrient spikes and seasonal temperature changes. The biochar tended to buffer the stress on the plants in R1 and R2.

The improved plant resistance to the shock from high nutrient loading is potentially an effect of biochar adsorption of some fraction of the NH_4^+-N, thereby decreasing its

concentration in the water column and plant rhizosphere. The effluents of the reactors were not measured, but it was possible to calculate the theoretical effect biochar adsorption of NH_4^+ had on the concentration of the wastewater in the full biochar (R1, ~15.7 kg biochar) and half biochar (R2, ~7.85 kg biochar) reactors using the theoretical sorption maximum that was calculated using the Langmuir isotherm. The biochar may have reduced the NH_4^+-N concentration to only ~570 mg L^{-1} in R1 and ~603 mg L^{-1} in R2. Secondly, the biochar is expected to significantly increase the number of bacteria and the microbial activity of denitrifiers in the bioreactor. It has also been shown that there are many species of microbes and fungi whose presence increases the ability of plants to withstand nutrient, salt, and heavy metal toxicity (Dimkpa et al., 2009). It is also likely that the onset of cold weather during this test (November) further decreased the plants' resilience to the nutrient shock.

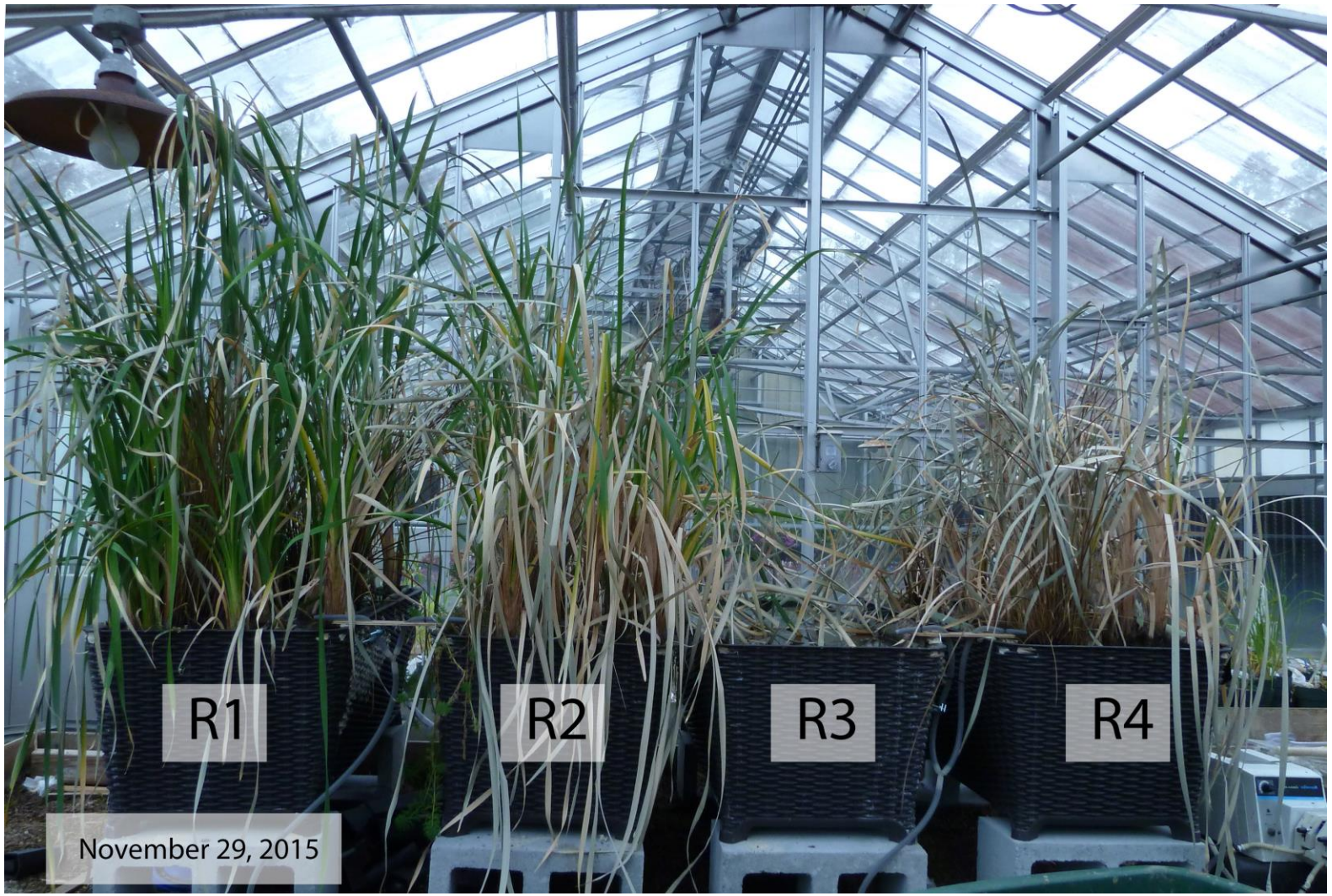

Figure 5. Photo of treatment wetland reactors in November after first swine wastewater test. Cattails showing evidence of yellowing and mortality due to shock caused by high initial nutrient loading. The no biochar reactor (R4) experienced 100% plant mortality, while the biochar reactors (R1 and R2) did not.

Greenhouse Constructed Treatment Wetland Test #2

At the start of the second test, all of the planted CTW reactors had approximately the same number of cattails (>30), a small clump of parrot's feather and a small clump of knotweed (Figure 6). The tallest cattails in each reactor were within 5 cm of each other at an average height of about 0.97 m (measured from the top of the reactor tank). Over the course of the 55 days of Test #2, all the plants in the reactor grew considerably, however growth was greater in R1 and R2 than in R4 (Figure 7). At the end of the test, the tallest cattails in R1 were 0.4 m and R2 0.5 m taller than in R4. The knotweed and parrot's feather clumps also grew larger in the biochar reactors than in the control. The dry weight of the harvested plant biomass from each reactor were as follows: R1-1.48 kg (32% greater than R4), R2-1.32 kg (18% greater than R4) and R4-1.12 kg.

The influent concentration (IC) of the monitored constituents in the wastewater and the amount of biochar both influenced the removal efficiency observed in the planted reactors which explains why R1 and R2 outperformed the other two reactors. Table 1 shows ANOVA results for all of the pollutants where either IC or biochar amount made a significant difference in the planted reactor performance. For all of the pollutants, the ANOVA performed showed that the IC and biochar amount had no significant interaction, so the interaction term was removed. IC significantly ($p<0.05$) affected TS removal from the planted reactors, measured in mg L^{-1} and percentage removal. Table 2 contains the TukeyHSD test results and shows the significance of the differences between pollutant removal due to IC or biochar amount. For TS, a significantly ($p<0.05$) higher amount was removed from the higher concentration wastewater. In contrast, biochar significantly ($p<0.05$) affected the percentage removal but *not* the mg L^{-1} removed (Table 1). This is

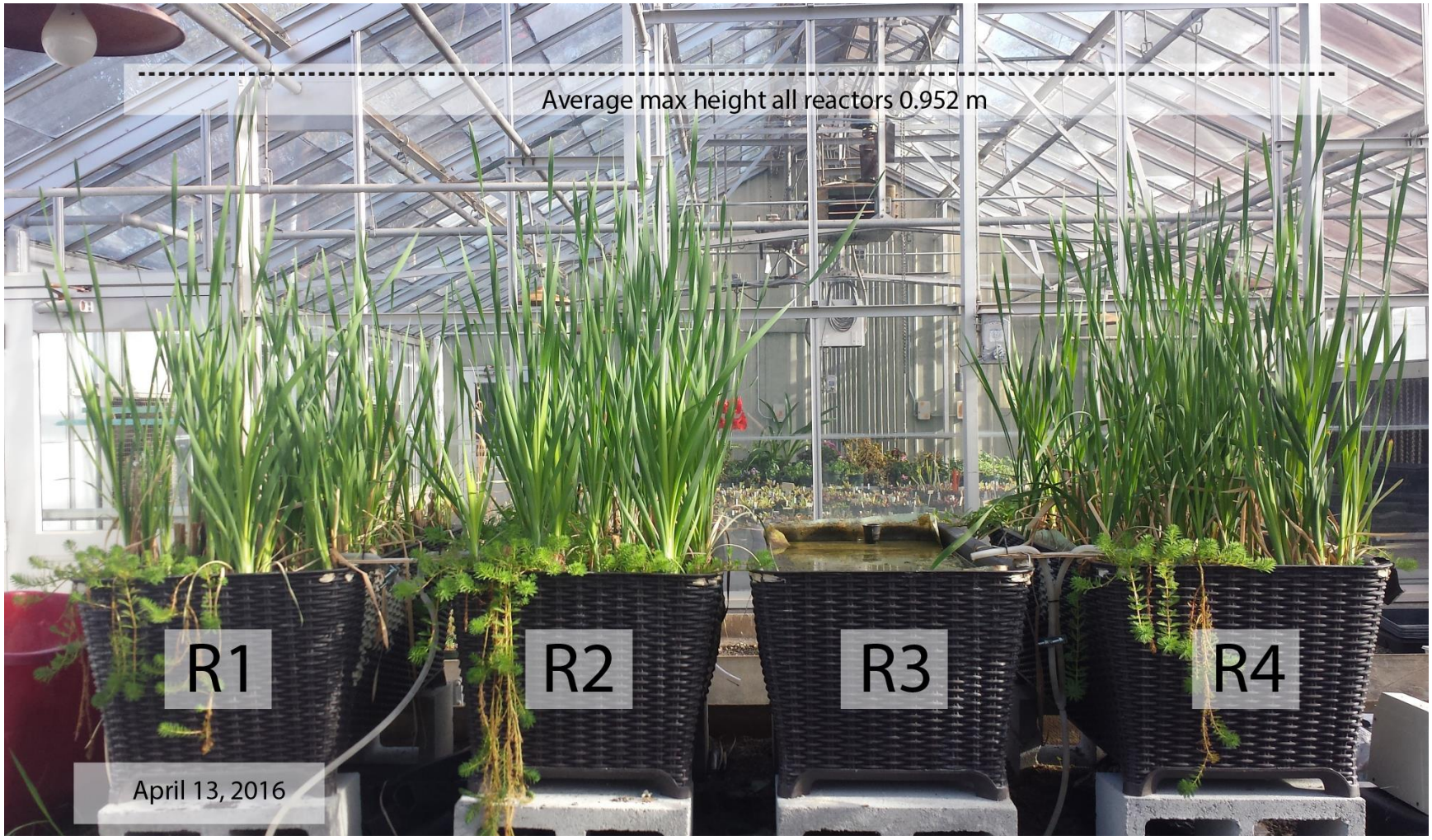

Figure 6. Photo of treatment wetland reactors at the beginning of second test with line indicating the average maximum height of the tallest plant in each reactor.

Figure 7. Photo of treatment wetland reactors at the end of second test with lines indicating the maximum height of the tallest plant in each reactor. The two biochar planted reactors (R1 and R2) had the tallest cattails, while R4 (no biochar) had the shortest.

probably due to the variation in influent concentrations (ICs) causing extra variation in removal concentrations if removal amounts by each reactor are effected by the IC. This extra variation may be minimized when the removal percentage (the percent of the IC removed by each reactor) is calculated.

Figure 8 shows the influent and effluent TS concentrations over time. The TS concentrations in R1 and R2 treated effluents were consistently lower than for R3 and R4. There was no significant difference between R1 and R2 TS removal percent (Table 2), suggesting that there is a threshold of biochar mass to realize optimum performance. Both did however, remove a significantly higher ($p<0.05$) percent of TS from the influent than R4. Overall R1's mean removal was 51% greater than R3 and 16% greater than R4. R3, with biochar only and no plants removed the least TS which is attributed mainly to sorption to or entrapment by the biochar and sand media.

For all four reactors, removal percentages increased with time and IC (Figure 9A), and the actual amount of solids removed more than doubled for each reactor (Figure 9B). The TS removal from the wastewater tends to be dominated by physical removal processes such as adsorption to plant root and biochar surfaces. Since the removal increased over time and as such the adsorption surfaces were not saturated, the biodegradable fraction of TS could have been metabolized by microorganisms as well. Other investigators have confirmed increases in microbial community abundance and composition due to biochar application to plant growth substrate (Lehmann et al., 2011) so enhanced microbial growth contributing to higher biotransformation may be one reason for R1 and R2's greater TS removal.

