

**EVALUATION OF BIOCHAR FOR REDUCTION OF
NITROGEN COMPOUNDS IN STORMWATER REMEDIATION SYSTEMS**

by

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ABSTRACT

As stormwater travels over impervious surfaces it collects a variety of contaminants, including nitrogenous compounds that can contribute to eutrophication if they reach water bodies. Engineering systems, such as bioretention facilities, can treat stormwater; however, these structures typically have inadequate removal of nitrogen compounds like ammonium (NH_4^+) and nitrate (NO_3^-). The objective of this study was to evaluate the potential for poultry litter (PL) or wood derived (WD) biochar to remove NH_4^+ and NO_3^- from artificial stormwater (ASW) and to evaluate any effects biochar may have on the microbial nitrogen cycle. Both PL and WD biochar removed more than 90% of the NH_4^+ from ASW solutions, while NO_3^- removal was negligible. WD biochar removed more NH_4^+ from ASW than PL biochar, and its NH_4^+ sorption capacity was not significantly affected by the presence of other cations in solution. Particle size of the biochar influenced adsorption. Smaller WD biochar had increased NH_4^+ adsorption, whereas smaller PL particle size increased NH_4^+ leaching into solution. In addition, PL biochar produced at 400°C had significantly higher leaching of NH_4^+ than PL biochar produced at 500°C. In column experiments, WD biochar slightly decreased the pH of the effluent, while PL biochar caused increase in effluent pH. Using the measured adsorption isotherms, the NH_4^+ sorption capacities of both PL and WD biochars were calculated for field conditions. At application rates of 2% by

weight, calculated PL and WD biochar removal of NH_4^+ was quickly depleted. However, for WD biochar at 10% application, removal efficiencies of over 50% can be achieved for inflows over 200,000 L. This equates to effectiveness for multiple storm events. The microbial results of the experiments were inconclusive, needing a much larger scale of research to get a better representation of the soil communities. These results suggest that biochar can be a useful amendment to bioretention facilities for the removal of NH_4^+ . However, source of the biochar and preparation techniques will influence sorption capacity and may induce different changes to the soil environment of the system.

Chapter 1

INTRODUCTION

1.1 Stormwater Background and Issues

After a precipitation event, water that does not infiltrate into the surrounding soil will accumulate on the surface and travel as runoff. This stormwater runoff will travel until it reaches a permeable surface and infiltrates, or flows into a sewer system or water body. Concerns arise from this movement of runoff as it can cause erosion or sedimentation problems to land and streams, contributing to flooding and water quality issues [15], [54]. Also, because runoff may leave the watershed before it has time to infiltrate, reduction in recharge of groundwater can occur [15].

Another detrimental quality of stormwater runoff is its pollutant content. As the water travels over various land surfaces, it collects sediment, oils, chemicals, such as pesticides, nutrients (including nitrogen and phosphorus) or metals [23], [54]. The high nutrient loads can have harmful effects on both environmental and human health. Particular nutrients of interest are nitrogen compounds, such as NH_4^+ and NO_3^- . Both NH_4^+ and NO_3^- can cause increased growth of plants and microbes [30]. This accelerated growth can deplete dissolved oxygen supplies, causing eutrophication that may be harmful to the ecosystem in the area [57]. Consequences of increased NO_3^- in groundwater include effects on human health [25], [37]. Children, especially infants

less than six months old, can become sick or even die from by high levels of NO_3^- . Infants have low levels of acid in their stomachs and rely on bacteria to help digest food. These bacteria may transform high levels of NO_3^- to nitrite (NO_2^-), which can enter the bloodstream. NO_2^- replaces oxygen in the blood, causing a condition called methemoglobinemia, or blue baby syndrome [25], [37]. A similar process can happen in ruminant livestock, such as cattle and sheep, causing serious health effects [37]. Furthermore, some researchers have hypothesized about the possibility of NO_3^- interaction with organic compounds to form potential cancer causing agents [25], [37]. In this case, compounds would be transformed to N-nitroamines, similar to pesticides. However, little has been done to understand this interaction with human health [25], [37]. NH_4^+ has also been found to affect the reproduction rates, health, and population of certain fish [35], [56]. There are a variety of standards in place to limit the loading of these nutrients in water bodies, but in order to meet these goals stormwater treatment systems need to be considered.

1.2 Stormwater Treatment Options

Currently, various methods are used in an attempt to control the volume of runoff after a storm event and influence the water's infiltration into the ground. The best method to control runoff is to slow it down and provide a permeable surface, allowing more time for infiltration [34]. This may be done using increased areas of vegetative cover or systems that capture water for slow release. Treatment options for

stormwater are more generally known as Best Management Practices (BMPs). In these engineered systems water is not only slowed down, but also physical, chemical and microbial processes are utilized for the removal of unwanted contaminants [34]. Some common BMPs include sandfilters, retention basins, and bioretention facilities. In these structures, stormwater flows through various media allowing for the removal of contaminants. Common media include sands and soils; however amendments that have enhanced removal efficiencies can also be utilized. Removal in media is influenced by adsorption or absorption capacities, as well as ion exchange, van der Waals forces, or surface binding sites [25]. Furthermore, if the retention time of flow is increased, removal of some elements can be increased due to microbial interactions [25].

1.3 Bioretention Facilities

Bioretention is a type of BMP that utilizes the collection and infiltration of stormwater runoff through vegetated soil media, which acts as a filter [22], [23]. Other names for this type of facility are rain gardens or biofilters [22], [15]. As water travels through the media, processes including sorption, fine filtration, sedimentation and precipitation, as well as biological activity and plant uptake remove pollutants before the treated water is collected in under-drains for either storage or discharge [15], [22], [23]. Typically, a bioretention system is designed for small volumes of stormwater that are allowed to pond on a surface before infiltration through the media, which

often contains a mix of mulch, top soil, sandy soils, organic compost material [15], [22].

1.3.1 Ammonium and Nitrate Removal

Although bioretention has been found to be very effective at the removal of heavy metals and suspended solids, retention of nitrogen compounds is often variable [22]. Laboratory column studies used to evaluate different bioretention media showed poor removal efficiencies of NH_4^+ ranging from 8-15% for sand mixes and up to 20% for a mixture of sand, soil, and mulch [23]. Field trials of existing bioretention facilities showed similar low removal rates of NH_4^+ [23]. In field bioretention studies, removal of NH_4^+ from stormwater reached 64% to 97%; however, these results were not consistent. NO_3^- had very low removal and often leached into effluent causing an increase from initial concentrations [22]. A study conducted at a bioretention facility focused on NO_3^- removal caused by microbial denitrification that was achieved through creating an anoxic zone with an over drain. The experiments achieved NO_3^- plus NO_2^- removals of up to 80% from a solution of synthetic stormwater containing calcium chloride, sodium nitrate, and dibasic sodium phosphate, but did not achieve NH_4^+ removal [30].

Hossain et al listed a variety of sorption medias that had been used by researchers for the removal of stormwater pollutants including limestone, compost, sawdust, newspaper, zeolites, wood fibers, glass, and ash among others, but chose to conduct their experiment using a mixture of 50% sand, 20% limestone, 15% sawdust,

and 15% tire pieces. The results of this test show a removal efficiency of 64% for ammonia (NH_3) from a stormwater concentration of 5 mgL^{-1} and removal near 80% for 0.5 mgL^{-1} . The life expectancy of NH_3 removal for a BMP with this particular amendment is 0.244 years, using 300 kg of media, assumed flow of 378.5 L/day and an ammonia concentration of 1.0 mgL^{-1} [25].

In another study, a variety of bioretention media of varying mulch, soil, and sand ratios was used in short-term column experiments to evaluate the differences in removal efficiencies from stormwater applied at varying rates. The mixtures were found to have removal efficiencies ranging from 2%-26% for ammonium and 1%-43% for NO_3^- , while removals for constituents like total suspended solids and lead were generally over 90% [23].

In Newark, DE, a bioretention facility put in place has been monitored for its removal of a variety of stormwater constituents. The BMP is an AbTech Ultra-Urban Filter with Smart Sponge plus Antimicrobial Additive and is found in a plaza off of Southbound I-95. The facility, located near a truck parking area, has an area of 0.8 ha and permeability of 80% [26]. During data collection, inflow and outflow rates as well as NH_3 and NO_3^- concentrations in the flow were monitored. The mean inflow of NH_3 as nitrogen to the system was 0.1 mg/L while maximum reached 3.53 mg/L. For NO_3^- and NO_2^- , mean inflow was 0.42 mg/L and maximum 2.44 mg/L [26]. Outflow values were mean of 0.1 mg/L and max 0.5 mg/L for NH_3 and mean of 0.35 mg/L and max of 2.32 mg/L for NO_3^- and NO_2^- [26]. This shows that although there is some removal, the removals are not always constant and can be minimal, especially for NO_3^- . In a

study from between December of 2006 and June 2009, NH_3 levels were found to increase between inflow and outflow, from 0.25 to 0.28 mg/L in the facility.

Evaluation the effectiveness of different media amendments for bioretention facilities can help to target those contaminants, such as NH_4^+ and NO_3^- that typically are not well removed. One possibility for this type of amendment is to use a biochar material. These materials have been studied for various scientific purposes, but have not been used as a stormwater treatment option.

Chapter 2

BIOCHAR CHARACTERISTICS AND POTENTIAL

2.1 Biochar Background

The incorporation of pyrolyzed biomass into soils is an ancient agricultural practice that can potentially affect the fertility and greenhouse gas emissions of soils [3], [17]. In the Amazon Basin, indigenous people added charred biomass for thousands of years to produce highly fertile soils called *Terra preta de Indino*, or Amazon Dark Earths [3]. These methods of adding charred biomass have been seen to increase crop production as well as maintain soil fertility long-term, even in areas that were known to previously have infertile soil. Although this method has been used for thousands of years, it is still an active area of research in soil fertility. This charred biomass material has taken on the name “biochar” and is being researched extensively. Its physical and chemical properties are thought to be responsible for many of its beneficial qualities. Experiments are being done to analyze the interactions of biochar with its environment and evaluate how these interactions can be controlled and manipulated to better improve environmental conditions.

The manufacturing process for biochar is very similar to that of charcoal, where a pyrolysis process is used. This process is commonly used for all biochars, even if the initial biomass is different. Biochar can be produced from a wide variety of feedstocks

including waste materials, such as wood, paper mill waste, crop and forest waste, or wastewater sludge [34], [38]. Although the production processes may have different mechanical set-ups, the overall procedure used is the same. During production, an organic material is burned with little or no oxygen. The process results in the production of syngas, bio-oils, and biochar, a solid material [34].

Altering the conditions of production can change the product characteristics. Pyrolysis processes at temperatures above 500°C produce biochars that are more resistant to weathering, due to their higher surface areas and aromatic structure [30]. Biochars produced at lower temperatures are able to better retain nutrients, and this coupled with their higher reactivity in soil, make them better at improving soil fertility [30]. Because there are benefits at both high and low temperatures, biochars can be produced at a varying range; however, the optimal temperature for biochar production is often set at 500 °C [11], [38].

2.2 Characteristics of Biochar

The starting material for biochar production will affect the physical structure, texture, porosity, particle size distribution and density of the final product [3]. The porous structure of biochar is a physical property that influences many of its benefits. Pore formation increases the surface area of the biochar, allowing more area for the binding of nutrients [38]. The large and small pore spaces also give areas for microorganisms to thrive [34], [38]. Also, the large surface area created by pores

allows for increased binding of cations and anions, which may be nutrients for plants or soil organisms. Pore formation also influences the binding of macronutrients such as nitrogen and phosphorus [3]. When incorporated into soils, biochar has a great effect on the cation exchange capacity (CEC). Because biochar is oxidized during production, there is a greater ability for organic matter to adsorb to its surface [11]. It has also been noted that as biochar weathers over time there is a further increase in CEC, which will allow for cations, including nutrients such as NH_4^+ , to be retained in the soils [11]. This chemical change not only helps with soil fertility, but also can help to reduce leaching and volatilization. The nutrients in biochar are less mobile than those in raw organic matter, so they may fertilize the soil for longer periods of time instead of leaching into groundwater and contributing to eutrophication of waterways [11], [12].