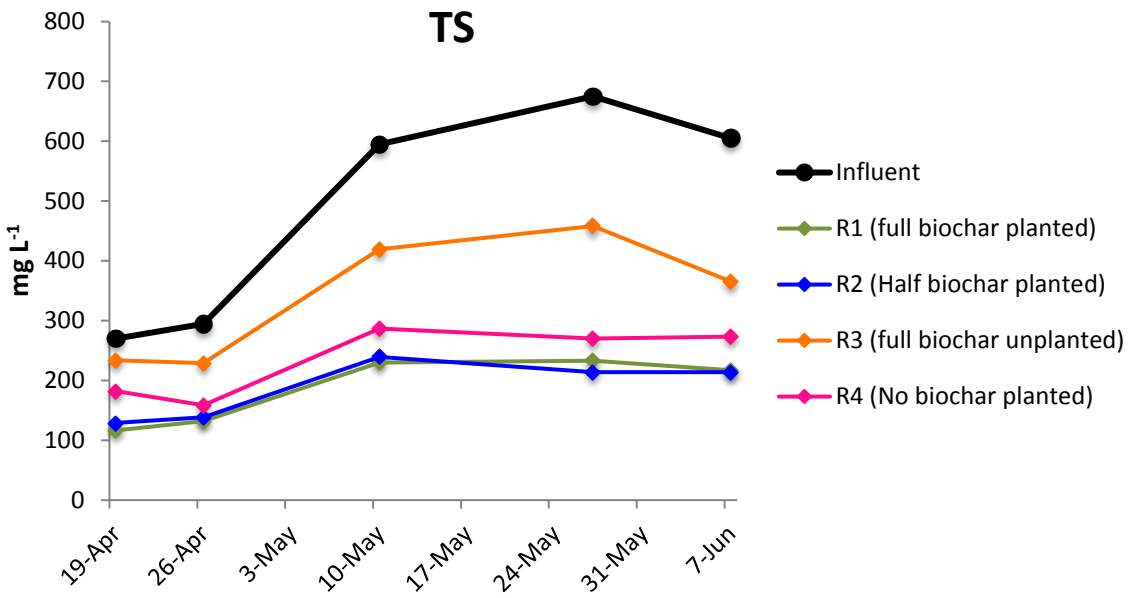

Figure 8. TS influent and effluent concentrations over the course of the study.

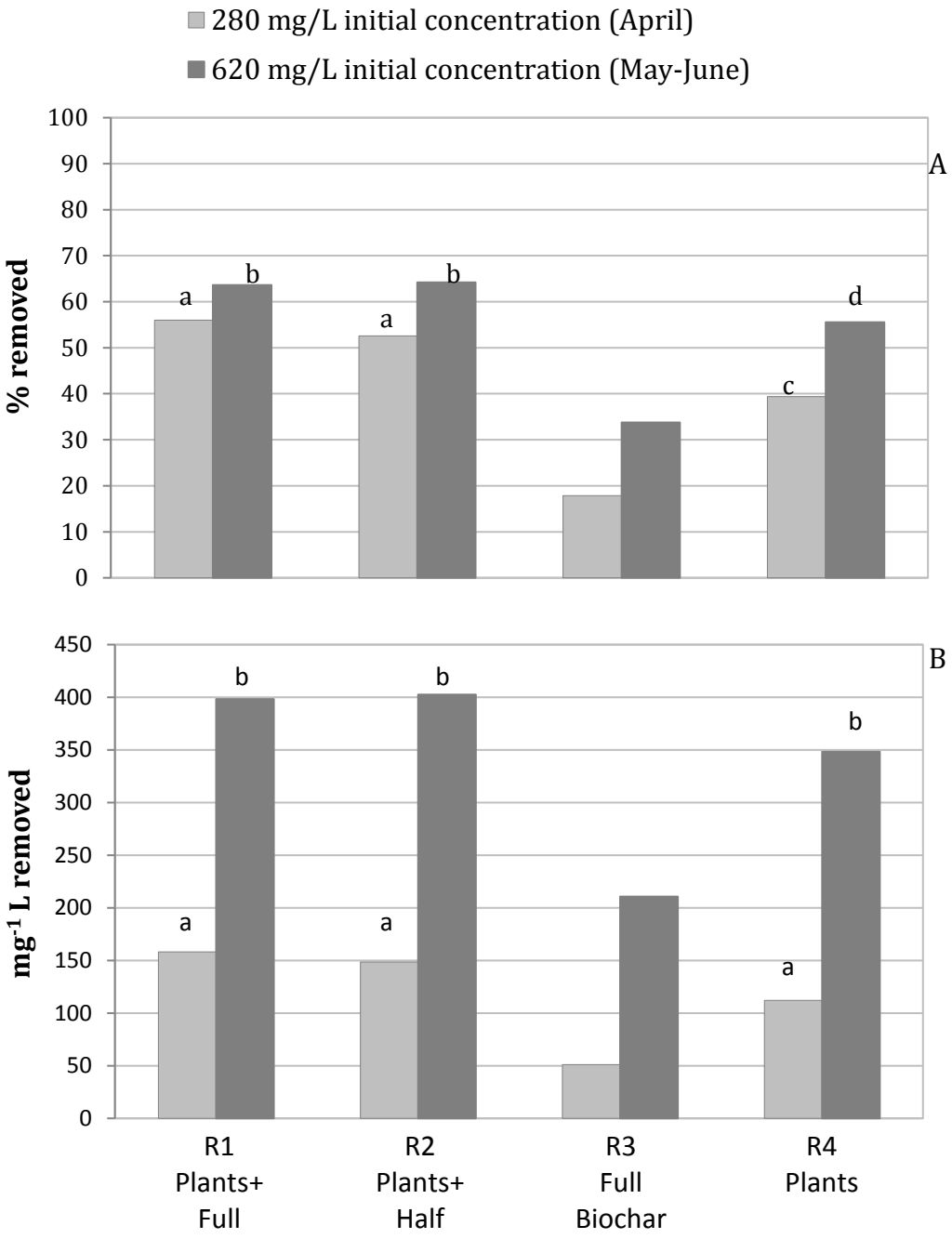

Figure 9. Total Solids removal from each reactor. Light colored bars represent average removal from lower initial concentration influent period. Dark colored bars represent average removal from higher concentration influent. Different letters represent a statistical difference between means of removal by the planted reactors.

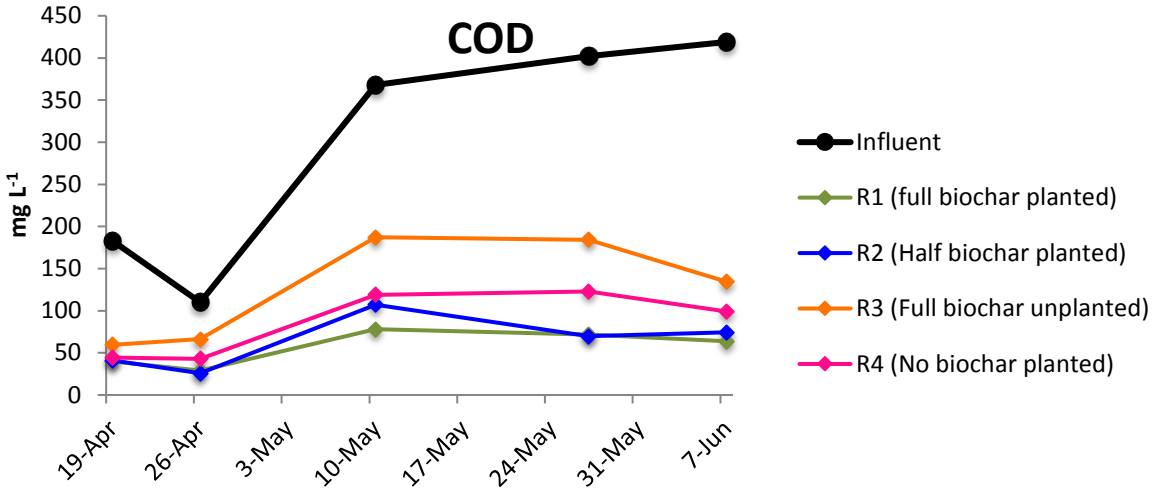

Figure 10. Chemical oxygen demand (COD) influent and effluent concentrations over the course of the study.

The trends for COD removal were similar to TS removal. Each reactor maintained a relatively steady effluent COD concentration (Figure 10). IC (influent concentration) significantly ($p<0.05$) affected COD removal in mg L^{-1} but not percentage (Table 1) suggesting that for each planted reactor, the overall COD removal capacity can be better described as a percent of the IC than a set removal mass or concentration. The reactors removed a significantly ($p<0.05$) higher concentration from the higher IC than the lower (Table 2) influent dose treatments. Biochar did not significantly affect removal concentration (in mg L^{-1}) by the planted reactors but it did effect the removal calculated as a percent of the influent ($p<0.05$, Table 1). Once again this may be due to variations in IC causing variations in the removal amount in concentration units. Although the COD removal efficiencies of R1and R2 were not significantly different from each other in terms

Table 1. Results of type III ANOVA on test 2 data with the significance (based on the p-value) of the effect of each factor on each pollutant's removal reported. "***" denotes significance past 0.001, "**" past 0.01, "*" past 0.05, and "." past 0.1. Pollutants which did not experience significant effects from either of the factors were excluded from the table in the interest of space.

	Pollutant											
Factor	TS ($mg\ L^{-1}$)	TS (%)	COD ($mg\ L^{-1}$)	COD (%)	NH_4 ($mg\ L^{-1}$)	NH_4 (%)	PO_4^{3-} ($mg\ L^{-1}$)	K ($mg\ L^{-1}$)	K (%)	K* ($mg\ L^{-1}$)	K* (%)	Na* ($mg\ L^{-1}$)
Influent Concentration	***	***	***		**	***	***		***		***	.
Amount of Biochar		**		*	.	**		*	**	.	*	

Table 2. Results of Tukey's Honestly Significant Difference test on greenhouse test 2 data, with the estimated difference between removal means and significance (based on the p-value) reported. "***" denotes significance past 0.001, "**" past 0.01, "*" past 0.05, "." past 0.1. K* and Na* denote results after having removed May 10th data. Pollutants which did not experience significant effects from either of the factors were excluded from the table in the interest of space.

	Pollutant											
Comparison	TS (mg L^{-1})	TS (%)	COD (mg L^{-1})	COD (%)	NH_4 (mg L^{-1})	NH_4 (%)	PO_4^{3-} (mg L^{-1})	K (mg L^{-1})	K (%)	K* (mg L^{-1})	K* (%)	Na* (mg L^{-1})
Influent Concentration												
High-Low	243 ***	11.8 **	197 ***		14.6 **	-18.7 ***	4.91 ***		-22.9 ***		-22.1 ***	2.78 ***
Amount of Biochar												
50-0		10.5 **		7.95 .		13.4 **		11.9 .	16.3 *	8.22 .	12.3 *	
100-0		11.5 **		9.50 *	12.8 .	15.4 **		12.3 *	17.7 *		11.1 .	
100-50												

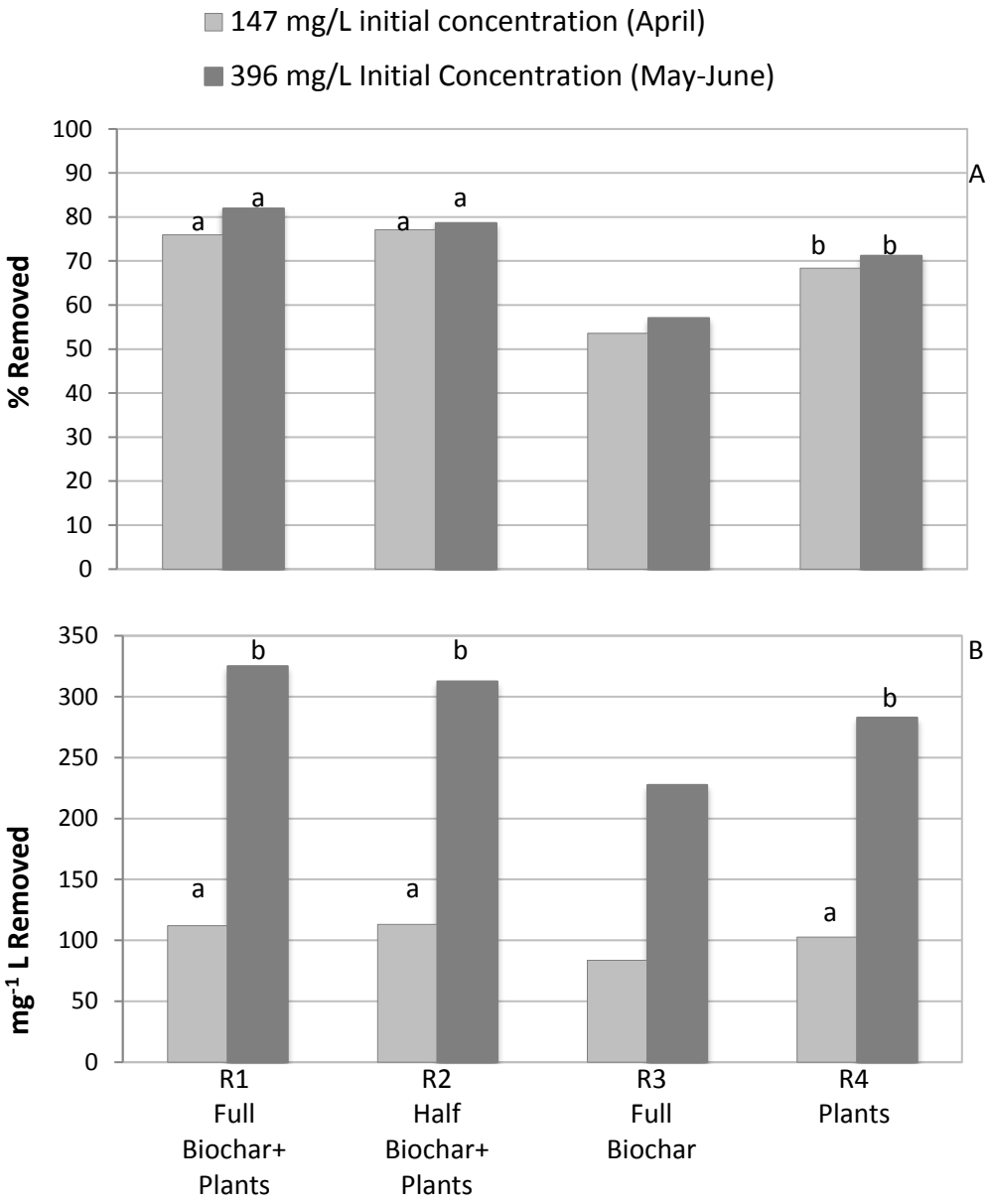

Figure 11. COD removal from each tank. Light colored bars represent average removal from lower initial concentration influent period. Dark colored bars represent average removal from higher concentration influent. Different letters represent a statistical difference between means of removal by the planted reactors.

of the percentages of COD (Table 2), R1 and R2 did remove a significantly higher ($p<0.05$ and $p<0.1$, respectively) percent than R4 and R3. R1 removed 29.1% and 12.2% more than R3 and R4, respectively.

Figure 11 shows that the percentage removal of COD in all the reactors increased slightly with time and an increase in IC, and that the COD removal in units of mg L^{-1} more than doubled over time when the IC was doubled. COD is a measure of the amount of oxygen required to degrade the organic material in the wastewater that can be oxidized by other chemicals as well as by microbes. In constructed wetlands, the primary COD removal mechanism is microbial oxidation, although plants are capable of removing some COD as well. Biochars have also been found to remove COD through adsorption (Berger, 2012). The COD removal in R3 is attributed to a combination of adsorption by biochar and microbial removal activity, meanwhile in R4 it is attributed to the plants and microbial activity. Since R1 and R2 removed the most COD, the increased plant biomass and biochar's large surface area available for adsorption and enhancement of microbial activity increased COD removal. This result is comparable to a similar study by De Rozari et al. (2015) which found planted wetlands with biochar amended sand media removed more biological oxygen demand than those with sand and plants only.

NH_4^+-N removal was different from COD removal. The IC of NH_4^+-N had a significant effect on both mass in mg L^{-1} ($p<0.05$) and percent ($p<0.05$) removal (Table 1) estimations. The higher concentration resulted in a significantly ($p<0.05$) higher mass removal but a significantly ($p<0.05$) lower percent removal for the planted reactors (Table 2). Biochar amount had a moderately significant ($p<0.1$) effect on NH_4^+-N mg L^{-1} removal and a significant ($p<0.05$) effect on the percentage removal of the planted reactors (Table 1).

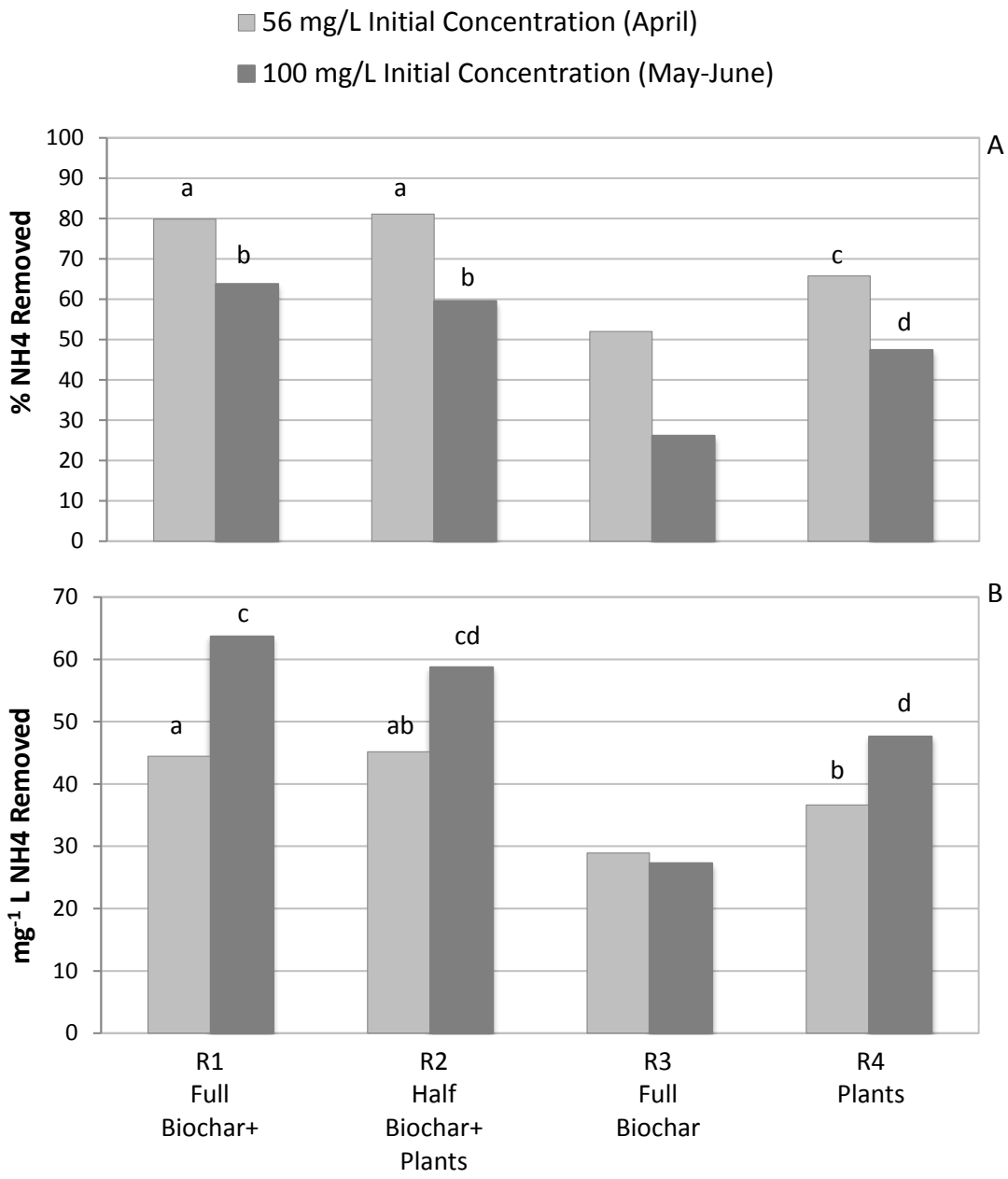

Figure 12. NH_4^+-N removal from each tank. Light colored bars represent average removal from the lower initial concentration influent period. Dark colored bars represent average removal from higher concentration influent. Different letters represent a statistical difference between means of removal by the planted reactors.

Removal by R1 and R2 was not significantly different, but R1 removed a moderately significantly ($p<0.1$) higher amount than R4 and both R1 and R2 removed a significantly ($p<0.05$) higher percent NH_4^+-N than R4 (Table 2). R1 overall NH_4^+-N removal was 50% greater than R3 and 23% greater than R4.

Figure 12 shows each reactor's removal of NH_4^+-N as both percent of the IC and in mg L^{-1} for high and low IC of NH_4^+-N treatments. The actual mass (in mg L^{-1}) of NH_4^+-N removed by each of the three planted reactors increased with increased IC and time although the increase in the mass removal was not proportional to the increase in IC (Figure 12a). R1's removal increased by 43.3%, R2 by 30.0% and R4 by 18.0% with increased IC and time. These increases can be attributed to the observed increase in plant growth corresponding to increased plant uptake of NH_4^+-N over time. R3's removal amount remained nearly at a steady state over the course of the test (Figure 12b); a removal mechanism attributed to in the peer-reviewed literature (Ghezzehei et al., 2014) is the adsorption of NH_4^+-N from the wastewater onto the biochar surface. If this was the only removal mechanism in the reactor, the removal capacity should decrease with increased loading over time until no removal was observed due to the saturation of sorption sites. The total mass of NH_4^+-N (17472 mg) added to R3 by the end of the second week of the test (calculated with equation 4) far exceeds the calculated theoretical Langmuir adsorption maximum of the mass of biochar in the reactor (4396 mg NH_4^+-N). Therefore, other removal mechanisms such as microbial oxidation or transformation of NH_4^+-N to ammonia and/or nitrogen gases were at work in this reactor. For a CTW reactor, the contribution of algae and microorganisms to the removal of NH_4^+-N is expected to be significant. R4

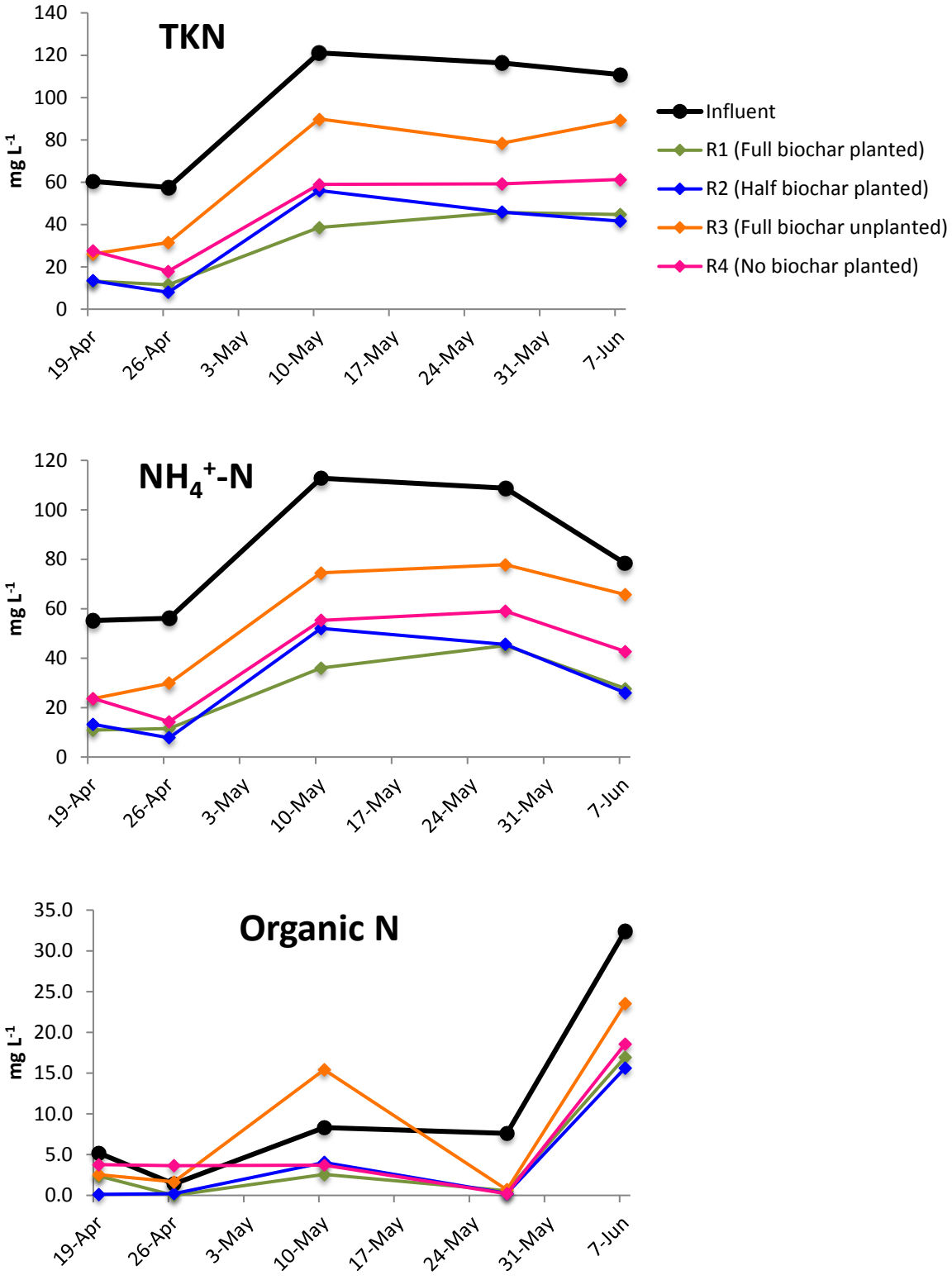

Figure 13. Total Kjeldahl Nitrogen, NH_4^+-N, and Organic N (calculated as TKN - NH_4^+-N) concentrations over the course of the study.

removed more NH_4^+-N than R3 indicating the importance of plants in NH_4^+-N removal and use as a plant nutrient. The fact that R1 and R2 removed the most NH_4^+-N then suggests that the interaction of biochar and plants enhances NH_4^+-N removal capacity. The more rapid increase in NH_4^+-N removal by R1 and R2 over that of R4 over time may be attributed to the biochar mediated increase in plant growth and plant uptake and root zone microbial activity.