Water retention and water holding capacity in biochar treated soils can be increased significantly due to the changes it causes in soil structure and pore space, especially in sandy soils [37]. This increased water retention is a beneficial effect on soil structure, plant growth, as well as possible contaminate removal from influent water.

2.3 Biological Effects of Biochar

A variety of mechanisms have been proposed for biochar's ability to change the microbial community in a soil system. One especially important mechanism is

adsorption. Biochar can also adsorb toxins that may inhibit growth of microorganisms [35]. Furthermore, bacteria may adsorb to biochar surfaces, including surfaces within pores. Biochar has the potential to increase the microbial community in a soil system by giving bacteria a habitat where they are protected from predators and the sorption to biochar particles makes microorganisms less susceptible to washing out of soil [35]. The sorption of microbes to biochar may become a problem; however, when trying to evaluate the microbial community in biochar-amended soils. There may be strong sorption of cell matter and microbial exoenzymes making the extraction of this matter difficult [35]. Therefore challenges may arise during DNA or biomass extractions due to strong sorption properties, making microbial experiments with biochar complex [35].

Limited studies have been completed on biochar's effect on microbial communities, but effects of similar compounds, such as charcoal or activated carbon, may be useful for comparison. *Terra preta* soil is the term used for ancient soils rich in carbon due to the addition of burned organic matter similar to biochar. The charred carbon in *terra preta* is different than biochar in that no specified starting material is known and its production was likely not carried out in a controlled way. Otherwise, the two materials have similar chemical and physical characteristics. A review by Akinson et al evaluated the differences between the microbial communities in *terra preta* soils compared to those of soils without the added charcoal in a similar area. It was found that the microbial communities in *terra preta* soils were more diverse and quite different from untreated soils. The communities with the incorporated charcoal

were about 25% richer than forest soil, with 14 phylogenetic groups compared to only nine in forest soils. From this study it was hypothesized that biochars' low inorganic-N can provide a good habitat for diazotrophs, which possess the enzyme nitrogenase and the ability to reduce atmospheric N₂ into NH₃ [3].

Similarly, Grossman et al compared Amazon anthrosols (charcoal containing soils) to adjacent soils to evaluate effects of the charcoal on microbial population. Their methods included use of denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (TRFLP) to assess microbial diversity. Their results showed an overall less diverse archaeal community than bacterial in all the soils. Archaea in anthrosols were 90% different from the archaeal population in adjacent soil, indicating that the incorporation of the charcoal may have been a factor to these differences. Bacterial communities were also compared at different depths between the different anthrosols, but found to not have notable differences. Most bacteria detected though the analysis were novel, and thus could not be identified or used for comparison. *Verrucomicrobia* was common in the anthrosols showing this species may be more dominant when charcoal is present. Sequences closely related to *Proteobacteria* and *Cyanobacteria sp.* were recovered only in untreated soil, showing that these may thrive better in soils without charred-carbon source. Sequences related to *Pseudomonas*, *Acidobacteria*, and *Flexibacter sp.* were recovered from both soils, showing these particular species does not seem to favor habitats with or without the carbon.

Kim et al compared bacterial diversity and community structures of an untreated forest soil and a *terra preta* from the Western Amazon. They used a method of oligonucleotide fingerprint grouping of 16S rRNA gene sequences for 1500 clones (OFRG) and DNA sequencing to evaluate the community differences at a genomic level. Sequences obtained were analyzed mathematically using Shannon and Chao 1 statistical methods to measuring diversity. The *terra preta* soils were found to have 25% greater diversity than what was present in the forest soil. This may indicate charcoal gives soil added benefits, allowing a greater variety of species to thrive, possibly due to more protected habitat or change in soil chemistry or nutrient content. A more diverse population is likely to change composition of the soils, by decomposition of organic matter, nitrogen cycling, or biodegradation of chemicals in soil [42]. These changes can be beneficial to plants, other soil organisms, and overall soil quality. Similarities between the soils were found as well. Common to both soils were *Proteobacteria*, *Actinobacteria*, *Planctomycetes*, and *Verrucomicrobia*. *Proteobacteria* (α , β , γ) were similarly abundant in *terra preta*; α *Proteobacteria* in forest soil. Two new groups of *Acidobacterium* were found in the *terra preta* that were previously unknown.

Some researchers have started to evaluate the effects that biochar itself has on the communities in a variety of soils. Kolton et al looked at bacterial communities at the roots of pepper plants grown in biochar-treated soils. Molecular fingerprinting (DGGE and TRFLP) of 16S rRNA gene fragments were used for identification purposes along with Pyrosequencing of the resulting 16S rRNA amplicons. The study

was able to generate a total of 20,142 sequences. The analysis of the resulting sequences showed *Bacteroidetes* phylum increased from 12% to 30% as a result of biochar amendment; however, *Proteobacteria* decreased from 71% to 47%. The *Bacteroidetes*-affiliated *Flavobacterium* was the strongest biochar-induced genus overall. The changes in community structure were attributed to pH changes, physical and chemical changes to soils, and soil structure differences.

The Table 1 combines the results of these microbial studies to show a better comparison of the findings from the three studies.

Table 1 Comparison of microbes identified from *terra preta* or biochar-treated soils

Charcoal/Biochar Soil Only	Control Soil Only	Both Soils
<i>Bacteroidetes</i> [33] <i>Flavobacterium</i> [33] <i>Proteobacteria</i> (β , γ) [33]	<i>Cyanobacteria</i> [21]	<i>Verrucomicrobia</i> [33] <i>Acidobacteria</i> [21] <i>Aciobacterium</i> [33] <i>Planctomycetes</i> [33] <i>Proteobacteria</i> (α) [21],[33] <i>Flexibacter</i> [21]

Comparing all of the studies, it can be seen that similar types of microbes exist in the treated and untreated soil types, but in different amounts that varied greatly among the studies. No distinct pattern could be seen and no strong conclusions about the communities drawn. There were also many novel species found that could not be accurately identified within the papers. Because soil communities are so diverse, more sequencing is necessary to comprehensively identify specific differences between

communities. Furthermore, even if more sequencing was completed, the results give a general idea of the population present, but do not give a quantifiable comparison. As more studies emerge, the community structures of biochar treated soils may be better understood.

2.4 Biochar and the Nitrogen Cycle

An important area of interest for biochar studies includes its effects on nitrogen cycling. In the nitrogen cycle, nitrogen is fixed, oxidized and reduced. N_2 present in the atmosphere is converted to NH_4^+ , which can be taken up by plants and microorganisms [8], [9]. Other microbes mediate the conversion of NH_4^+ and NO_3^- via several pathways. These processes are mediated by a variety of microbes in the soil, and have intermediate products such as nitrous oxide (N_2O), NH_3 , and hydrazine (N_2H_4) [28]. Studies using agricultural soils show biochar derived from plant matter, such as pine and bamboo, has the potential to alter the rates of nitrogen cycling by modifying nitrification and denitrification rates and adsorption of NH_3 [1], [11]. Treated soil has an increased cation exchange capacity, resulting in the possibility of increased NH_4^+ sorption [11], [45]. These changes would contribute reduction of gaseous nitrogen emissions, such as N_2O and NH_3 , as well as decrease NO_3^- or NH_4^+ leaching from soil [11]. Furthermore, the incorporation of biochar into the soil system can increase soil aeration or water holding capacity. These changes have the potential to decrease the frequency of areas of low oxygen where microbiological nitrification

processes may take place [2]. This may lead to reduced emission of nitrous oxide from soils.

2.5 Benefits of Biochar on Sustainability and Energy

When biomass goes through the pyrolysis process, the carbon is sequestered in the biochar formed. This carbon is stable when applied to soil, making biochar a viable option for carbon sequestration where the release of carbon dioxide due to decomposition is greatly reduced [11]. In this way, using biochar as a soil amendment can help to offset anthropogenic carbon dioxide emissions through this sequestration [1].

The use of biochar has the potential to significantly alter energy use in a variety of ways. Poultry litter, paper mill sludge, and other organic matter are currently sent to landfills for disposal, and this transport is both money- and fuel-intensive. If these products are instead used to produce biochar, it becomes a valuable resource rather than an expensive waste product. The other products of the manufacturing of biochar manufacturing, syngas and bio oils, can be used to produce electricity or to replace a variety of fossil fuels [17], [34]. Application of biochar to increase soil fertility may also reduce the need for nitrogen fertilizer. Industrial reduction of atmospheric N_2 to NH_4^+ for fertilizer production is an energy intensive operation. Any reduction in fertilizer use can have an immediate effect on world energy use.

2.6 Biochar for Remediation

Biochar has been seen to significantly affect metal cycling in soil environments. Aluminum and lead, toxic at high concentrations, can be adsorbed to biochar particles, limiting toxicity to both plant roots and soil bacteria [36], [38]. A study was done by Park et al 2011, to evaluate the effectiveness of biochar at immobilizing metals in soil. Different soils were used that had naturally high concentrations of copper, lead, and cadmium, and also soil that had these contaminants added was studied. Incorporation of 5% chicken manure biochar or green waste biochar into soils with heavy metal contamination resulted in the immobilization of cadmium, copper, and lead and reduced the levels of these metals in plants growing in the contaminated soils. Biomass of the plants grown in chicken manure biochar amended soils was also greater than in control soils, potentially from the reduction in heavy metal concentrations due to the biochar [44]. Although biochar is effective in the uptake and immobilization of heavy metals, competitive sorption when multiple metals are present at one time may limit overall metal reductions [10], [53]. Cadmium had the greatest affinity for sorption in the presence of biochar when single metals were introduced. However the presence of other metals, such as lead, copper, and zinc, caused cadmium sorption to decrease 5-fold. Furthermore, in the presence of other metals, zinc desorbed, although it sorbed when it was the only metal present [53]. This shows different contaminants present in soil, including metals or ions, can compete for sorption sites on biochar. In addition to removing metals from soil, batch studies

showed biochar can remove lead, copper, cadmium, and nickel from aqueous solutions [27]. Mechanisms that contributed to this heavy metal removal were precipitation after reaction with carbonate and phosphate on the crystalline surface of the biochar, as seen through SEM imaging and XRD spectra analysis [27]. Different biochars have been found to have different affinities for metal compounds, likely caused by the difference in surface area or active sites and that possible aggregation of biochar particles can further affect available sorption sites on the material [10].

Another remediation potential mediated by biochar is for the removal of organic compounds. These compounds, such as pesticides or herbicides, can sorb to biochar, reducing chemical mobility [28]. Biochars from a variety of feedstocks are noted to sorb an array of organic pollutants, including Diuron, Atrazine, acetochlor, and terbuthylazine [4].

Some studies completed to assess the possible removal of nutrients from water due to biochar addition have shown promising results. When used as an amendment to soil greenroofs with ryegrass cover, 7% biochar composed of agriculture waste and tires reduced leaching of NO_3^- (97%), phosphate (38%), total phosphorus (52%), total nitrogen (87%), and organic carbon (71%), as well as reduce the suspended solids present in the effluent [5].

From these studies, it can be seen that biochar has a great potential for energy, sustainability, and remediation purposes. With the current problem with stormwater pollutant removal, many different media types are being investigated for more efficient treatment, especially in regard to nitrogen compounds. The variable physical

structure of biochar along with its chemical properties may allow it to be a useful media for more successful removal of NO_3^- and NH_4^+ from stormwater.