TKN is a measure of the total amount of NH_4^+-N and organic N. The primary source of TKN in most swine wastewater is NH_4^+-N. Figure 13 shows influent and effluent concentrations of TKN, NH_4^+-N, and organic N (calculated as TKN - NH_4^+-N) over time. For the most part, all the reactors reduced the amount of organic N in the wastewater, with one noticeable spike in organic N concentration in the effluent of R3. Organic nitrogen can be released into a wetland system by a variety of processes including microbial mineralization of solid organic material (e.g., organic TS), desorption from wetland media, nitrogen fixation as well as release from plants and organisms (Kadlec, 2009). It is unclear exactly which process caused this release in R3. Overall concentrations of organic N in either the influent or the effluent were significantly lower than NH_4^+-N.

The concentration of NO_3^--N in the influent CAFO wastewater was low (<1.0 mg L^{-1}) and fluctuated in the effluent of all four reactors (Figure 14). The increase in NO_3^--N tended to be relatively higher in the biochar planted reactors. The increased levels of NO_3^--N in reactor effluent (over that of the influent) probably indicate periods of nitrification that were greater than any NO_3^--N removal by plant uptake by plants and denitrification by microorganisms.

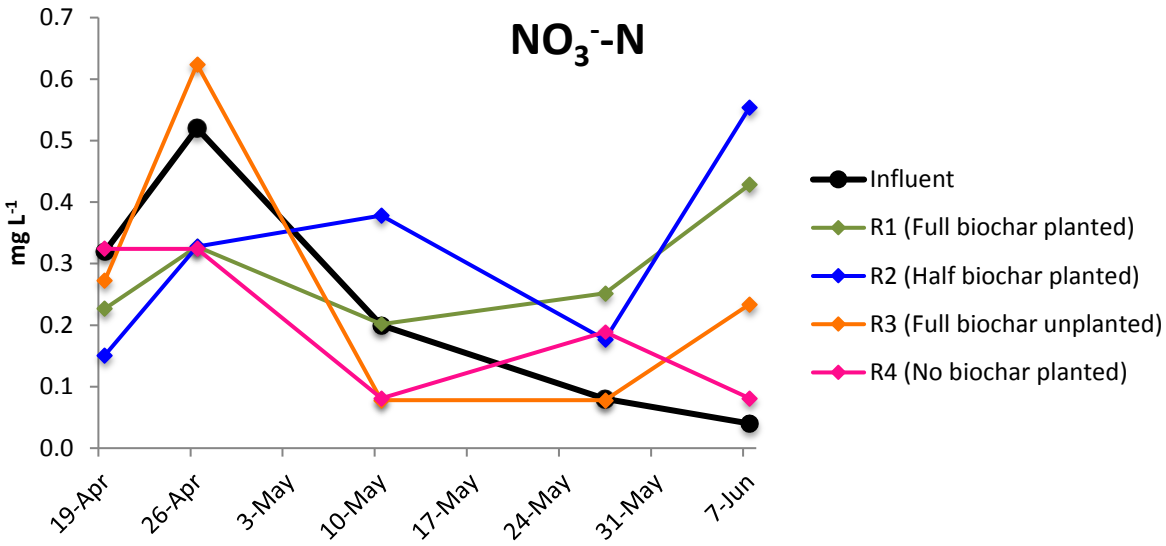

Figure 14. NO_3^--N influent and effluent concentrations over the course of the study.

PO_4^{3-} is the primary source of P in swine wastewater, and the difference between Total P and PO_4^{3-} is probably organic P. Figure 15a shows PO_4^{3-}-P influent and effluent concentrations over the course of the test. The effluent PO_4^{3-}-P concentrations remained steady over time, despite changes in the IC. R1 and R2 effluents consistently had lower PO_4^{3-}-P concentrations than R4 and especially R3. This was not the case for organic P. Figure 15b shows organic P (calculated as *Total P - PO_4^{3-}-P*) concentrations over time. The effluents of all four reactors had organic P levels that were higher than the influent for some of the weeks, and all had some increases, but R1 and R2 had relatively steady levels of organic P (<4 mg L^{-1}) while R3 and R4 both had sudden spikes. The spikes in R3 and R4 are most likely due to changes in microbial and plant root zone activity as both utilize P for growth and can convert PO_4^{3-}-P to organic P. Plants can release organic P back into a wetland and can release large amounts in a short period of time when they are dying

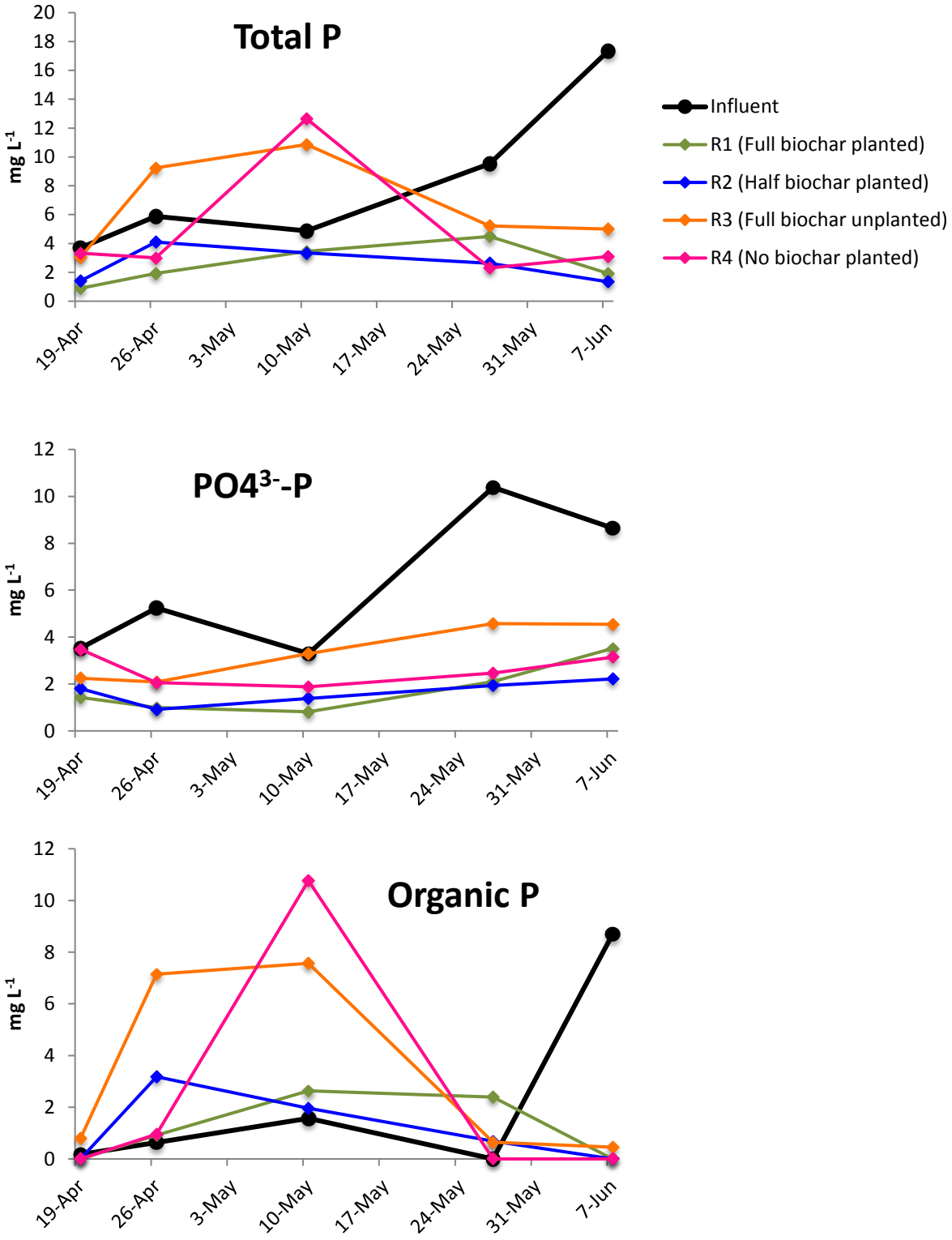

Figure 15. Total P, PO_4^{3-}-P and organic P (Total P – PO_4^{3-}-P) influent and effluent concentrations over time.

(Dunne and Reddy, 2005), however there was no indication of dying plants in R4 during the time of its P spike. Microbial activity or die off, potentially related to changes in redox potential within these reactors can also potentially cause a release of P into the system, as well as the reduction of ferric to ferrous iron. There were corresponding releases of Fe in the effluents of the two control reactors (R3 and R4) on the same dates as the P releases that may provide evidence of this (Figure 17). The results of R1 and R2 suggest that the interaction between biochar and plants in the CTW minimizes the organic P release from the wetland reactors by stabilizing the redox potential.

Although the effluent PO_4^{3-}-P concentration was significantly ($p<0.05$) influenced by the IC, the percent removal was not (Table 1). This indicates that for the planted reactors, removal is better described as a percentage of the IC than as a set mass removal capacity. As a result, for the planted reactors, the mg L^{-1} removed from the higher IC loading was significantly ($p<0.05$) higher than from the lower IC loading. Figure 16 shows that for all four reactors, the amount of inorganic P removed increased with time and the IC. R3 successfully removed PO_4^{3-}-P from the wastewater and since this biochar was not found to adsorb PO_4^{3-}, this removal can be attributed to algae and other microbes living in the reactor's media and adsorption to the layers of sand and gravel. R4 removed PO_4^{3-}-P better than R3 indicating that plants and the many microbes growing in the gravel and associated with the plants were more efficient at PO_4^{3-}-P removal than just biochar alone. The biochar had no statistically significant effect on the planted reactors' PO_4^{3-}-P removal (Table 2). Despite the biochar's ability to remove PO_4^{3-}-P on its own in R3, it does not appear to enhance plant uptake of PO_4^{3-}-P in R1 and R2. This is comparable to results in a similar study by De Rozari et al. (2016) where planted wetlands with only sand media removed

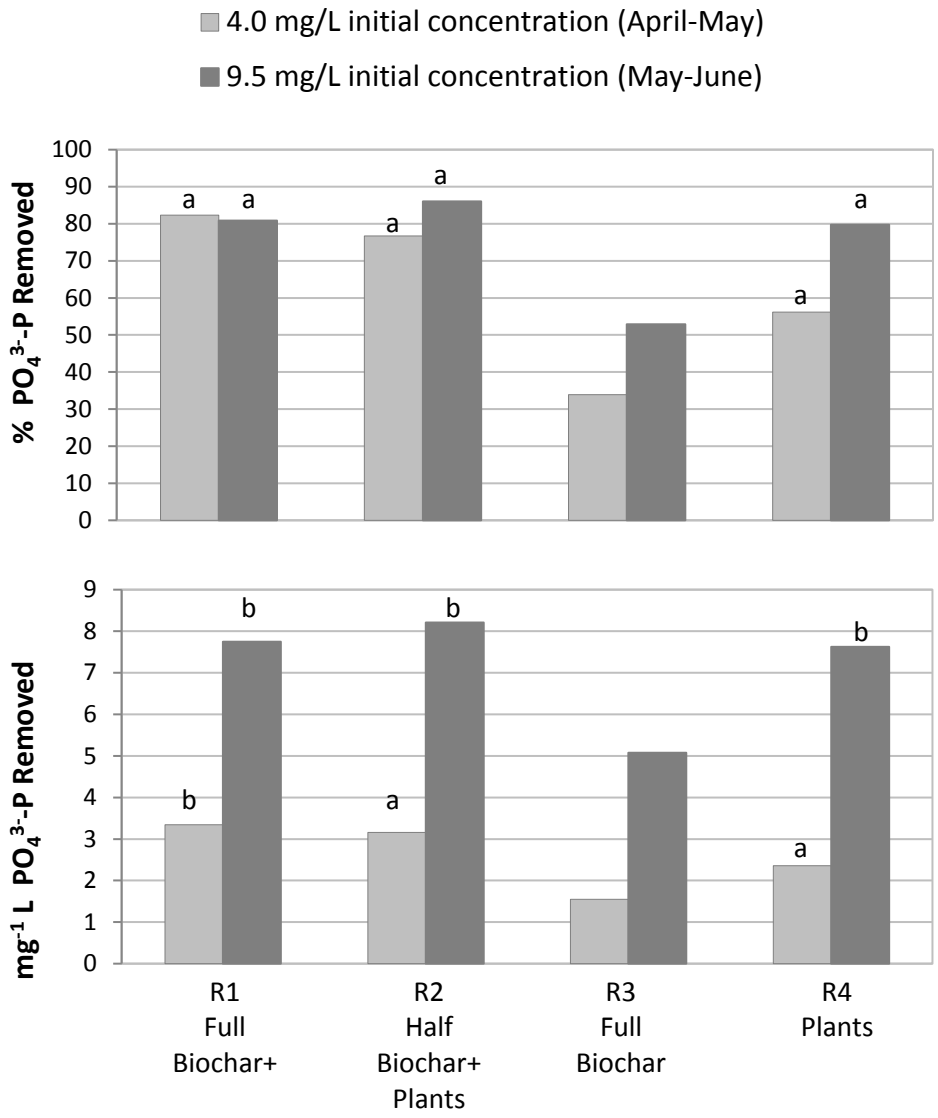

Figure 16. PO_4^{3-}-P removal from each tank. Light colored bars represent average removal from lower initial concentration influent period. Dark colored bars represent average removal from higher concentration influent. Different letters represent a statistical difference between means of removal by the planted reactors.

more P than ones amended with biochar. This is likely due to the fact that sand media has more surface area for P adsorption than gravel, and an overall neutral charge compared to the negative charged surfaces of the softwood biochar used in this test.

The reactors were also somewhat able to decrease K, S and Na concentrations in the wastewater, some more than others (Figure 17). The IC of most of these parameters varied in a way inconsistent with the wastewater dilution, although they all increased over time. Figure 17 also shows spikes in K, S and Na effluent concentrations from one or more of the reactors on May 10th, the same date of the spike in Organic N and Organic P. For K, the IC was consistent enough with the dilution to perform the ANOVA the same way as the previous parameters, but it was performed twice, once with all the data and once without the data from May 10th (Tables 1 and 2) because Figure 17 shows a release of K in R3 as well as potentially in R4. The amount of biochar in the planted reactor significantly ($p<0.05$) affected the amount of K removed by the planted reactors when the May 10th data was included, however, the significance went down to only moderate ($p<0.1$) when the May 10th data were not included in the statistical analysis. The IC of K did not affect the amount removed. Both the IC and biochar significantly affected the percent removed with and without the May 10th data (Table 1). R1 and R2 did not remove significantly different percentages of K, but both removed significantly ($p<0.05$) more than R4 when including May 10th and moderately ($p<0.1$ R1) to significantly ($p<0.05$ R2) more when discarding May 10th. This suggests that the biochar and plants together both enhanced K removal as well as stabilized the mechanisms responsible for K release.

Figure 18 shows that the average mass of K removed increased with time and the IC in R1 and R2 but not in R4. In R3, more K was released into the wastewater than was in the

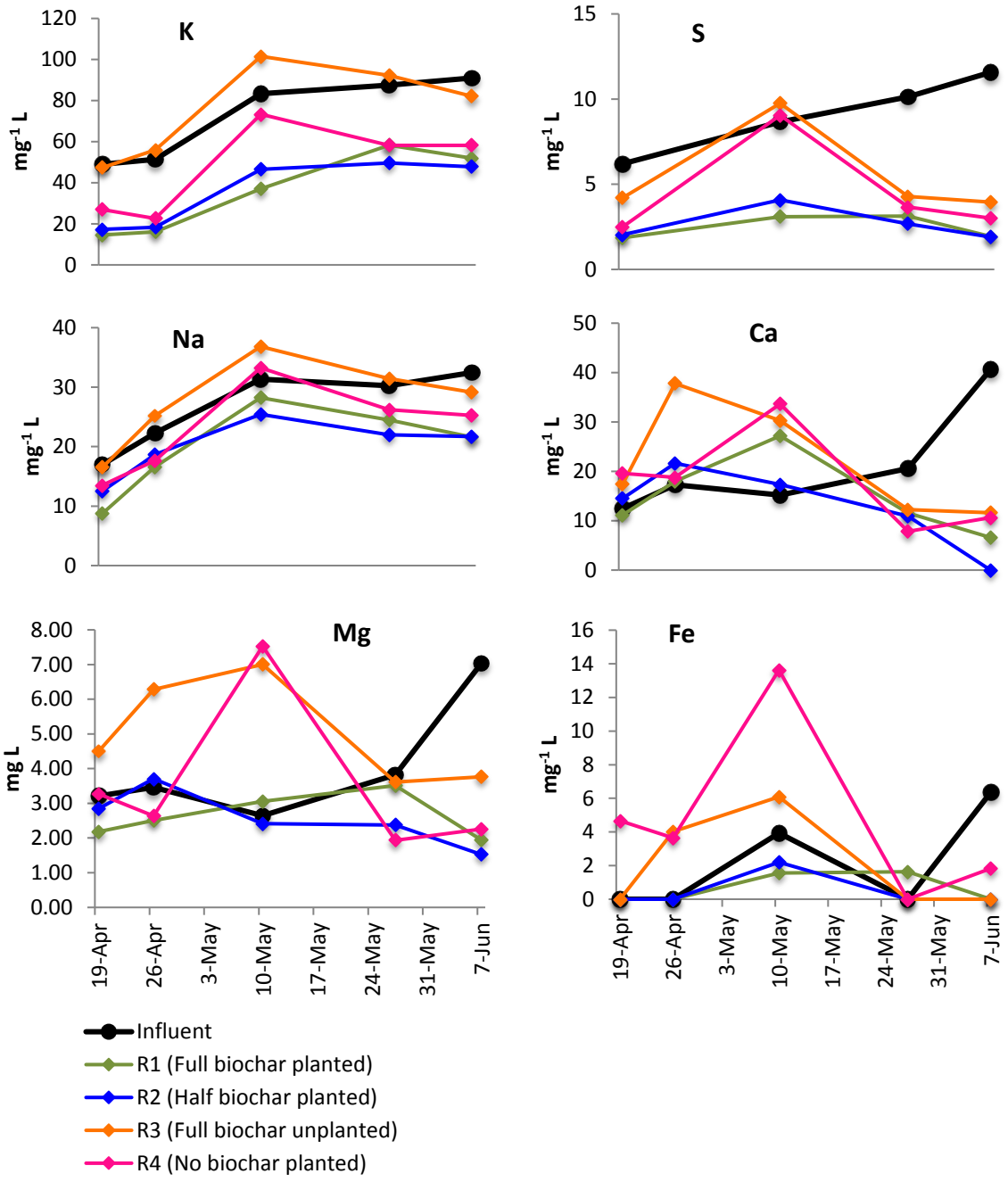

Figure 17. Influent and effluent concentrations over time for K, S, Na, Ca, Mg and Fe.

influent (suggesting leaching of K from the biochar media). The results observed in R3 suggest that the primary K removal mechanism in the other reactors was through plant uptake. The higher K removal in R1 and R2 over that of R4 can then be attributed to the positive effect of biochar on plant growth and microbial abundance. Figure 19 shows no Na removal from R3, indicating that any Na removal is entirely due to plant uptake. There was no statistically significant effect of biochar on the removal of Na in the planted tanks (Table 1).

The ICs of S during the test were not consistent with the wastewater dilutions (the S concentration in the influent rose steadily over time instead of being "low" during April and "high" during May-June when the wastewater was less diluted (Figure 17), so S data was not separated into "high" and "low" IC sections for analysis. In addition, the IC on April 29th was measured as an order of magnitude higher than any of the others which seemed highly unlikely and possibly the result of a measurement error. One-way ANOVA was performed without IC as a factor and with April 29th and May 10th data removed as well because Figure 17 shows a S release in R3 and R4 on that date. The biochar had no significant direct effect on S removal in the planted biochar CTW reactors (R1 and R2) despite the reported capability of biochar alone (R3) to remove S (Figure 17). The biochar and plants together limited the release of S into solution in R1 and R2.

The release of nutrients from the CTW observed in the effluents were generally higher for R3 and R4 than R1 or R2. R3 released the most organic N (Figure 13), P (Figure 15), K, S, Na, Ca, Mg and Fe (Figure 17). R4 also released to a lesser extent P, K (although not over influent levels), S, Na, Ca, Mg and Fe. R1 released only small amounts of Organic P, Ca and Mg and R2 only some Organic P, Ca, Mg and Fe. Nutrient releases in wetlands can be

due to redox and pH changes or cell decay due to microbe or plant death. If the releases were just due to a change in redox, the COD should also change significantly, however Figure 10 does not show any decrease in the COD. Since most of the recorded releases were observed on May 10th when influent and effluent pH were at their highest (Figure 20), it is unlikely that the spikes were due to a sudden dissolution of adsorbed nutrients. Moreso, the pH of R3 that registered the highest effluent concentrations remained high during the week of May 10.

Greenhouse Constructed Treatment Wetland Test #3

The ANOVA performed with data from both test 2 and 3 provided evidence of the effect of time (aging of the CTWs) on the performance of each reactor. All of the reactors removed significantly ($p<0.05$) more NH_4^+-N and PO_4^{3-}-P during the third test (Tables 3 and 4) indicating the importance of microbial growth and acclamation, as well as increased root mass in the reactor media over time on the treatment efficiencies of the reactors. NH_4^+-N removal was moderately significantly ($p<0.1$, Table 5) influenced by the biochar in the reactor with no significant difference between R1 and R2 but R1 removed significantly ($p<0.05$ for removal in mg L^{-1} and $p<0.1$ for removal as a percent) more NH_4^+-N than R4 (Table 6). This is consistent with the results from the second test. The overall increase in NH_4^+-N removal from test 2 to test 3 was 22 mg L^{-1} for R1 (34% increase since test 2), 18 mg L^{-1} for R2 (31% increase), 25 mg L^{-1} for R3 (91% increase) and 20 mg L^{-1} for R4 (41% increase). It is possible that the more dramatic increase in R3 (the unplanted biochar reactor) compared to the other planted reactors was due to more rapid microbial growth due to lack of competition for nutrients with plants. In the period between test #2 and #3,

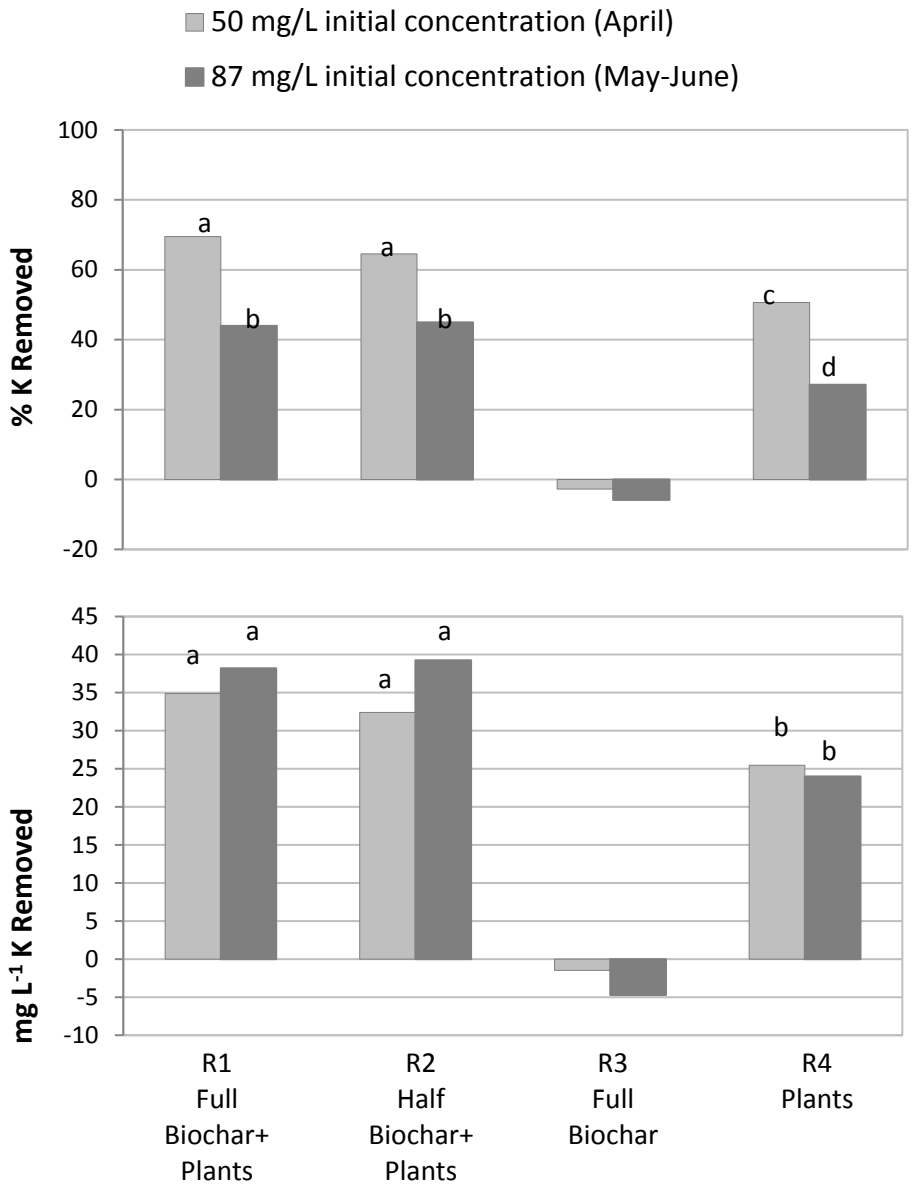

Figure 18. K removal from each tank. Light colored bars represent average removal from lower initial concentration influent period. Dark colored bars represent average removal from higher concentration influent. Different letters represent a statistical difference between means of removal by the planted reactors.