Chapter 3

RESEARCH OBJECTIVES

3.1 Hypothesis

Addition of biochar to stormwater treatment structures will change the physical properties and microbial activity present, leading to increased retention of nitrogen compounds. The difference in initial biomass, pyrolysis temperature, and particle size will change the biochar properties, and thus biochar's effectiveness for stormwater remediation.

3.2 Goals of Research

Through the knowledge about the potential interactions of biochar in an environmental setting and the need for increased stormwater remediation, combining these two research areas is a logical research direction. We assume that due to its sorption properties, as well as its positive effect on both soil structure and microbial habitat, biochar will be able to increase the immobilization of nitrogen compounds from stormwater, thus increasing the remediation potential in new or existing stormwater treatment facilities. We also believe that biochars originating from

different biomasses will have a different potential for this kind of remediation. The research will aim to:

- 1.) Quantify compositional differences between poultry liter (PL) and wood derived (WD) biochars
- 2.) Estimate the ability for biochar to remove nitrogen compounds from applied stormwater solutions
- 3.) Evaluate the differences between the microbial communities of biochar-amended and untreated soils

Methods to achieve these goals include:

- 1.) Physical and elemental analysis of PL and WD biochars
- 2.) Column experiments to evaluate the potential for nitrogen compound adsorption to biochar and batch experiments to create an isotherm further describing this adsorption by PL and WD biochars
- 3.) Microbial analysis including PCR/16S DNA sequencing, identification of genes present involved in nitrogen cycling, and fluorescent cell counts from biochar treated soil samples

Chapter 4

MATERIALS AND METHODS

4.1 Biochar Preparation

Biomass was pyrolyzed at Delaware State University. The WD biochar was made from Pennington Nature's Heat hardwood pellets manufactured for household heating. Poultry litter, consisting of a combination of manure and bedding material resulting from the poultry industry, was commercially produced by Perdue AgriRecycle, LLC in Seaford, DE and used for the PL biochar. The equipment to pyrolyze the biomass to biochar included an oven connected to a condenser. This allowed for collection of volatiles produced during the production of the biochar resulting in condensation of bio-oils. Biomass was packed into cylindrical aluminum containers 11 cm in diameter by 13 cm in height. A lid with a 5mm diameter hole was placed on the canister before heating. The temperature in the oven was increased to 500°C at an average rate of 20°C min⁻¹. The biomass was pyrolyzed until no visible smoke was seen from the oven vent. For WD this was approximately two hours and for PL near six hours. After the process was complete, the canisters with prepared biochar were removed from the oven and the ventilation hole in the lid sealed while the biochar cooled.

Upon return to University of Delaware, the biochars were baked at 100°C overnight to remove surface oils, washed to remove any soluble oils or surface precipitates, and dried at 100°C overnight. The biochars were then ground using mortar and pestle and sieved to a size range of 0.85mm to 1mm. All analyses and experiments were performed used prepared in this way.

4.2 Characteristics of Biochar

To evaluate chemical properties and composition of the WD and PL biochar for use in experiments, samples were sent to the University of Delaware Soil Testing Program for analysis. Mehlich 3 extraction was used to extract the putative plant available nutrients in the biochar, including P, K, Ca, Mg, Zn, Cu, Fe, B, S, and Al, and samplers were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES) using Thermo Iris Intrepid II XSP Duo View ICP (Thermo Elemental, Madison, WI) [57]. The same set of elements was evaluated using the EPA3051 Digestion Procedure using a CEM MARs5 microwave digestion system (CEM, Matthews, NC) to quantify the total amount of these metals in the biochar [55]. Digests for this method were analyzed for total adsorbed metals by ICP-OES as well. The EPA method gives a good estimate of the total concentration of the metals in biochar, although some constituents like Al may not completely dissolve from the biochar using this method.

The Soils Testing Laboratory also measured organic matter, CEC, exchangeable ions (K, Ca, Mg and Na), NH_4^+ as N, NO_3^- as N, total nitrogen and total carbon in the biochars [46]. Organic matter content was measured by loss on ignition using a Blue-M High Temperature Furnace model CW6680F (SPX Thermal Product Solutions, White Deer, PA) [47]. CEC at pH 7.0 and exchangeable cations were determined by NH_4^+ saturation with 1N $\text{NH}_4\text{CH}_3\text{CO}_2$ buffered at pH 7.0 [46]. Ammonium-N and NO_3^- -N were extracted with 2M KCl (1:10 w:V) and measured colorimetrically using a Bran&Luebbe AutoAnalyzer 3 (Bran&Luebbe, Buffalo Grove, IL) [20], [41]. Total carbon and total nitrogen were measured by combustion using an Elementar VarioMax CN Analyzer (Elementar Americas, Mt. Holly, NJ) [7], [43]. All analyses were done on two samples of the same biochar type and the average value calculated.

The biochar surface area and pore size estimations were analyzed with the Brunauer-Emmett-Teller method using a Quantachrome Nova 2000 Gas Analyzer. Biochar samples were degassed and analyses completed under N flow.

4.3 Unsaturated Column Experiments

Sand columns were set up to evaluate the effect of biochar on pH, NH_4^+ concentration, and NO_3^- concentration in artificial stormwater solutions (ASW). Ace Glass chromatography columns with 25mm ID and 300mm length were used. Columns were filled with 20/30 sand, which was washed with DI water and oven

dried. One sand-only column, as a control, and two test columns with 10% of either PL or WD biochar mixed with sand were set up. Approximately 300g of media (sand or sand/biochar) was added to each column.



Figure 1 Unsaturated column set-up. Columns present were a sand only control, 10% PL biochar with sand, and 10% WD biochar with sand. Flow of 1 mL min^{-1} was applied to the top of the columns using a peristaltic pump and effluent collected from tubing connected to the bottom of the columns.

4.3.1 Initial DI Test

DI water was applied to the unsaturated columns over a 6-hour period to monitor the change in pH due to the biochar addition as well as any leaching of NH_4^+ or NO_3^- from biochar into solution. A Fisher Scientific Accumet Basic AB15 pH meter was used to evaluate pH change. A Dionex ion chromatograph with ED40

electrochemical detector, GP50 gradient pump, AS40 automated sampler, and IonPac AS20 Analytical 4x250mm column was used to detect NO_3^- from the samples and NH_4^+ analyzed using HACH Test 'N Tube Vials Method 10031 Salicylate Method, 0.4-50.0 mg/L $\text{NH}_3\text{-N}$ (Cat No. 26069-45). The HACH kit detects ammonia; however, the concentration of NH_4^+ could be calculated from this value.

4.3.2 Intermittent Stormwater Column Experiments

Remaining tests were done applying ASW to the unsaturated columns. First an initial test was done to evaluate potential removal of NH_4^+ and NO_3^- . Then four additional ASW applications for ~8hrs at a time were completed over a two month period. Similar to other studies, the ASW solution contained 120 mg/L CaCl_2 , 2 mg L^{-1} (as N) NaNO_3 , and 3 mg L^{-1} (as P) NaHPO_3 , and the 2 mg L^{-1} NH_4^+ (as N) of NH_4Cl , in DI water with a pH adjusted to 7 [13], [23], [30]. The ASW was applied to the columns using a peristaltic pump at a flow rate of 1mL min^{-1} . The effluent from the columns was collected in acid washed 50mL screw-capped tubes containing 1mL 1N HCl. This adjusted the solution pH to below 2 so loss of NH_4^+ would be reduced. Sample tubes were collected and replaced every 45 minutes.

The change in effluent NH_4^+ and NO_3^- concentrations was measured during the initial test to evaluate the changes due to biochar, but for the remaining four trials, only the change in NH_4^+ concentration was monitored. The change of the pH in solution was measured during the third trial. The NH_4^+ concentration was first

measured using HACH Test 'N Tube Vials Method 10023 Salicylate Method, 0.02 to 2.5 mg L⁻¹ NH₃-N (Cat No. 26045-45) and later with Thermo Scientific 9512 Standard Ammonia ISE probe for ease of sampling and broader detection range.

4.4 Batch Experiments

Batch experiments were conducted to evaluate the maximum potential of biochar to remove NH₄⁺ from solution under different conditions. Using the change in NH₄⁺ concentrations, isotherms were generated for NH₄⁺ sorption by PL and WD biochars from both the DI and ASW NH₄⁺ solutions.

4.4.1 Leaching Experiments

A leaching experiment was conducted to see the amount of NH₄⁺ leached from the biochars into solution and to determine the appropriate volume of sample to be used in continuing batch experiments. A ratio of 1g biochar to 50 mL solution was set up in three different volumes: 0.2g in 10mL, 1g in 50mL, and 3g in 150 mL. All experiments were performed in screw-capped glass containers acid washed with 0.1N HCl before use. The ratio of liquid to headspace in the containers was approximately equal for all samples. The solutions used in the experiments were either DI water or ASW with no NH₄⁺ added. The biochar used was either PL or WD. The samples were set up with two replicates of each sample type and shaken at 150rpm for 24 hours to reach equilibrium. After shaking, 10-15mL of sample was transferred to a test tube,

centrifuged at 3000rpm for 15 minutes, then filtered through a 0.2 μm syringe filter and analyzed using a Dionex ion chromatograph with CD20 conductivity detector, GP40 gradient pump, AS3500 autosampler, and IonPac CS16 analytical 5x250 mm column with Dionex cation self-regenerating suppressor.

4.4.2 Adsorption Batch Experiments

To evaluate the potential for biochar to remove NH_4^+ from solutions of different concentrations, batch experiments were set up in 20mL glass vials with screw caps. Each vial received 0.2g of either PL or WD biochar. Then 10mL of a solution containing NH_4Cl was added. The concentration of NH_4Cl in the solutions ranged from 10ppm to 100ppm. NH_4Cl was dissolved either in DI water or in the ASW mixture described previously. Vials containing biochar and DI water or ASW were shaken horizontally for 24hrs at 150rpm, to reach equilibrium. Two replicates of each vial were used. Control vials without biochar were also prepared, and were treated the same way.

Table 2 Samples and solutions for batch adsorption experiment

Biochar Type	Solution Type	Ammonium Concentrations (mg L ⁻¹)					
		10	20	30	50	70	100
PL	DI +NH ₄ Cl	10	20	30	50	70	100
PL	ASW	10	20	30	50	70	100
WD	DI +NH ₄ Cl	10	20	30	50	70	100
WD	ASW	10	20	30	50	70	100
No Biochar	DI +NH ₄ Cl	10			50		100
No Biochar	ASW	10			50		100



Figure 2 Batch experiment shaker set-up. Vials with 0.2g biochar and 10mL of NH₄Cl solutions were prepared and shaken horizontally at 150 rpm for 24 hours to reach equilibrium of NH₄⁺ adsorption.

After 24hrs the samples were removed from the shaker, transferred to 15mL test tubes and centrifuged at 3000rpm for 15 minutes to separate biochar particles from solution. The solutions were vacuum filtered through No. 5 Whatman filter paper (2.5 μ m nominal pore size) to remove any remaining biochar particles. The pH of the solutions was adjusted to below 2 with 70 μ L 1N HCl to allow for preservation stored at 4°C until analysis.

The NH₄⁺ in the samples was quantified using a Thermo Scientific 9512 Standard Ammonia ISE probe. The probe was connected to a pH meter with a voltmeter attached to allow a millivolt display of the electrode output. A calibration curve was made to allow for conversion of the millivolt output to mg L⁻¹ of NH₄⁺.