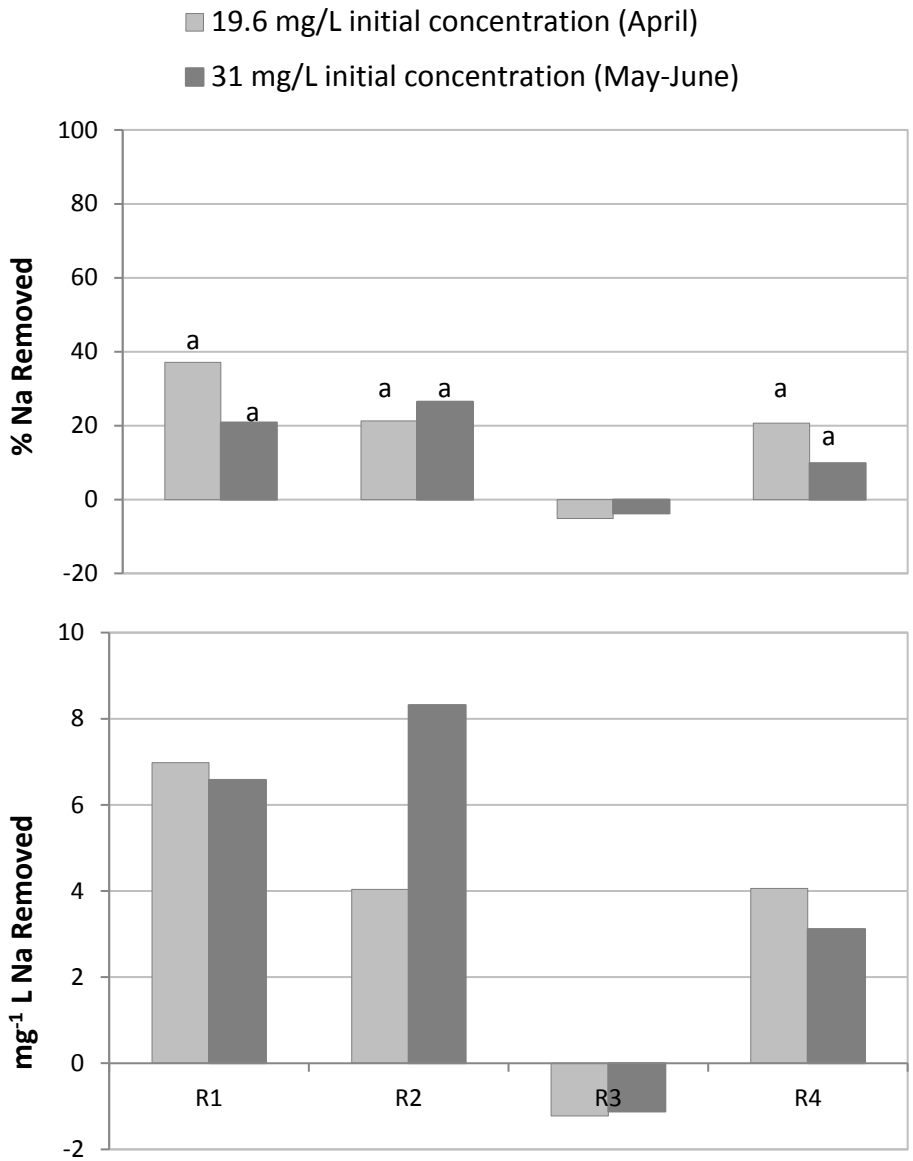

Figure 19. Na removal from each tank. Light colored bars represent average removal from lower initial concentration influent period. Dark colored bars represent average removal from higher concentration influent. Different letters represent a statistical difference between means of removal by the planted reactors.

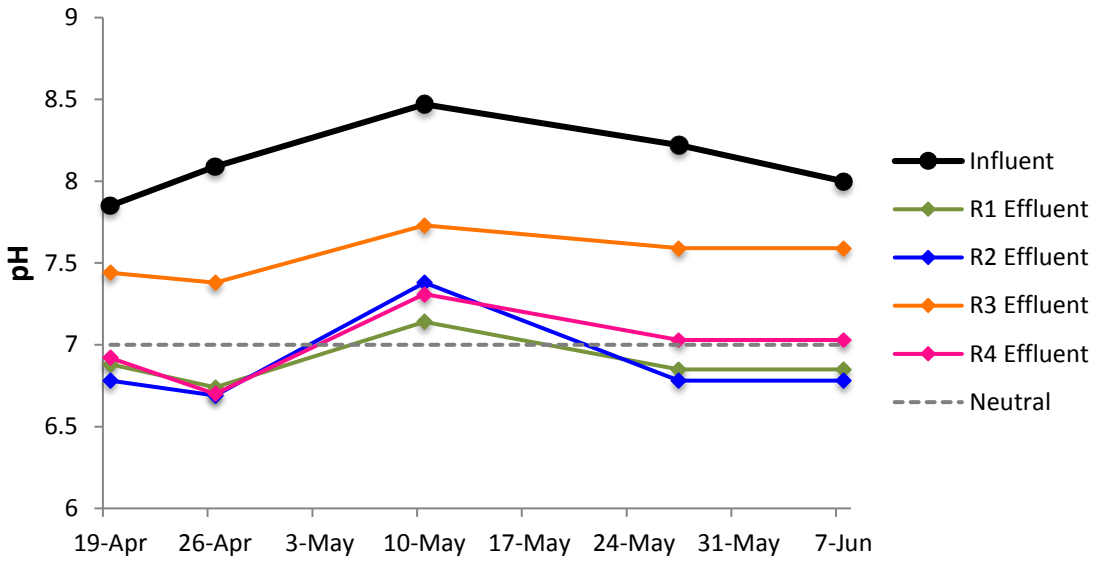

Figure 20. Influent and effluent pH over the course of test 2.

Table 3. Results of type I ANOVA on tests 2 and 3 data with the significance (based on the p-value) of each factor's effect reported. "***" denotes significance past 0.001, "**" past 0.01, "*" past 0.05, and "." past 0.1.

	Pollutant			
Factor	NH_4 (mg L^{-1})	NH_4 (%)	PO_4^{3-} (mg L^{-1})	PO_4^{3-} (%)
Reactor	***	***		***
Test	***	***	***	**

Table 4. Results of Tukey's Honestly Significant Difference test on greenhouse tests 2 and 3 data, with the estimated difference between removal means and significance reported. "***" denotes significance past 0.001, "**" past 0.01, "*" past 0.05, and "." past 0.1.

	Pollutant			
Comparison	NH_4 (mg L^{-1})	NH_4 (%)	PO_4^{3-} (mg L^{-1})	PO_4^{3-} (%)
Reactor				
4-3	17.3 *	17.0 **		27.7 **
2-3	27.8 ***	27.5 ***		33.7 **
1-3	34.5 ***	33.3 ***		34.2 ***
2-4		10.5 .		
1-4	17.1 *	16.3 **		
1-2				
Test				
3-2	21.2 ***	13.4 ***	34.5 ***	16.4 **

Table 5. Results of type I ANOVA on test 3 data for the planted reacors. The significance (based on the p-value) of the effect of the amount of biochar on each pollutant's removal is reported. "***" denotes significance past 0.001, "**" past 0.01, "*" past 0.05, and "." past 0.1.

	Pollutant			
Factor	NH_4 (mg L^{-1})	NH_4 (%)	PO_4^{3-} (mg L^{-1})	PO_4^{3-} (%)
Biochar	.	.		

Table 6. Results of Tukey's Honestly Significant Difference test on greenhouse test 3 data for the planted reactors (T1=100% biochar, T2=50%, T4=0%). The estimated difference between removal means by each tank and significance of this difference (based on the p-value) is reported. "***" denotes significance past 0.001, "**" past 0.01, "*" past 0.05, and "." past 0.1.

	Pollutant	
Comparison	NH_4 (mg L^{-1})	NH_4 (%)
Amount of Biochar		
50-0		
100-0	18.2*	16.2 .
100-50		

the reactors received clean water only, so some nutrients may have become limited in the planted reactors as the plants regrew. It is likely that if the nutrient-rich wastewater had continued to run through the reactors instead, that the planted reactors might have experienced even more rapid microbial and plant growth and increases in nutrient removal.

During the 3rd test, the removal of PO_4^{3-}-P from the reactors was similar to the results of the 2nd test (Table 5). All the planted reactors each experienced similar increases in removal from the 2nd to the 3rd test: R1 removed 35 mg L^{-1} (22% increase), R2 34 mg L^{-1} (24%), R3 28 mg L^{-1} (18%) and R4 34 mg L^{-1} (23%). R3 showed the lowest increase in PO_4^{3-}-P removal which again confirms the role of plants in the performance of biochar treatment systems for CAFO and similar wastewater.

CHAPTER 4

CONCLUSIONS

The constructed treatment wetland (CTW) bioreactors amended with soft wood performed better than the CTW with plants alone, and the no-plant bioreactor containing only biochar. Plant growth in the biochar reactors was significantly faster than in the planted gravel reactor, and at the end of the experiment had produced larger amounts of aboveground plant biomass. The biochar tended to increase plant tolerance of high nutrient concentrations and cold weather, potentially increasing the capacity of a wetland system to treat wastewater during the winter months. The presence of biochar in a CTW may also decrease the need to pretreat wastewater prior to its addition to a constructed wetland. This effect, however, was dependent on the amount of biochar in the system as the plants in the reactor with the most biochar had the highest survival rate when the reactors were overloaded with nutrients.

The CTW amended with biochar was most effective in removing nutrients from the CAFO wastewater due to the combined contributions of nutrient adsorption by the biochar, microbial transformation and uptake by the plants. Interestingly, the amount of biochar in the reactor appears to have made little difference in the reactors' removal capacity of most of the parameters tested. This suggests that the biochar's enhancement of the nutrient removal by plants and microbes was more important than the adsorption contributed by the biochar. The presence of biochar, at an undetermined threshold quantity significantly enhanced biological removal of the nutrients from the CAFO wastewater.

The overall removal efficiencies of the reactors increased over time for many of the constituents monitored, although for some the increase was observed only in the planted reactors (NH_4^+). This is attributed to improved plant growth and the increase in plant biomass in each reactor leading to increased uptake. For the parameters for which their removal efficiency increased both the planted and unplanted bioreactors (TS, COD, PO_4^{3-}), it is assumed that increased microbial growth and activity over time significantly influenced the treatment efficiency. For many constituents (e.g., organic P, K, S, Na, Mg, Fe) the biochar improved treatment efficiency of the planted CTWs by decreasing the frequency and volume of nutrients re-released into the effluent by plants and microbes. It is possible that some nutrient releases were neutralized by adsorption to the biochar but it is also possible that the biochar contributed to a reduction of the mechanisms responsible for their release.

Compared to many other biochars, this biochar showed a relatively low adsorption capacity for NH_4^+ so it is possible that the use of a biochar with a much higher sorption capacity would have greatly improved the efficiency of the CTWs. There are also biochars with a high anion adsorption capacity and there are also several feedstock modifications ('designer' biochars) and biochar activation methods (using steam or acid, for example) that have been found to increase cation as well as anion adsorption onto the biochar surface (Borchard et al., 2012; Liu et al., 2015). The use of such biochars in conjunction with plants for constructed wetland water treatment needs to be studied.

Since it was observed that the CTW performance may not always be influenced by the amount of biochar, more detailed studies are needed to determine the optimum ratio of biochar to gravel or sand in CTW systems. It would also be useful to investigate the effects of using biochar in conjunction with other filter media used in wetlands, for example

calcium carbonates or peat. A greater understanding of biochar-microbe and biochar-microbe-plant interactions within a biochar wetland system is needed in order to fully understand the specific mechanisms of the enhanced wastewater treatment observed in this study.

Since biochar is quite recalcitrant, it offers a very promising media for treatment for long-term application in treatment wetlands where the improved plant growth will increase treatment capacity over many years. This study was performed at a relatively short, small scale in a greenhouse, so in order to understand the large-scale potential of a biochar-planted wetland, long-term field trials need to be conducted.

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APPENDIX A

BIOCHAR SORPTION SAMPLES

Ammonia Kinetics

Sample	Minutes	Conc. (mg/L)	pH
1a	5	111.9	7.81
1b	5	110.9	7.74
2a	10	116.5	7.89
2b	10	111.9	7.85
3a	33	105.4	7.98
3b	33	101.7	7.86
4a	120	108.9	8.03
4b	120	97	8
4c	120	105.9	7.97
5a	624	95.9	8.26
5b	624	95.6	8.24
6a	1560	89.7	8.16
6b	1560	95.7	8.09
6c	1560	95	8.18
IC	0	119.8	6.22
FC	1560	115.2	6.31
BC1	1560	0.9	8.92
BC2	1560	1.3	8.8

Phosphate Kinetics

Sample	Minutes	Conc. (mg/L)	pH
1a	5	102.76	8.06
1b	5	104.22	8.03
2a	10	101.74	7.94
2b	10	103.8	8.01
3a	30	109.23	8.24
3b	30	112.43	8.36
4a	120	104.23	8.06
4b	120	107.57	7.99
5a	820	102.75	8.32

5b	820	104.48	8.12
6a	1560	107.9	8.15
6b	1560	104.84	8.1
6c	1560	107.38	8.09
6d	1560	106.93	8.15
IC	0	102.22	8.18
IC 2	0	103.1	8.23
FC	1560	100.21	8.12
BC1	1560	1.09	8.83
BC2	1560	1.22	8.55

Nitrate Kinetics

Sample	Minutes	Conc. (mg/L)	pH
1a	5	6.12	7.69
1b	5	6.53	8.23
2a	10	6.31	8.36
2b	10	6.38	8.8
3a	30	6.47	7.74
3b	30	6.3	7.89
4a	120	6.19	7.88
4b	120	6.45	8.05
5a	820	6.52	8.35
5b	820	6.91	8.53
6a	1560	6.16	8.43
6b	1560	6.12	8.79
IC	0	5.29	6.21
FC	1560	5.35	6.12
BC1	1560	1.51	8.41
BC2	1560	0.985	8.6

IC and FC are initial and final solution controls, BC is biochar control in distilled water

Ammonia Equilibrium

Sample	IC (mg/L)	FC. (mg/L)	I pH	F pH
1a	0.6	1.1	6.45	8.52
1b	0.6	1	6.45	8.79
2a	19.1	10	5.91	8.81
2b	19.1	8.8	5.91	8.3
3a	40.3	19.6	5.57	8.4
3b	40.3	22.2	5.57	8

4a	60.6	40.6	5.52	8.05
4b	60.6	43.7	5.52	8.02
5a	77.4	59.3	5.53	7.97
4b	77.4	51.2	5.53	8.37
7a	98	77.9	5.91	7.85
7b	98	74	5.91	8.07
8a	152.8	128.5	6.32	8.27
8b	152.8	134.5	6.32	7.99
9a	194.4	191	5.69	7.88
9b	194.4	166.6	5.69	7.83
9c	194.4	154	5.69	7.88

IC and FC are initial and final concentrations, I pH and F pH are initial and final pH

Ammonia Desorption

Sample	S mg L	g char	Conc. (mg/L)
1	23.87	5.1	23.8
2	23.87	5.26	24.6

S is average mass ammonia sorbed per 5g char from an equal mixture of samples 9a,9b and 9c from ammonia equillibrium test. Conc. is mass of ammonia in solution after desorption process.

APPENDIX B

GREENHOUSE SAMPLES

Greenhouse Test 2 Wastewater Samples

		ppm									
Date	Sample	COD	TKN	NH_4-N	NO_3-N	Al	B	Ca	Cu	Fe	K
4/13/16	Influent	189	63	56	2.7	<2	0.51	15.9	<1	<2	52.8
4/15/16	Influent	183	60	55	0.3	<2	0.40	12.5	<1	<2	49.0
4/19/16	R1	63	21	17	0.4	<2	0.33	17.7	<1	<2	23.1
4/19/16	R2	65	21	21	0.2	<2	0.34	23.0	<1	<2	27.5
4/19/16	R3	61	27	24	0.3	<2	0.32	17.8	<1	<2	48.6
4/19/16	R4	66	41	35	0.5	<2	0.28	29.0	<1	6.85	39.8
4/22/16	Influent	110	58	56	0.5	<2	<0.2	17.3	<1	<2	51.4
4/26/16	R1	46	18	18	0.5	<2	<0.2	28.5	<1	<2	25.5
4/26/16	R2	41	13	12	0.5	<2	<0.2	34.3	<1	<2	29.0
4/26/16	R3	68	32	31	0.6	4.42	<0.2	38.7	<1	4.11	57.4
4/26/16	R4	64	27	21	0.5	2.81	<0.2	27.9	<1	5.36	33.5
5/6/16	Influent	368	121	113	0.2	<2	<0.2	15.2	<1	<2	83.3
5/10/16	R1	124	61	57	0.3	2.66	<0.2	43.1	<1	2.46	58.7
5/10/16	R2	170	89	83	0.6	3.27	<0.2	27.5	<1	3.49	73.9
5/10/16	R3	192	92	76	0.1	8.49	<0.2	31.1	<1	6.22	104.1
5/10/16	R4	176	87	82	0.1	26.58	<0.2	49.8	<1	20.04	108.7
5/23/16	Influent	402	116	109	0.1	<2.00	<0.2	20.6	<1	<2.00	87.5
5/27/16	R1	114	73	72	0.4	2.84	<0.2	18.4	<1	2.59	92.5
5/27/16	R2	111	73	72	0.3	<2.00	<0.2	17.3	<1	<2.00	78.7
5/27/16	R3	189	81	80	0.1	<2.00	<0.2	12.6	<1	<2.00	94.7
5/27/16	R4	182	88	87	0.3	<2.00	<0.2	11.6	<1	<2.00	86.2
6/3/16	Influent	419	111	78	0.0	7.27	<0.2	40.6	<1	6.36	91.0
6/7/16	R1	101	71	44	0.7	<2	<0.2	10.5	<1	<2	82.4
6/7/16	R2	118	66	41	0.9	<2	<0.2	<10	<1	<2	75.9
6/7/16	R3	138	92	67	0.2	<2	<0.2	11.9	<1	<2	84.2
6/7/16	R4	147	91	63	0.1	2.21	<0.2	15.8	<1	2.69	86.3

Greenhouse Test 2 Wastewater Samples

		ppm							TS	
Date	Sample	Mg	Mn	Na	P	S	Zn	PO_4^{3-}-P	(g/L)	pH
4/13/16	Influent	3.82	<1	17.8	6.09	7.97	<1	2.81	0.325	8.05

4/15/16	Influent	3.21	<1	17.0	3.70	6.20	<1	3.52	0.27	7.85
4/19/16	R1	3.47	<1	13.9	1.42	2.95	<1	1.43	0.185	6.88
4/19/16	R2	4.52	<1	19.9	2.23	3.22	<1	1.80	0.205	6.78
4/19/16	R3	4.62	<1	16.9	3.12	4.30	<1	2.25	0.24	7.44
4/19/16	R4	4.84	<1	20.0	4.93	3.69	<1	3.48	0.27	6.92
4/22/16	Influent	3.46	<1	22.3	5.88	7.17	<1	5.24	0.295	8.09
4/26/16	R1	3.98	<1	26.3	3.04	2.63	<1	1.00	0.21	6.74
4/26/16	R2	5.89	<1	29.7	6.50	6.83	<1	0.92	0.22	6.69
4/26/16	R3	6.45	<1	25.9	9.47	6.17	<1	2.09	0.235	7.38
4/26/16	R4	3.91	<1	26.2	4.46	3.36	<1	2.05	0.235	6.7
5/6/16	Influent	2.64	<1	31.4	4.87	8.66	<1	3.30	0.595	8.47
5/10/16	R1	4.86	<1	44.8	5.48	4.92	<1	0.82	0.365	7.14
5/10/16	R2	3.84	<1	40.4	5.31	6.48	<1	1.39	0.38	7.38
5/10/16	R3	7.18	<1	37.8	11.14	10.00	<1	3.29	0.43	7.73
5/10/16	R4	11.15	<1	49.3	18.74	13.41	<1	1.88	0.425	7.31
5/23/16	Influent	3.82	<1	30.2	9.53	10.13	<1	10.39	0.675	8.22
5/27/16	R1	5.58	<1	38.9	7.12	4.97	<1	2.10	0.37	6.85
5/27/16	R2	3.76	<1	34.9	4.16	4.26	<1	1.94	0.34	6.78
5/27/16	R3	3.71	<1	32.2	5.36	4.39	<1	4.57	0.47	7.59
5/27/16	R4	2.87	<1	38.8	3.41	5.42	<1	2.47	0.4	7.03
6/3/16	Influent	7.04	<1	32.5	17.34	11.57	1.06	8.65	0.605	8
6/7/16	R1	3.08	<1	34.3	3.05	3.08	<1	3.52	0.345	6.85
6/7/16	R2	2.42	<1	34.4	2.13	3.02	<1	2.22	0.34	6.78
6/7/16	R3	3.86	<1	29.9	5.13	4.05	<1	4.54	0.375	7.59
6/7/16	R4	3.34	<1	37.4	4.58	4.46	<1	3.15	0.405	7.03

Greenhouse Test 3 Wastewater Samples

		ppm		
Date	Sample	NH_4-N	PO_4^{3-}-P	pH
10/3/16	Influent	107.2	27.8	8.43
10/7/16	R1	36.3	4.2	7.72
10/7/16	R2	55.2	4.0	7.44
10/7/16	R3	61.8	11.3	8.02
10/7/16	R4	69.3	8.3	7.68
10/5/16	Influent	116.1	34.2	8.48
10/9/16	R1	41.6	5.6	6.97
10/9/16	R2	56.8	8.2	7.71
10/9/16	R3	51.0	10.8	7.91
10/9/16	R4	68.2	7.6	7.54
10/10/16	Influent	98.1	76.1	8.75
10/14/16	R1	35.5	8.5	8.02
10/14/16	R2	51.3	8.0	7.35

10/14/16	R3	51.9	15.8	7.69
10/14/16	R4	57.9	7.8	7.24

Test 2 Effluent Flow Rates (ml/min)

Date	Time	R1	R2	R3	R4
Influent		16.7	16.7	16.7	16.7
Effluent					
5/27/16	6:00 AM	10	10	16	13
5/27/16	6:00 PM	11	11	16.5	9.5
4/26/16	6:00 AM	10.5	11	17	11
4/26/16	6:00 PM	10	10	16	11.5
Effluent	Average	10.5	10.5	16.25	11.25

Test 3 Effluent Flow Rates (ml/min)

Date	Time	R1	R2	R3	R4
Influent		16.7	16.7	16.7	16.7
Effluent					
10/4/16	6:00 AM	11.5	11	16.5	12.5
10/4/16	6:00 PM	7.5	7.5	16.5	9.00
10/13/16	6:00 AM	11	11	16.5	12
10/13/16	6:00 PM	8.5	7.5	16.5	7.5
Effluent	Average	9.63	9.3	16.5	10.3