4.4.3 Effect of pH on Adsorption

To evaluate the effect of pH on adsorption, vials with 0.2g WD biochar with 10mL of ASW with pH adjusted to ~9 were shaken, filtered, and preserved in the same way as previous experiments. Initial concentrations of 50 mg L⁻¹ or 100 mg L⁻¹ NH₄⁺ were used. Before preservation, the pH of the samples was taken. Each vial was analyzed three times to get an average NH₄⁺ removal from each.

4.4.4 Effect of Particle Size and Pyrolysis Temperature on Adsorption

Batch experiments were completed to evaluate the effect of size and pyrolysis temperature on NH₄⁺ adsorption. WD and PL biochars from the same material as the

previous experiments with particle size below 0.85mm were used. Also, PL biochar pyrolyzed at 400°C was washed, baked and sieved in the same manner as the 500°C biochar and used. Size fractions of unsieved (no particle grinding or separation), 0.85mm to 1.0mm, and sizes below 0.85mm were utilized from 400°C PL biochar. As before, 0.2g of each sample was shaken with 10mL of DI + NH₄Cl and analyzed for removal of NH₄⁺ with Dionex ion chromatograph. The BET surface area and pore volume of the 400°C PL biochars were also analyzed.

4.5 Biological Methods

4.5.1 16S PCR and Sequencing

Soil samples were collected from Delaware State University Research Farm from plots with and without biochar amendment. The biochar present in the plots was PL biochar produced at 400°C and had been incorporated three years previously. DNA was extracted from 0.25g of soil using a MO BIO Laboratories, Inc. PowerSoil DNA Isolation kit (Catalog No: 1288-100) for untreated soil samples and Epicentre SoilMaster DNA Extraction Kit (Cat Nos. SM02050 and SM02005) for biochar treated samples. The MO BIO kit used with the biochar treated samples did not result in a large enough quantity of DNA, but the Epicentre kit was used successfully. Manufacturer's instructions were followed for both kits. A total of four samples of both treated and untreated soils were used for DNA extraction. The resulting sample was used to amplify the 16S gene from the DNA in a PCR reaction. Reaction mixtures

contained 0.25 μ L each primer (8F [18] and 1492R [51] 25mM each), 0.5 μ L dNTP (10mM each), 5 μ L 5x reaction buffer, 0.1 μ L Thermo Scientific Phusion polymerase (Cat# F-530S) in a total reaction volume of 25 μ L. PCR amplification conditions were 95°C, 3 min; 34 cycles of 95°C 30s, 51°C 30s, 68°C 1min 30s; 68°C 5min; final hold at 12°C. Successful amplifications were seen in one of the biochar treated samples and three of the untreated soil samples. Blunt-ended PCR products were ligated into pRC®4Blunt-TOPO® from the Zero Blunt TOPO PCR kit and TOP-10 chemically competent cells were transformed with the resulting plasmids according to manufacturers' instructions. The positive control for the transformation was pUC19 and the negative control was sterile DI water. Transformed cells were plated on LB plates amended with 100 μ g mL⁻¹ ampicillin and 100 μ g mL⁻¹ 5-bromo-4-chloro-indolyl- β -D-galactopyranoside (X-gal) and incubated at 37°C overnight. White colonies were transferred to liquid LB amended with ampicillin at a concentration of 100 μ g mL⁻¹ and grown for 12 hours at 37°C with shaking at 250 rpm. Plasmid DNA was extracted by alkaline lysis [6] and followed by a restriction digest to identify plasmids with inserts of the correct size. Plasmids with 16S rDNA inserts were sent for sequencing at the University of Delaware Sequencing and Genotyping Center.

4.5.2 Fluorescence Cell Counting

Initial cell counts were done on freshly collected soils with and without biochar treatment. Soils were collected from Delaware State University plots and

consisted of soil with no amendment, with 2% PL, and 2% WD biochar additions. The soils were taken to University of Delaware's Lewes campus for analysis. To prepare the soils, 1g of each sample type was measured into a sterile 1.5 mL tube and 1mL of filtered phosphate-buffered saline solution added. PBS was prepared with, 8g NaCl, 0.2g KCl, 1.44g NaH₂PO₄, 0.24g KH₂HPO₄, pH adjusted to 7.4, in a total volume of 1L distilled H₂O and sterilized by autoclaving. The samples were vortexed thoroughly to mix. Samples were diluted 1:500 in sterile PBS then sonicated on ice (20 V with 1 second pulse) 3 times for 30 seconds each, with a 30 second rest between pulsing. New tubes were prepared with 500μL sonicated sample and 2.5 mL PBS. These samples were filtered on to 0.22μm black polycarbonate membranes using a hand pump vacuum filter. Stock 10,000x SYBER Green stain was diluted in PBS then to a final dilution of 1:400. The polycarbonate filters were placed shiny side up on a Petri dish and 100μL of the diluted stain added. Dishes were closed and covered with foil to protect from light. After 15min incubation time, filters were dried on a Kimwipe. Filters were then placed on a slide and a drop of Citifluor added before the cover slip. A Nikon epifluorescence microscope was used with 490/20nm (center wavelength/bandwidth) used for excitation and 528/38 nm to detect the SYBR signal [40].

4.5.3 Gene Identification

The presence of different genes involved in the nitrogen cycle was compared between the untreated soil and 2% PL biochar treated soil samples from Delaware State University. Soil DNA was extracted using MO BIO Laboratories, Inc. PowerSoil DNA Isolation kit (Catalog #: 1288-100). This DNA was used in PCR reactions with selected primers to amplify regions containing genes of interest. The primers *niaDO1F* and *niaDO4R* were used to target nitrate reductase, the enzyme that reduces nitrate to nitrite [19] *Cd3F* and *Cd4R* primers were used to target the *nirS* gene encoding nitrate reductase, the enzyme that reduces nitrite to nitric oxide [39], *ZF* and *ZR* primers were used to target the *noz* gene, which encodes nitrous oxide reductase, the enzyme that reduces nitrous oxide to nitrogen gas [32]. For all PCR reactions, 1µL of DNA was mixed with 2.5µL 10x reaction buffer, 0.3µL dNTP mix (10mM each), 0.2µL forward and reverse primers (25µM each), 2.0µL MgCl₂ (25mM), 0.1µL New England Biolabs Taq polymerase (Cat No. MO320S), and DI water to a final volume of 25µL. For each primer pair the PCR conditions varied, as seen in Table 3.

Table 3 Targets and conditions for selected primers to identify presence of genes involved in the nitrogen cycle from soil bacteria

Primers	Target Organism	Target Gene	Annealing T	Extension T	Reference
niaD01F niaD04R	fungi	nitrate reductase	52°C	68°C, 30s	[19]
cd3 F cd4R	Bacteria	<i>nirS</i>	50°-43°C decreasing	68°C, 23s	[39]
ZF ZR	Bacteria	<i>nosZ</i>	68°-58°C decreasing	68°C, 7s	[32]

RESULTS

5.1 Characteristics of Biochars

The PL biochar used in this thesis, when compared to that of others produced at the same pyrolysis temperature [49], had a similar surface area. It does however have a higher surface area, $3.6 \text{ m}^2 \text{ g}^{-1}$ compared to $3.0 \text{ m}^2 \text{ g}^{-1}$ for WD biochar. Table 4 compares the BET surface area and pore size of the biochars produced at varying pyrolysis temperature and with different particle size. The PL biochar produced at 500°C has the same surface area at all particle sizes. However, for WD biochar smaller particle size results in a lower surface area.

Pore size was affected by pyrolysis temperature and particle size. 500°C WD biochar has smaller particle size, smaller pore volume and increases pore radius. This means there are fewer pores in smaller WD biochar particles, possibly due to loss of pore spaces during grinding. This would also account for the reduction in average pore radius if larger pore spaces were lost during grinding. For 500°C PL biochar, both the pore volume and radius decrease with smaller pore size. This shows that like WD biochar, smaller PL particles have fewer pores.

PL biochar produced at 400°C has greater surface area than the PL biochar produced at 500°C . The 400°C PL biochar has a smaller average pore size than the

500°C biochar. The differences in pore sizes can be used as an estimate of water holding capacity and available space for microbial habitats. Larger the pore volumes can better retain stormwater in a bioretention facility. This would allow more time for NH_4^+ in the water to adsorb to biochar surfaces. A higher surface area provides more sites for the NH_4^+ to adsorb.

The PL and WD biochars had a similar CEC, 40.8 meq/100g and 43.5 meq/100g, both with a region of error slightly above 2 meq/100g.

The compositional analysis of the biochars shows PL biochar had a higher concentrations of most elements, including metals, nitrogen, and phosphorus (Tables 5-7). The only notable differences to this are for extractable Al, organic matter, NO_3^- , and total carbon. These differences in composition become a factor for biochar incorporation into soils. If the biochar has a higher concentration of elements, these can possibly leach from the biochar into the system. This can be a positive attribute if elements are needed for biological growth or can be negative if excess leaching leads to unwanted contamination.

Table 4 Comparison of biochar with different pyrolysis temperature and particle size. WD biochar has lower pore volume and radius than PL biochar (Row 1 vs 2). As WD particle size decreases, so does surface area (Row 1 vs 2). Decreasing particle size of PL biochar produced at 500 °C does not affect surface area (Row 3 vs 4), but causes decreases in pore volume and radius. 400°C PL biochar has greater surface area than 500°C, and also shows decrease in pore volume and radius with smaller particle size (Row 6&7 vs 3&4).

Row	Pyrolysis T °C	Particle Size mm	Specific Surface Area m ² g ⁻¹	Pore Volume mL g ⁻¹	Average Pore Radius Angstrom
1	WD 500	0.85-1.0	2.97	0.48 e-3	3.27
2	WD 500	<0.85	0.33	0.19 e-3	11.7
3	PL 500	0.85-1.0	3.60	13.7 e-3	76.2
4	PL 500	<0.85	3.68	11.61 e-3	67.9
5	PL 400	Unsieved	5.84	14.3 e-3	48.9
6	PL 400	0.85-1.0	5.69	12.6 e-3	44.3
7	PL 400	<0.85	3.87	7.60 e-3	39.3

Table 5 Biochar characteristics by Mechlich 3 extractable nutrients method (mg kg⁻¹). The plant available forms of elements are higher in PL biochar than WD biochar, with the exception of Al.

	P	K	Ca	Mg
PL	3768.37±225.66	13675.16±325.52	2441.49±138.35	2446.52±169.37
WD	15.93±3.22	220.25±33.6	475.29±26.38	30.37±5.44
	Mn	Zn	Cu	Fe
PL	86.98±6.26	66.85±4.76	15.02±1.00	31.48±2.38
WD	4.81±0.57	0.83±0.09	1.00±0.12	15.42±1.22
	B	S	Al	
PL	16.93±0.40	2822.46±74.41	1.79±0.33	
WD	0.46±0.07	6.53±2.67	67.6±0.9	

Table 6 Biochar characteristics by EPA3051 digestion procedure (mg kg^{-1}). The total elemental contents are higher in PL biochar than WD biochar for all elements

	Al	B	Ca	Cu
PL	4721.24±163.92	106.83±5.67	51032.69±1231.22	871.67±46.14
WD	706.60±25.36	10.82±0.28	8504.74±69.68	10.99±0.13
	Fe	K	Mg	Mn
PL	2556.62±205.51	43079.78±1605.77	16063.70±523.28	1023.77±39.64
WD	661.89±52.26	3330.19±11.76	971.00±8.47	212.73±1.17
	P	S	Zn	P
PL	26710.42±799.44	9743.36±381.08	1114.93±49.3	26710.42±799.44
WD	212.33±19.97	132.17±0.66	19.88±0.85	212.33±19.97

Table 7 Additional biochar characteristics. WD biochar has a higher OM, TC, and NO_3^- content than PL biochar. Similar values are seen for both biochars' exchangeable Ca and CEC. Other values are higher in the PL biochar.

	OM (%) by LOI	TC (%)	NO3-N (mg/kg)	Exch Mg (meq/100g)
PL	40.35±1.65	18.8±0.67	1.55±0.28	6.1±0.1
WD	80.1±0.1	25.38±0.48	3.64±0.90	1.36±0.14
	Exch Ca (meq/100g)	CEC pH7 (meq/100g)		
PL	19±0.3	40.78±2.86		
WD	19.03±0.42	43.49±2.40		
	Exch K (meq/100g)	Exch Na (meq/100g)	NH4-N (mg/kg)	TN (%)
PL	90.5±2.7	38.8±0.8	13.00±0.07	2.81±0.24
WD	7.36±0.14	0.32±0.02	2.96±0.07	0.27±0.03

5.2 Column Experiments

Table 8 is a summary of the column experiments discussed below in the order completed.

Table 8 Column Experiment Summary

Experiment Name	Applied Solution	Test Duration	Analyzed Constituents
Initial DI Test	DI Water	6 hrs	pH NH_4^+ , NO_3^- (leaching)
Initial SW Test	Stormwater Mix	6 hr	NO_3^- (adsorption)
ASW Set: 1	Stormwater Mix	~8 hr	NH_4^+ (adsorption)
ASW Set: 2	Stormwater Mix	~8 hr	NH_4^+ (adsorption)
ASW Set: 3	Stormwater Mix	~8 hr	pH NH_4^+ (adsorption)
ASW Set: 4	Stormwater Mix	~8 hr	NH_4^+ (adsorption)

5.2.1 Initial DI Test

Biochar had different effects on solution pH. Figure 3 shows the pH of all samples changed within the first sampling time and remained fairly consistent throughout the duration of the experiment. The control sand column showed a pH lowered from the initial pH of 8.2 to between 6 and 7. The initial pH of the DI water was unusually high, but adjusted to a more typical level during the experiment. The pH from the WD column effluent was slightly lower than the control column. The PL biochar had the greatest effect, increasing effluent pH to near 10. This finding is similar to other reports that explain that PL biochar raises the pH of surrounding soils

when introduced [49]. The results show the addition of biochar will change the pH of solution, and thus affect the conditions in soil. For areas sensitive to pH change, the use of PL biochar may increase the pH excessively, reducing plant or microorganism survival. For bioretention facilities the change in pH may or may not be an issue depending on the initial soil conditions, plants incorporated, and the amount of biochar added.

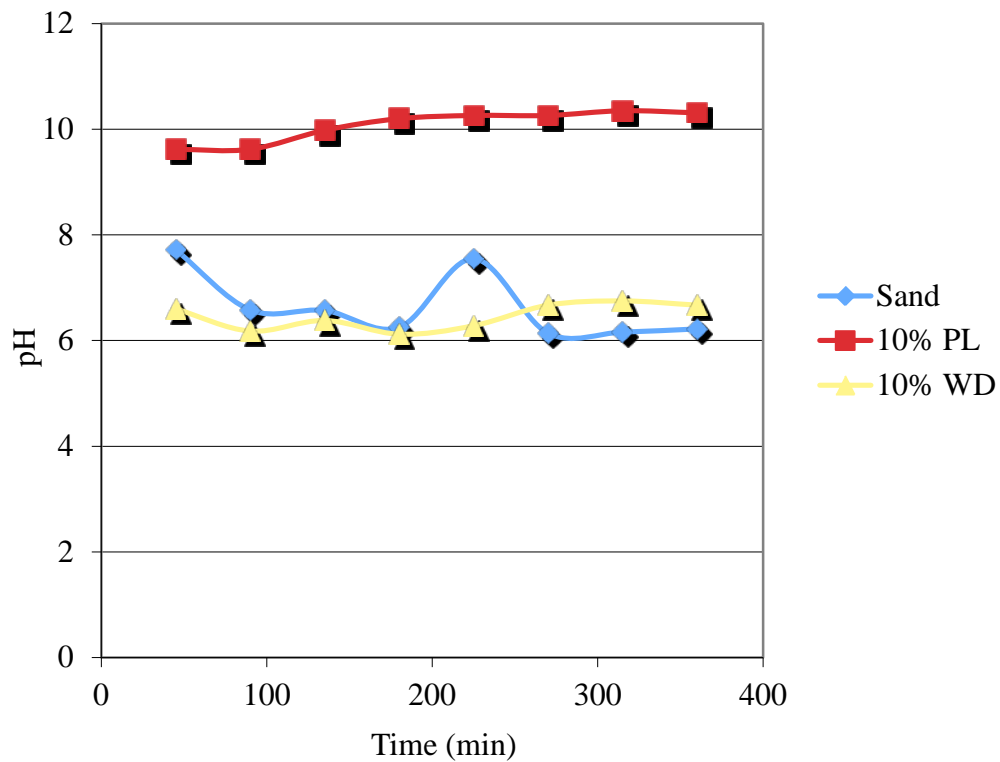


Figure 3 Change in pH of column effluent from initial DI water application. The sand control column had little change in pH during DI water application. The WD biochar caused a slight decrease in pH, while the PL biochar increased the effluent pH to near 10.

Leaching of NO_3^- and NH_4^+ was monitored in the effluent during the initial DI test as well. All samples maintained NO_3^- concentrations below the detection limit of the IC except for the first sample from the WD column. The lowest concentration used for calibration was 0.1ppm; all but the first WD sample were well below this calibration point. The 0.2ppm reading from the WD sample is a low enough concentration to conclude the NO_3^- leaching from the columns is minimal. All of the samples had NH_4^+ concentrations below the detection limit of the HACH kit.

Table 9 Leaching of nitrogen compounds from columns during initial DI water application

Column Media	Time (hr)	Nitrate (mg L⁻¹)	Ammonium (mg L⁻¹)
Sand	0.75	below detection	below detection
	6	below detection	below detection
10% PL Biochar	0.75	below detection	below detection
	6	below detection	below detection
10% WD Biochar	0.75	0.2	below detection
	6	below detection	below detection

5.2.2 Initial Stormwater Test and Intermittent Stormwater Set

During the initial application of ASW to the columns, the effluent was analyzed for both NO_3^- and NH_4^+ . Both biochar types had little effect on NO_3^- concentration. Table 10 shows percent removal of NO_3^- from effluent. The sand control, PL and WD columns fluctuated between some removal and some accumulation of NO_3^- . These percentages were similar for all columns and overall

showed no significant change in NO_3^- concentration. For this reason, NO_3^- was not analyzed during the remaining three trials. The NH_4^+ results could not be used from the initial ASW application to the columns because of an error with the analysis kit. Percent removal of NH_4^+ from the columns during the first run of the set of four column experiments is shown for comparison to the NO_3^- removal.

Both PL and WD biochar greatly reduced the amount of NH_4^+ in ASW, Table 11. The sand control column had little NH_4^+ removal, while biochar columns removed upwards of 90% NH_4^+ . During this first run, the WD biochar had greater NH_4^+ removal than the PL biochar.

Table 10 Sorption of NO_3^- in columns during initial stormwater application. Little change in NO_3^- concentration was noted from the control column and columns with biochar added. This showed low potential for NO_3^- removal with these media.

Column Media	Time (hr)	Nitrate Removal (%)
Sand	3.75	-0.72
	5.25	-2.13
	6.75	8.96
	8.25	-0.02
10% PL Biochar	3.75	2.32
	5.25	1.05
	6.75	1.72
	8.25	1.97
10% WD Biochar	3.75	1.69
	5.25	1.94
	6.75	0.97
	8.25	-4.63

Table 11 Sorption of NH_4^+ in columns during first run of stormwater set. Control column had little effect on the NH_4^+ concentration, whereas addition of PL and WD biochar resulted in high NH_4^+ removal for duration of experiment.

Column Media	Time (hr)	Ammonium Removal from ASW (%)
Sand	3.75	3.23
	4.5	3.29
	5.25	0.44
	6	-5.22
10% PL Biochar	3.75	89.9
	4.5	91.7
	5.25	91.7
	6	93.6
10% WD Biochar	3.75	100
	4.5	96.7
	5.25	95.2
	6	96.4

The remaining three trials in the stormwater application set were completed and results analyzed together. Columns were flushed with one pore volume of fluid (~three hours of flow) between runs. This removed any residual stormwater in the media between experiments. Figure 4 shows the percent removal of NH_4^+ vs the total stormwater volume added. Biochar continued to adsorb significant amounts of NH_4^+ over the four trials, while the sand control column did not adsorb NH_4^+ . The WD column adsorbed more NH_4^+ than the PL column during the first two runs; however, during the final two runs, NH_4^+ removal by the WD and PL biochars were similar. The removal of NH_4^+ from biochar columns remained fairly consistent over two runs, but

lowered during the final two runs (after about 1 liter of total stormwater had been added). This showed biochar began to lose its ability to adsorb NH_4^+ with time.

The ability to remove NH_4^+ from the ASW solutions and to retain this ability over time confirms that both PL and WD biochar are viable options for incorporation into bioretention facilities for NH_4^+ removal, but would not be effective at the removal of NO_3^- .

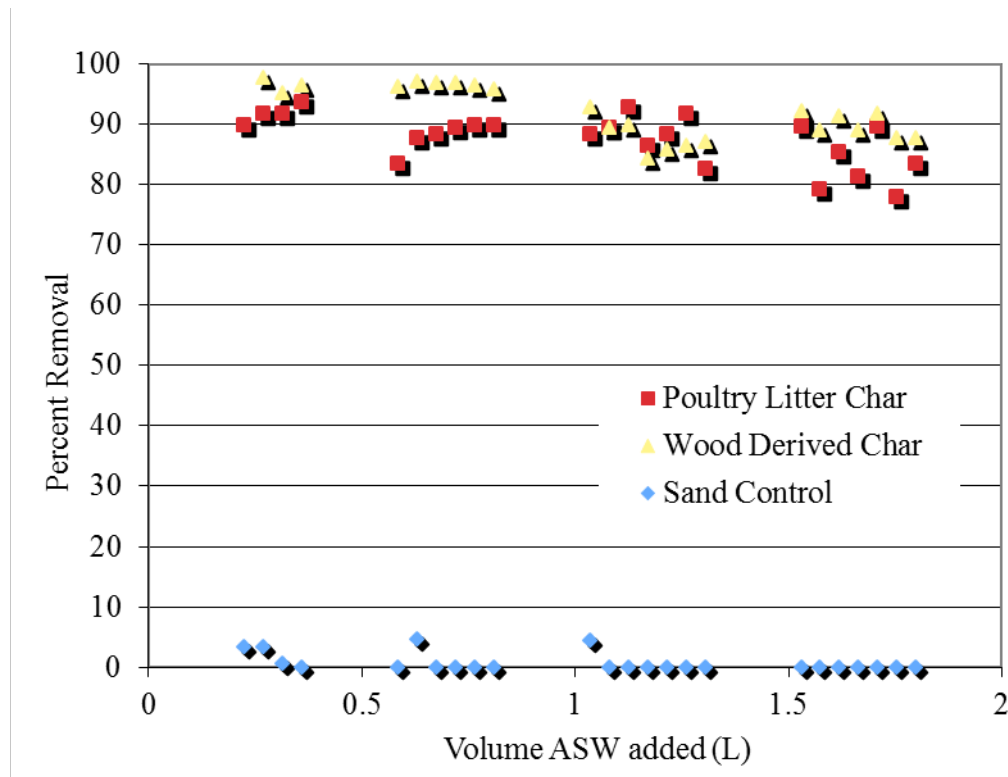


Figure 4 Percent removal of NH_4^+ from columns over the four intermittent runs. As ASW was intermittently applied to the columns, the sand control column consistently had little removal while the columns with biochar added showed high levels of NH_4^+ removal. The WD biochar initially had greater removal than PL biochar, however with the continued addition of ASW this difference became minimal.