APPENDIX C

R CODE

```
#Two way ANOVA on spring greenhouse treatment data
data <- read.table(file="Spring.data.csv", header=TRUE, sep=',')
attach(data)

#making the 2 independent variables (Char and Inf.Conc) into
ordered factors
Char <- ordered(Char)
Int.Conc <- ordered(Inf.Conc,
levels = c('low', 'high'))

#using the Anova function with the library "car" to easily
denote Type 3 Anova
library(car)

#assigning simpler symbols to each factor and using only data
from planted tanks
a <- Inf.Conc[planted=='yes']
a
b <- Char[planted=='yes']
b

############################################################remo
val of NH4 in mg/L
#Type 3 anova using a, b and a*b interaction
Anova(lm(NH4.mg[planted=='yes']~a*b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#if interaction not significant do anova without it

#Type 3 anova using a and b, no interaction
Anova(lm(NH4.mg[planted=='yes']~a+b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#a and b are significant, do TukeyHSD to see significance of
differences between factor levels

#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
```

```
re1 <- aov(formula = NH4.mg[planted == "yes"] ~ a + b, contrasts
= list(a = contr.sum, b = contr.sum))
summary(re1)

TukeyHSD(re1,c("a","b"),order=TRUE)

##########################################################removo
val of NH4 as percentage of influent
#Type 3 anova using a, b and a*b interaction
Anova(lm(NH4.p[planted=='yes']~a*b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#if interaction not significant do anova without it

#Type 3 anova using a and b, no interaction
Anova(lm(NH4.p[planted=='yes']~a+b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#a and b are significant, do TukeyHSD to see significance of
differences between factor levels

#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
re2 <- aov(formula = NH4.p[planted == "yes"] ~ a + b, contrasts
= list(a = contr.sum, b = contr.sum))
summary(re2)

TukeyHSD(re2,c("a","b"),order=TRUE)

#############################################################
#removal of PO4 in mg/L. Influent PO4 concentrations did not
match wastewater dilutions, the first 3 samples were from low
conc. and the last to were high
Inf.Conc.PO4 <- ordered(Inf.Conc.PO4,
levels = c('low', 'high'))
levels(Inf.Conc.PO4)
a1 <- Inf.Conc.PO4[planted=='yes']
a1

#Type 3 anova using a1, b and a*b interaction
Anova(lm(PO4.mg[planted=='yes']~a1*b,
contrasts=list(a1=contr.sum, b=contr.sum)),type=3)
#if interaction not significant do anova without it

#Type 3 anova using a1 and b, no interaction
Anova(lm(PO4.mg[planted=='yes']~a1+b,
contrasts=list(a1=contr.sum, b=contr.sum)),type=3)
#a and b are significant, do TukeyHSD to see significance of
differences between factor levels
```

```
#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
re3 <- aov(formula = PO4.mg[planted == "yes"] ~ a1 + b,
contrasts = list(a1 = contr.sum, b = contr.sum))
summary(re3)

TukeyHSD(re3,c("a1","b"),order=TRUE)

############################################################
#removal of PO4 as percentage of influent
#Type 3 anova using a1, b and a*b interaction
Anova(lm(PO4.p[planted=='yes']~a1*b,
contrasts=list(a1=contr.sum, b=contr.sum)),type=3)
#if interaction not significant do anova without it

#Type 3 anova using a and b, no interaction
Anova(lm(PO4.p[planted=='yes']~a1+b,
contrasts=list(a1=contr.sum, b=contr.sum)),type=3)
#a and b are significant, do TukeyHSD to see significance of
differences between factor levels

#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
re4 <- aov(formula = PO4.p[planted == "yes"] ~ a1 + b, contrasts
= list(a1 = contr.sum, b = contr.sum))
summary(re4)

TukeyHSD(re4,c("a1","b"),order=TRUE)

############################################################
#removal of COD in mg/L
#Type 3 anova using a, b and a*b interaction
Anova(lm(COD.mg[planted=='yes']~a*b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#if interaction not significant do anova without it

#Type 3 anova using a and b, no interaction
Anova(lm(COD.mg[planted=='yes']~a+b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#a and b are significant, do TukeyHSD to see significance of
differences between factor levels

#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
re5 <- aov(formula = COD.mg[planted == "yes"] ~ a + b, contrasts
= list(a = contr.sum, b = contr.sum))
```

```
summary(re5)

TukeyHSD(re5,c("a","b"),order=TRUE)

############################################################
#removal of COD as percentage of influent
#Type 3 anova using a, b and a*b interaction
Anova(lm(COD.p[planted=='yes']~a*b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#if interaction not significant do anova without it

#Type 3 anova using a and b, no interaction
Anova(lm(COD.p[planted=='yes']~a+b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#a and b are significant, do TukeyHSD to see significance of
differences between factor levels

#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
re6 <- aov(formula = COD.p[planted == "yes"] ~ a + b, contrasts
= list(a = contr.sum, b = contr.sum))
summary(re6)

TukeyHSD(re6,c("a","b"),order=TRUE)

############################################################
#removal of S in mg/L. Using 1 way anova because influent
concentration does not match wastewater dilutions, also
excluding data from May 10, due to S release by T3 and T4
#Type 3 anova using b1
b1 <- Char[planted=='yes' & Date!='10.May']
b1
Anova(lm(S.mg[planted=='yes'& Date!='10.May']~b1,
contrasts=list(b1=contr.sum)),type=3)

#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
re7 <- aov(formula = S.mg[planted == "yes"& Date!='10.May'] ~
b1, contrasts = list(b1 = contr.sum))
summary(re7)

TukeyHSD(re7,"b1",order=TRUE)

############################################################
#removal of S as percent. Using 1 way anova because influent
concentration does not match wastewater dilutions, also
excluding data from May 10, due to S release by T3 and T4
```

```
#Type 3 anova using b1
Anova(lm(S.p[planted=='yes'& Date!='10.May']~b1,
contrasts=list(b1=contr.sum)),type=3)

#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
re8 <- aov(formula = S.p[planted == "yes"& Date!='10.May'] ~ b1,
contrasts = list(b1 = contr.sum))
summary(re8)
TukeyHSD(re8,"b1",order=TRUE)

############################################################
#removal of K in mg/L
#Type 3 anova using a, b and a*b interaction
Anova(lm(K.mg[planted=='yes']~a*b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#if interaction not significant do anova without it

#Type 3 anova using a and b, no interaction
Anova(lm(K.mg[planted=='yes']~a+b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#a and b are significant, do TukeyHSD to see significance of
differences between factor levels

#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
re9 <- aov(formula = K.mg[planted == "yes"] ~ a + b, contrasts =
list(a = contr.sum, b = contr.sum))
summary(re9)
TukeyHSD(re9,c("a","b"),order=TRUE)

#In the graph showing influent and effluent conc over time, it
looks like T4 may have a release of K May 10 that may affect the
anova, see without:
a2 <- Inf.Conc[planted=='yes' & Date!='10.May']
a2
b2 <- Char[planted=='yes' & Date != '10.May']
b2

#Type 3 anova using a2, b2 and a2*b2 interaction
Anova(lm(K.mg[planted=='yes' & Date != '10.May']~a2*b2,
contrasts=list(a2=contr.sum, b2=contr.sum)),type=3)

#Type 3 anova using a2 and b2, no interaction
Anova(lm(K.mg[planted=='yes' & Date != '10.May']~a2+b2,
contrasts=list(a2=contr.sum, b2=contr.sum)),type=3)
```

```
#if a or b are significant, do TukeyHSD to see significance of
differences between factor levels

#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
re10 <- aov(formula = K.mg[planted == "yes" & Date != '10.May']
~ a2 + b2, contrasts = list(a2 = contr.sum, b2 = contr.sum))
summary(re10)
TukeyHSD(re10,c("a2","b2"),order=TRUE)

############################################################
#removal of K as percent
#Type 3 anova using a, b and a*b interaction
Anova(lm(K.p[planted=='yes']~a*b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#if interaction not significant do anova without it

#Type 3 anova using a and b, no interaction
Anova(lm(K.p[planted=='yes']~a+b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#a and b are significant, do TukeyHSD to see significance of
differences between factor levels

#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
re11 <- aov(formula = K.p[planted == "yes"] ~ a + b, contrasts =
list(a = contr.sum, b = contr.sum))
summary(re11)
TukeyHSD(re11,c("a","b"),order=TRUE)

#In the graph showing influent and effluent conc over time, it
looks like T4 may have a release of K May 10 that may affect the
anova, see without:
#Type 3 anova using a2, b2 and a2*b2 interaction
Anova(lm(K.p[planted=='yes' & Date != '10.May']~a2*b2,
contrasts=list(a2=contr.sum, b2=contr.sum)),type=3)

#Type 3 anova using a2 and b2, no interaction
Anova(lm(K.p[planted=='yes' & Date != '10.May']~a2+b2,
contrasts=list(a2=contr.sum, b2=contr.sum)),type=3)
#if a or b are significant, do TukeyHSD to see significance of
differences between factor levels

#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
re12 <- aov(formula = K.p[planted == "yes" & Date != '10.May'] ~
a2 + b2, contrasts = list(a2 = contr.sum, b2 = contr.sum))
```

```
summary(re12)
TukeyHSD(re12,c("a2","b2"),order=TRUE)


############################################################
#removal of Na in mg/L
#Type 3 anova using a, b and a*b interaction
Anova(lm(Na.mg[planted=='yes']~a*b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#if interaction not significant do anova without it

#Type 3 anova using a and b, no interaction
Anova(lm(Na.mg[planted=='yes']~a+b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#a and b are significant, do TukeyHSD to see significance of
differences between factor levels

#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
re13 <- aov(formula = Na.mg[planted == "yes"] ~ a + b, contrasts
= list(a = contr.sum, b = contr.sum))
summary(re13)
TukeyHSD(re13,c("a","b"),order=TRUE)

#In the graph showing influent and effluent conc over time, it
looks like T4 may have a release of K May 10 that may affect the
anova, see without:
#Type 3 anova using a2, b2 and a2*b2 interaction
Anova(lm(Na.mg[planted=='yes' & Date!='10.May']~a2*b2,
contrasts=list(a2=contr.sum, b2=contr.sum)),type=3)
#if interaction not significant do anova without it

#Type 3 anova using a2 and b2, no interaction
Anova(lm(Na.mg[planted=='yes' & Date!='10.May']~a2+b2,
contrasts=list(a2=contr.sum, b2=contr.sum)),type=3)
#a and b1 are significant, do TukeyHSD to see significance of
differences between factor levels

#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
re14 <- aov(formula = Na.mg[planted == "yes" & Date!='10.May'] ~
a2 + b2, contrasts = list(a2 = contr.sum, b2 = contr.sum))
summary(re14)

TukeyHSD(re14,c("a2","b2"),order=TRUE)

############################################################
```

```
#removal of Na as percent
#Type 3 anova using a, b and a*b interaction
Anova(lm(Na.p[planted=='yes']~a*b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#if interaction not significant do anova without it

#Type 3 anova using a and b, no interaction
Anova(lm(Na.p[planted=='yes']~a+b, contrasts=list(a=contr.sum,
b=contr.sum)),type=3)
#a and b are significant, do TukeyHSD to see significance of
differences between factor levels

#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
re15 <- aov(formula = Na.p[planted == "yes"] ~ a + b, contrasts
= list(a = contr.sum, b = contr.sum))
summary(re15)

TukeyHSD(re15,c("a","b"),order=TRUE)

#In the graph showing influent and effluent conc over time, it
looks like T4 may have a release of K May 10 that may affect the
anova, see without:
#Type 3 anova using a2, b2 and a2*b2 interaction
Anova(lm(Na.p[planted=='yes' & Date!='10.May']~a2*b2,
contrasts=list(a2=contr.sum, b2=contr.sum)),type=3)
#if interaction not significant do anova without it

#Type 3 anova using a2 and b2, no interaction
Anova(lm(Na.p[planted=='yes' & Date!='10.May']~a2+b2,
contrasts=list(a2=contr.sum, b2=contr.sum)),type=3)
#If a2 and b2 are significant, do TukeyHSD to see significance
of differences between factor levels

#TukeyHSD requires use of aov function, cannot be done with
Anova. Do results from aov and Anova match?
re16 <- aov(formula = Na.p[planted == "yes" & Date!='10.May'] ~
a2 + b2, contrasts = list(a2 = contr.sum, b2 = contr.sum))
summary(re16)

TukeyHSD(re16,c("a2","b2"),order=TRUE)

############################################################
detach(data)

############################################################
#One way ANOVA on Fall 2016 greenhouse treatment data
```

```
data2 <- read.table(file="Fall.data.csv", header=TRUE, sep=',')
attach(data2)

#making the independent variable (Char) into an ordered factor
Char <- ordered(Char)

#assigning simpler symbol to the factor and using only data from
planted tanks
c <- Char[planted=='yes']
c

############################################################
#removal of NH4 in mg/L
#Regular anova because no unbalanced data
fallANOVA1 <- aov(NH4.mg[planted=='yes']~c)
summary(fallANOVA1)

TukeyHSD(fallANOVA1, "c", ordered=TRUE)

############################################################
#removal of NH4 as percent of the influent
#Regular anova because no unbalanced data
fallANOVA2 <- aov(NH4.p[planted=='yes']~c)
summary(fallANOVA2)

TukeyHSD(fallANOVA2, "c", ordered=TRUE)

############################################################
#removal of PO43-P in mg/L
#Regular anova because no unbalanced data
fallANOVA3 <- aov(PO4.mg[planted=='yes']~c)
summary(fallANOVA3)

TukeyHSD(fallANOVA3, "c", ordered=TRUE)

############################################################
#removal of PO43-P as percentage of influent
#Regular anova because no unbalanced data
fallANOVA4 <- aov(PO4.p[planted=='yes']~c)
summary(fallANOVA4)

TukeyHSD(fallANOVA4, "c", ordered=TRUE)


############################################################detac
h(data2)
```

```
#############################################################
#2 way ANOVA Comparison of NH4 and PO4 removals from "high"
influent concentration test 2 vs test 3

data3 <- read.table(file="SpringvsFall.data.csv", header=TRUE,
sep=',')
attach(data3)

#making the independent variables (Tank and Test) into factors
Tank <- factor(Tank)
Test <- ordered(Test)
levels(Test)

#############################################################
#removal of NH4 in mg/L
#Regular 2-way anova because no unbalanced data
svsfANOVA <- aov(NH4.mg~Tank*Test)
summary(svsfANOVA)

svsfANOVA2 <- aov(NH4.mg~Tank+Test)
summary(svsfANOVA2)

TukeyHSD(svsfANOVA2, c("Tank", "Test"), ordered=TRUE)

#############################################################
#removal of NH4 as percent of the influent
#Regular 2-way anova because no unbalanced data
svsfANOVA3 <- aov(NH4.p~Tank*Test)
summary(svsfANOVA3)

svsfANOVA4 <- aov(NH4.p~Tank+Test)
summary(svsfANOVA4)

TukeyHSD(svsfANOVA4, c("Tank", "Test"), ordered=TRUE)


#############################################################
#removal of PO43-P in mg/L
#Regular 2-way anova because no unbalanced data
svsfANOVA5 <- aov(PO4.mg~Tank*Test)
summary(svsfANOVA5)

svsfANOVA6 <- aov(PO4.mg~Tank+Test)
summary(svsfANOVA6)

TukeyHSD(svsfANOVA6, c("Tank", "Test"), ordered=TRUE)
```

```
############################################################remo
val of PO43-P as percent
#Regular 2-way anova because no unbalanced data
svsfANOVA7 <- aov(PO4.p~Tank*Test)
summary(svsfANOVA7)

svsfANOVA8 <- aov(PO4.p~Tank+Test)
summary(svsfANOVA8)

TukeyHSD(svsfANOVA8, c("Tank", "Test"), ordered=TRUE)


############################################################
detach(data3)
```

r