During the third run of the stormwater application set, pH of the effluent was monitored to evaluate the change in pH caused by biochar, Figure 5. Similar results were seen as in the first pH study where DI water was applied. The control column pH remained fairly constant, the pH of the WD column was slightly decreased, and the pH of the PL column was increased. The pH was more constant between samples during the ASW experiments than it was for the initial set of DI experiments. This shows the biochar retains its pH adjusting characteristics over time and has the same effect with the additional ions present in ASW as compared to DI water.

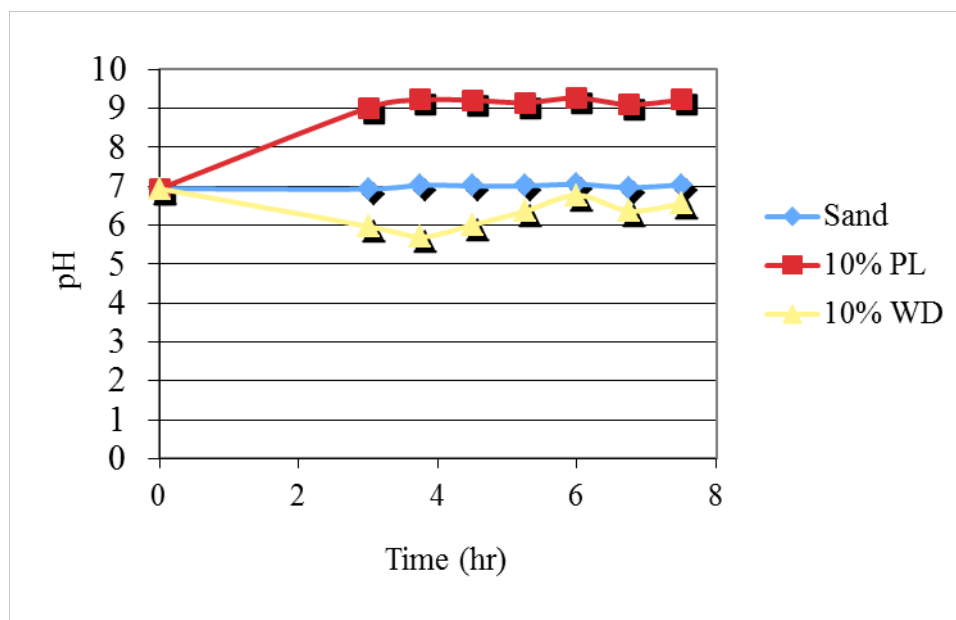


Figure 5 Change in pH of effluent from third run of stormwater set. The control column had little change in pH during ASW application. The WD biochar caused a slight decrease in pH while the PL biochar increased the effluent pH to 9.

5.3 Batch Experiments

5.3.1 Leaching Experiments

There is little difference in the leaching of NH_4^+ between the different volumes of biochar and solution applied as long as the ratio of the two remained the same, Table 12. More NH_4^+ leached from the PL biochar than the WD. There was little difference between the leaching of NH_4^+ into the DI or SW solutions. Because the leaching of NH_4^+ was constant regardless of total volume, the smallest volume of 0.2g of biochar to 10mL of solution was used for the batch sorption experiments.

Table 12 NH_4^+ leached from PL and WD biochars by various volumes of DI or ASW solutions. Leaching of NH_4^+ from biochars remained consistent within three standard deviations, independent of the sample volume if the solid to liquid ratio remained constant.

Sample Mix	NH_4^+ leached (mg g^{-1})			
	PL/DI	WD/DI	PL/ASW	WD/ASW
0.2g/10mL A	0.061	0.017	0.043	0.025
0.2g/10mL B	0.050	0.006	0.058	0.021
1g/50mL A	0.050	0.007	0.044	0.011
1g/50mL B	0.052	0.006	0.043	0.007
3g/150mL A	0.070	0.007	0.050	0.015
3g/150mL B	0.061	0.003	0.047	0.009
Std Dev	0.008	0.005	0.006	0.007

5.3.2 Isotherm Results from Batch Experiments

Adsorption isotherms were created from the batch experiments relating the equilibrium concentration of NH_4^+ in mgL^{-1} of solution to the mass of NH_4^+ adsorbed per gram of biochar. Figures 6 and 7 represent individual biochar types, WD or PL, and show NH_4^+ adsorption results from solutions of DI containing NH_4Cl and ASW. The error bars indicate the range of systematic error due to variability of the NH_4^+ detection probe, as evaluated from control samples.

PL and WD biochars removed NH_4^+ from both solution types, with similar adsorption trends are seen between the biochars. PL biochar was affected by the additional ions in solution and therefore removed less NH_4^+ from the ASW than the DI with NH_4Cl . The WD biochar removed NH_4^+ from both solutions similarly. The PL biochar removed more NH_4^+ from the DI solution than WD; however, the WD had greater removal when ASW was present. Larger error bars at higher NH_4^+ concentrations reflect larger variability in the probe readings in this range.

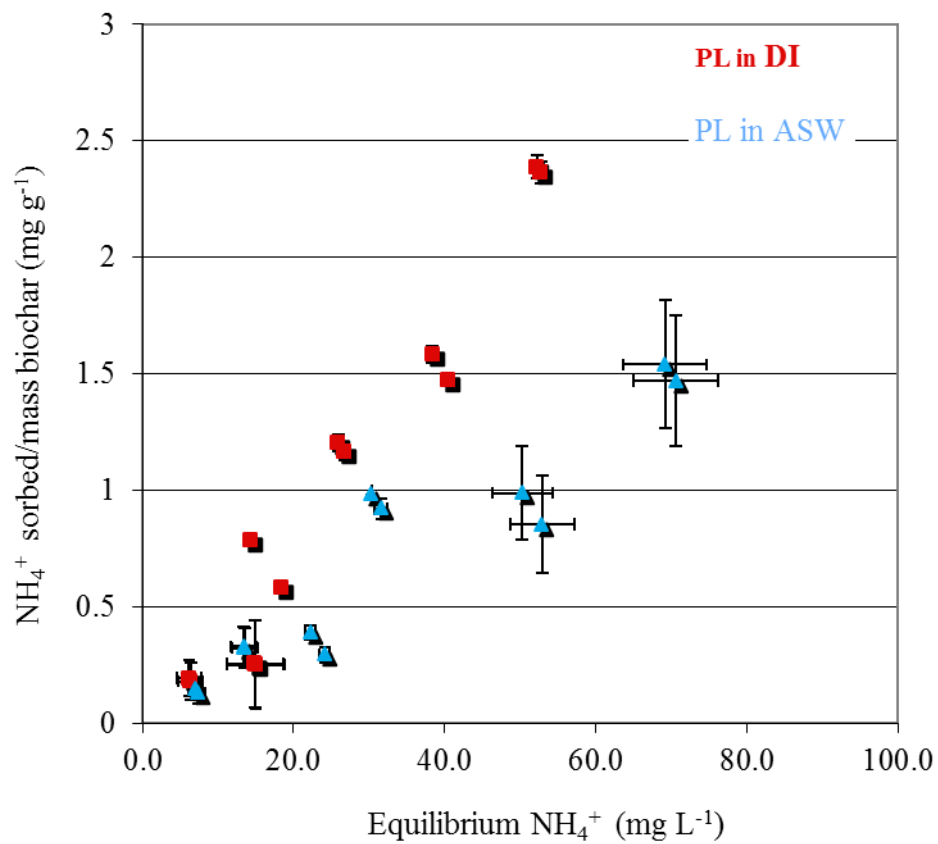


Figure 6 Isotherm for NH_4^+ adsorption to PL biochar. PL biochar removes NH_4^+ from both solutions, however removal is reduced in the presence of additional ions in ASW. The sorption of NH_4^+ by biochar is linearly related to the NH_4^+ concentration in solution.

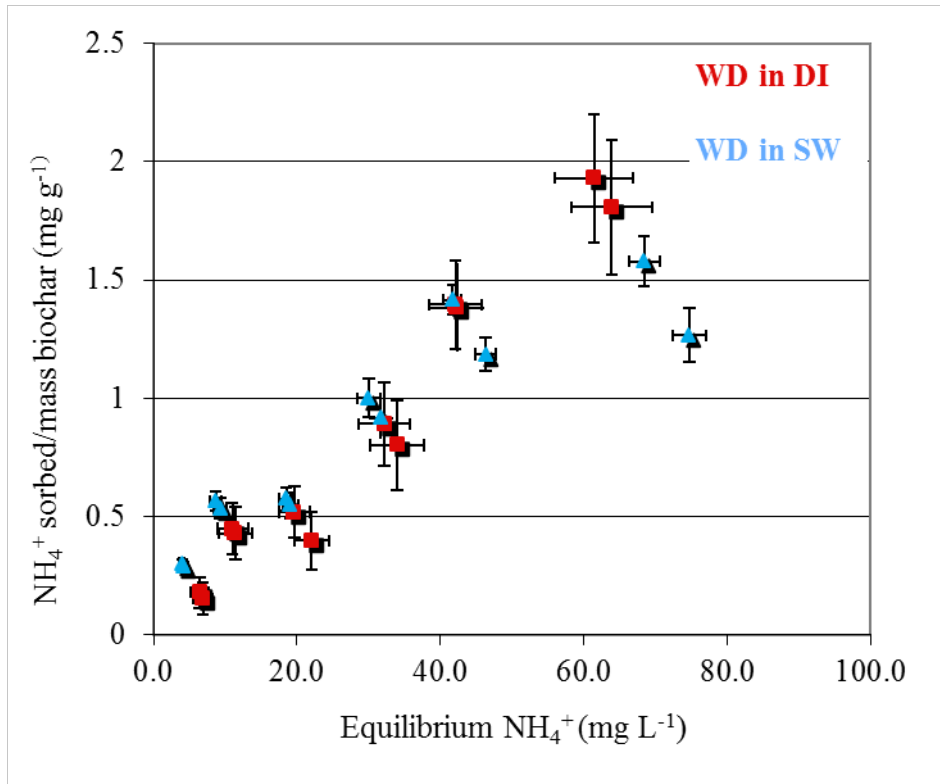


Figure 7 Isotherm for NH_4^+ adsorption to WD biochar. WD biochar removes NH_4^+ from both solutions and retains efficient removal in the presence of additional ions in ASW. The sorption of NH_4^+ by biochar is linearly related to the NH_4^+ concentration in solution.

5.3.3 Effect of pH on Sorption

The data from the ASW with pH of 9 shaken with WD biochar were compared to data from the ASW at a pH of 7. Figure 8 compares the adsorption of NH_4^+ when the pH of the solution is 9 versus the original data where the pH was 7. There is no significant difference between the sorption between the pH 7 and pH 9 solutions, showing sorption is not dramatically affected by changing pH. This is important to note because different biochars affect the pH of a solution. From these results we can

hypothesis the difference in sorption between the PL and WD biochars is not due to the fact PL biochar raises pH to 9, but is due to other factors, likely biochar composition and physical properties.

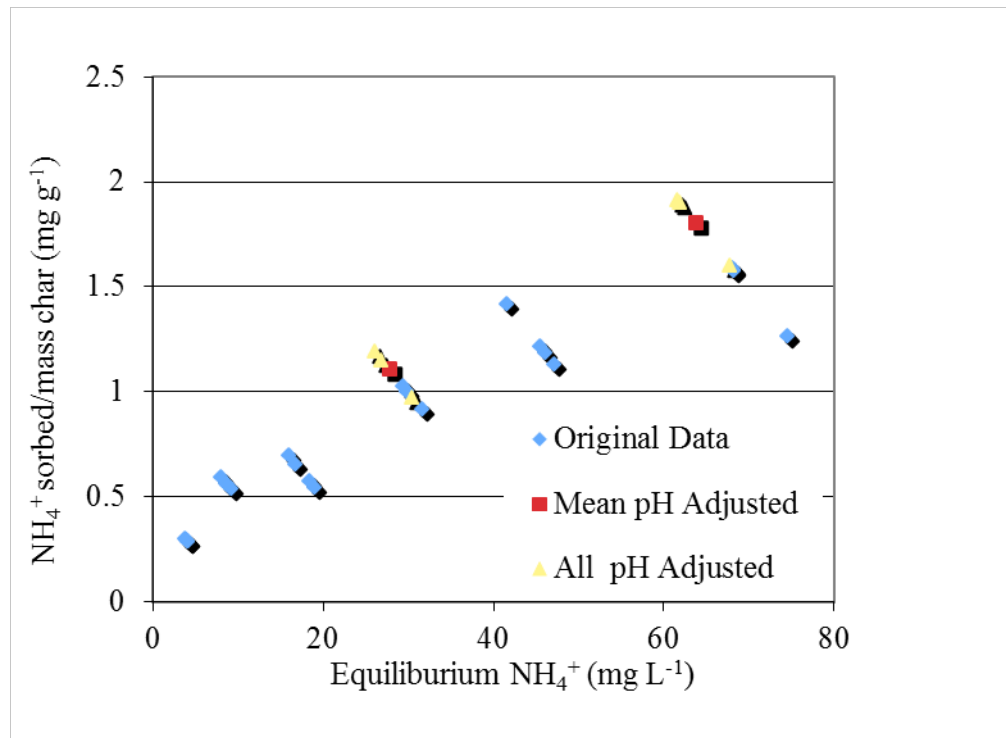


Figure 8 Isotherm of original WD biochar data at pH 7 and data of WD biochar with pH 9 adjusted ASW. The sorption of from ASW adjusted to a pH of 9 is similar to that of the sorption when the pH of solution is 7. Therefore sorption is essentially independent of pH in this range.

5.3.4 Effect of Particle Size and Pyrolysis Temperature on Sorption

Particle size affects NH_4^+ adsorption by biochar, Table 13. For WD biochar produced at 500°C , a particle size of $<0.85\text{mm}$ adsorbed more NH_4^+ from both 10 mgL^{-1} and 50 mgL^{-1} NH_4Cl solutions than a biochar particle size of 0.85mm -

1.0mm (5.8 mgL⁻¹ vs 3.3 mgL⁻¹ and 29.4 mgL⁻¹ vs 16.9 mgL⁻¹). PL biochar produced at 500°C with particle size of <0.85mm leached of over 7 mgL⁻¹ NH₄⁺ in 10 mgL⁻¹ solution. NH₄⁺ adsorption from 50 mgL⁻¹ was very low with smaller particles of PL char compared to PL char with a particle size of 0.85mm-1.0mm (12.9 mgL⁻¹ vs 23.6 mgL⁻¹).

When incorporating biochar into soils for NH₄⁺ reduction, these results show particle size selection is critical. For WD biochar, using smaller particle size may increase NH₄⁺ removal rates while for PL biochar smaller fractions have the potential to increase NH₄⁺ leaching thus hindering stormwater remediation.

Table 13 Sorption results for biochar produced at 500°C. WD biochar of smaller particle size adsorbs more NH₄⁺. PL biochar of smaller particle size leaches NH₄⁺ in 10 mg L⁻¹ solutions and has reduced removal in 50 mg L⁻¹ solution.

Biochar Type	Particle Size (mm)	Initial NH ₄ ⁺ (mg L ⁻¹)	Final NH ₄ ⁺ (mg L ⁻¹)	Adsorbed NH ₄ ⁺ (mg L ⁻¹)
WD	<0.85	10.0	4.2	5.8
WD	0.85-1.0	10.0	6.7	3.3
WD	<0.85	50.0	20.6	29.4
WD	0.85-1.0	50.0	33.1	16.9
PL	<0.85	10.0	17.4	(7.4) Leached
PL	0.85-1.0	10.0	6.3	3.7
PL	<0.85	50.0	37.1	12.9
PL	0.85-1.0	50.0	26.4	23.6

The pyrolysis temperature also changes the NH_4^+ sorption capability of PL biochar. Table 14 shows NH_4^+ sorption from PL biochar produced at 400 °C and compares this sorption from varying particle sizes. The 400 °C PL biochar has high NH_4^+ leaching in 10 mg L⁻¹ solution and either the <0.85mm or 0.85-1.0mm particle size removes significantly less NH_4^+ from 50 mg L⁻¹ NH_4Cl than PL biochar produced at 500°C (9.0 mg L⁻¹ vs 12.9 mg L⁻¹ and 9.3 mg L⁻¹ vs 23.6 mg L⁻¹ in Table 13 vs Table 12). Furthermore, there is little difference in removal with different particle size of 400 °C PL biochar. The results indicate the PL biochar pyrolyzed at a lower temperature has more leaching than sorption and thus may not be an affective remediation amendment.

Table 14 Sorption results for PL biochar produced at 400°C. There is significant leaching of from biochar when present in an initial NH_4^+ concentration of 10mg L⁻¹ and little overall removal when in 50 mg L⁻¹ solution. There is little difference between the results related to particle size.

Particle Size (mm)	Initial NH_4^+ (mg L⁻¹)	Final NH_4^+ (mg L⁻¹)
Unsieved	10.0	22.4
0.85-1.0	10.0	22.0
<0.85	10.0	21.3
Unsieved	50.0	42.1
0.85-1.0	50.0	40.7
<0.85	50.0	41.0

5.4 Biological Results

5.4.1 16S PCR and Sequencing

The University of Delaware Sequencing Center analyzed DNA sequences from the 16S PCR of the biochar treated and untreated soil samples. These resulting sequences were compared to known sequences using NCBI Nucleotide BLAST database to evaluate which strains were present in the soil samples. Table 15 shows the resulting BLAST sequence match for the untreated and PL treated soil samples. This list of bacteria only represents a portion of what is present in the soil, thus these results do not give a clear picture of soil community differences.

Table 15 BLAST results from sequencing of 16S DNA of untreated soil and PL biochar treated soil

BLAST Result: Untreated Soil
<i>Beta Proteobacterium</i>
<i>Acidobacteriaceae Bacterium</i>
<i>Acidobacteria Bacterium</i>
<i>Rhodocyclaceae Bacterium</i>
<i>Rhodocyclaceae Bacterium</i>
<i>Acidobacteriales</i>
<i>Burkholderiales Bacterium</i>
<i>Acidobacteria Bacterium</i>
<i>Bacillus Niacini Strain</i>
<i>Gamma Proteobacterium</i>
<i>Nitrosomonadales Bacterium</i>
BLAST Result: PL Biochar Treated Soil
<i>Bacillus Megaterium</i>
<i>Bacillus Megaterium</i>
<i>Acidobacteria Bacterium</i>

5.4.2 Fluorescence Cell Counting

Microscopically, the bacterial cells were not easily distinguished from the soil matter and differences between the treated and untreated soils were not pronounced. Some problems arose because cells were not fixed. Cell growth was actively occurring, which made visualization of individual cells difficult. Another issue came from the selection of stain. With soil samples, SYBER Green often has background fluorescence, which impedes visualization. The background may be reduced with different staining techniques, perhaps with or without fixation.

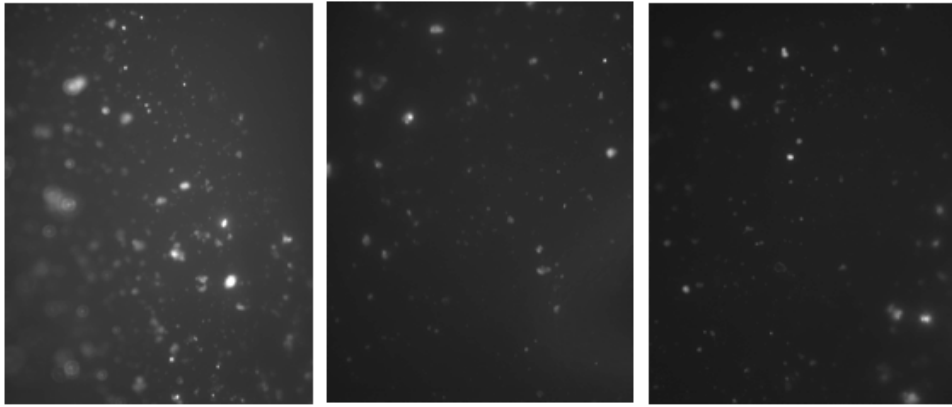


Figure 9 Three fields of view from stained control soil under microscope (100X magnification)

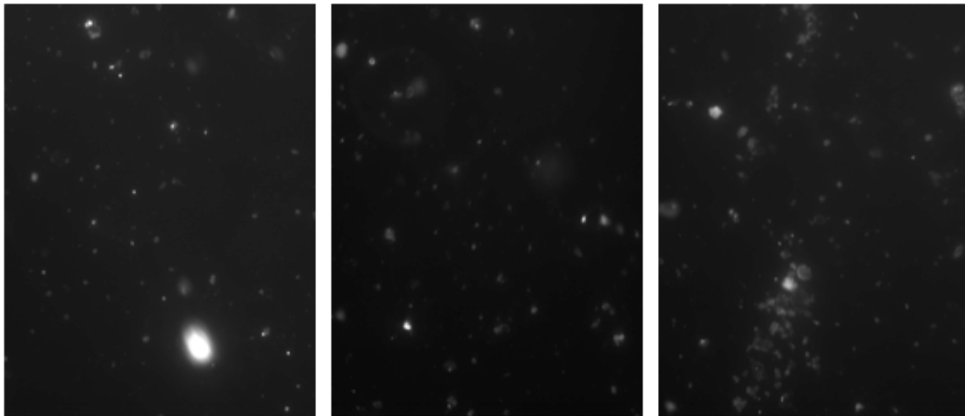


Figure 10 Three fields of view from stained PL biochar amended soil under microscope (100X magnification)

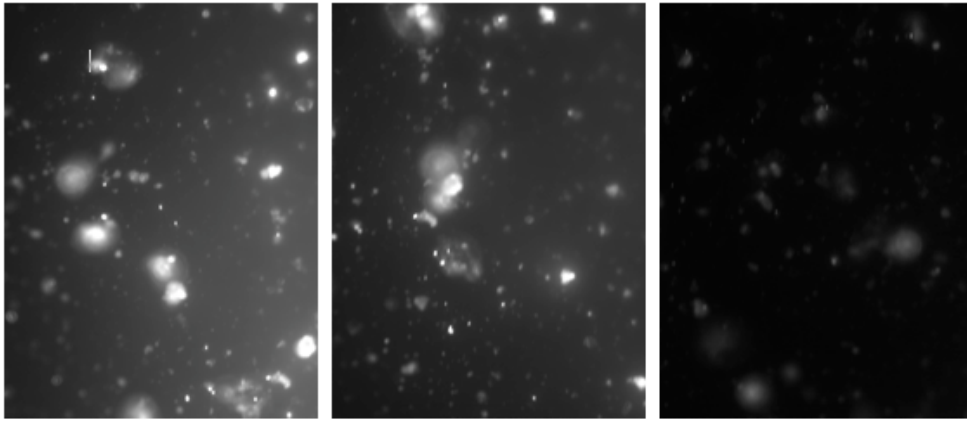


Figure 11 Three fields of view from stained WD amended soil under microscope (100X magnification)

5.4.3 Gene Identification

Each reaction was performed with the untreated soil DNA, the PL biochar treated soil DNA, and a negative control of DI water. No positive control was used. Treated and untreated samples showed presence of the nitrate reductase gene. A faint band was present in both the treated and untreated samples showing the presence of the *nirS* gene. The PCR for the *nosZ* gene was not successful. These results show there are genes involved in the nitrogen cycle present in both biochar treated and untreated soil samples, but no conclusive relationships can be drawn.

Chapter 6

DISCUSSION

The work described in this thesis demonstrates that biochar effectively removes NH_4^+ from ASW solution. The mechanism of removal is primarily adsorption, which depends on the composition of the biochar. In bioretention facilities, biochar has the potential for extended NH_4^+ removal that can be calculated using the experimental results from this paper. Microbial activities may also come into effect, but results from these studies were inconclusive in the current experiments.

6.1 Adsorption by Biochar

Both PL and WD biochars adsorbed NH_4^+ . Linear isotherms (Figure 6 and 7) for NH_4^+ removal from ASW by PL and WD biochars imply that adsorption is the primary mechanism of action. The adsorption is dependent on the biochar surface properties. Because both PL and WD biochars have high CEC and a negative surface charge, both adsorb NH_4^+ , but not NO_3^- . Differences in the adsorption capacities in the char vary with biochar characteristics, like surface area, pore spaces, particle size, and elemental content. The difference in surface area between PL and WD biochar may affect adsorption of NH_4^+ from solution or in soil. PL char had a greater surface area than WD, which is likely related to its higher pore volume and pore size (Table 4).

Elemental composition and physical characteristics of PL and WD biochar are related to the composition of the starting material (Tables 5-7). Because poultry litter contains nutrients as well as a heterogeneous mixture of shavings, manure, and animal feed, the biochar produced from this feedstock is also rich carbon, phosphorus, nitrogen, and metals. After the pyrolysis process, the elemental contents are reduced and more recalcitrant, but a portion can leach from the biochar. WD biochar has higher Al and NO₃⁻ content than PL biochar, likely due to manufacturing processes, but overall its composition mostly carbon. Because of the higher concentrations of NH₄⁺ in PL biochar, more NH₄⁺ leaches from the PL char than from WD biochar. PL biochar with smaller particle size and lower pyrolysis temperature leaches greater amounts of NH₄⁺.

6.2 Biochar Incorporation into Bioretention Facilities

6.2.1 Predicted Removal Potential

The potential of biochar to remove NH₄⁺ from stormwater (SW) in a bioretention facility was calculated (see Figure 12) from the isotherm data. The specifications for size and NH₄⁺ concentration were estimated from the AbTech Ultra-Urban Filter in Newark, DE. Using the isotherm data maximum NH₄⁺ removal by PL and WD biochar was estimated for an inflow NH₄⁺ concentration of 0.32 mg L⁻¹ or - (mean concentration to this BMP from past research, equivalent to 0.32ppm: [26]). The mass of biochar added was calculated assuming a volume of 42.21ft³ and bulk density of 600 kg/m³. From the calculations, Figure 13 was generated to relate percent

removal of NH_4^+ in the facility to increasing SW flow. Figure 13 shows 2% PL biochar application has low removal efficiency, becoming exhausted after application of approximately 200,000L of SW. After the same SW volume, 2% WD biochar can remove 5% of incoming NH_4^+ concentration. If 10% biochar is used, ammonium sorption sites on the PL char are not exhausted until nearly 350,000 L SW is applied. The WD char at 10% has the greatest removal potential, maintaining over 50% NH_4^+ capacity after filtering of 200,000 L of SW.

A minimum storm event creates 9,500L and maximum event 71,000L of stormwater to the AbTech facility [26]. Biochar added to the bioretention facility would make it capable of removing NH_4^+ from SW for multiple storm events. Furthermore, because a past study showed an increase in NH_4^+ concentration over time in the bioretention facility [26] any increased NH_4^+ removal due to biochar adsorption is an improvement.

The above calculation did not factor the bioavailability of the NH_4^+ once adsorbed to the biochar. Once adsorbed, the NH_4^+ is available to plants as a nitrogen source [52]. As plants use biochar adsorbed NH_4^+ as a nutrient, the biochar is recharged, regenerating NH_4^+ sorption potential. Microbial interactions with the biochar can alter the nitrogen cycle, giving the potential for additional NH_4^+ removal via modifications to nitrification and ammonification rates [1], [11]. Estimates of NH_4^+ removal by PL and WD biochars from the calculations alone are an underestimate of actual field removal when plant recharging of biochar and modification in nitrification and ammonification rates occur.

Average AbTech Stormfilter data:

-Volume of Facility: 42.21 ft³ -approximate bulk density of material: 600 $\frac{kg}{m^3}$

-Mean stormwater NH_4^+ concentration: 0.25 $\frac{mg}{L}$ as N or 0.32 $\frac{mg}{L}$ as NH_4^+

-Minimum Storm Event: 9,500 L -Maximum Storm Event: 71,000L

Removal of NH_4^+ from stormwater from equation of line for Isotherm

PL:

$$\begin{aligned}\frac{\text{mg } NH_4^+ \text{ Sorbed}}{\text{g biochar}} &= 0.0204 \left(\frac{\text{mg}}{\text{L}} NH_4^+ \text{ in SW} \right) + 0.0263 \\ &= 0.0204 \left(0.32 \frac{\text{mg}}{\text{L}} NH_4^+ \right) + 0.0263 \\ &= 0.0328 \frac{\text{mg } NH_4^+ \text{ Sorbed}}{\text{g biochar}}\end{aligned}$$

WD:

$$\begin{aligned}\frac{\text{mg } NH_4^+ \text{ Sorbed}}{\text{g biochar}} &= 0.0168 \left(\frac{\text{mg}}{\text{L}} NH_4^+ \text{ in SW} \right) + 0.3484 \\ &= 0.0168 \left(0.32 \frac{\text{mg}}{\text{L}} NH_4^+ \right) + 0.3484 \\ &= 0.354 \frac{\text{mg } NH_4^+ \text{ Sorbed}}{\text{g biochar}}\end{aligned}$$

Figure 12 Calculations of NH_4^+ removal potential by WD and PL Biochar in AbTech Stormfilter Sized Bioretention Facility

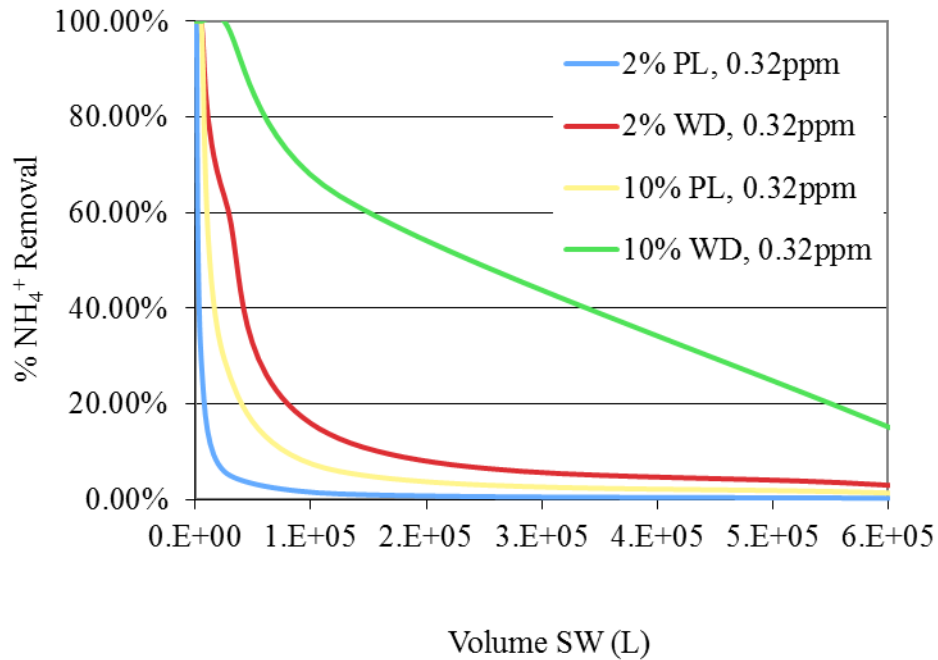


Figure 13 NH_4^+ Removal Potential of Biochar from Accumulating SW in a bioretention basin modeled from the Newark, DE Ab-Tech Facility

6.2.2 Effect of pH of Bioretention Facilities

PL and WD biochar alter pH. The WD biochar slightly decreased pH while the PL increased the pH. Typical soil pH ranges from 5.5 to 8.5 [42]. An optimum pH range for bioretention design is 5.5 to 6.5 in order to increase pollutant adsorption and support microbial activity [34]. Optimal pH for a system will help dictate what type of biochar is used. These experiments used sand with biochar, so the pH changes noted may be more pronounced than if biochar and soil were used. However, the change in pH due to biochar addition should be considered before choosing a biochar for

stormwater remediation purposes. Future experiments with buffers at different pH values may help further define the pH changes.

6.3 Biochar and Microbial Communities

Many unidentified microorganisms were present in soil used in these experiments. The results did not define the differences in populations between the biochar treated and untreated soil samples. The strains identified are bacteria common to a multitude of soils types (see Table 15). Although a Betaproteobacterium, potentially capable of N fixation and nitrification, and *Bacillus megaterium*, involved in denitrification, were found in the untreated and biochar treated soils respectively, their identification does not necessarily mean the strains are more common those soils. However, the different functions of the microbes may cause variability between nitrogen cycling in the biochar-treated and untreated soils. A better idea of the community differences will require a larger scale sequencing effort.

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

Biochar can successfully remove NH_4^+ from stormwater and would provide a beneficial addition to new or existing stormwater remediation facilities. Although biochar did not remove NO_3^- , the adsorption of NH_4^+ from stormwater will have environmental benefits. The NH_4^+ removal is dependent on biochar characteristics, including starting biomass, particle size, porosity, and pyrolysis temperature.

7.1.1 Initial Biomass

Initial biomass contributes to the effectiveness of biochar to adsorb NH_4^+ . WD biochar removed more NH_4^+ from ASW than PL biochar. PL biochar leached NH_4^+ and can potentially leach other elements due to its high elemental content. Biochar types alter pH, thus soil conditions and optimal pH should be evaluated before selecting biochar for amendment. Using a waste product, like PL, to produce biochar can help repurpose an unwanted material in addition to creating a method for stormwater treatment. The desire to utilize these wastes can be a

determining factor in biochar selection. The use of a less effective biochar, such a PL, may be appropriate if larger quantities of the unwanted material are available.

7.1.2 Particle Size

Depending on elemental content, biochar particle size can have positive or negative effects on NH_4^+ removal. Smaller particle size increased NH_4^+ adsorption in WD biochar, but increased NH_4^+ leaching in PL biochar. Using a range of WD biochar sizes including fine particles may be more effective than coarser particles alone. However, smaller particles of PL biochar should be avoided to prevent NH_4^+ leaching.

7.1.3 Pyrolysis Temperature

Biochars produced at higher temperatures have less nutrient and metal content because these elements are easily lost during pyrolysis. The elements remaining are better retained in the biochar structure and thus may result in less NH_4^+ leaching. Thus a biochar produced at 500°C is more effective at NH_4^+ removal than that produced at 400°C.

7.2 Recommendations

Successful incorporation of biochar into a stormwater remediation facility requires analysis of current conditions of the system and determination of

overall goals. If excess NH_4^+ needs to be controlled, biochar is a viable option. To use a waste product like PL, a higher pyrolysis temperature, larger particle size, and incorporation into an area that can withstand an increase in pH is important. WD biochar can be used effectively in a smaller particle size and has less effect on pH. Because N adsorbed to biochar is bioavailable, incorporating biochar into a stormwater remediation facility with plants, like bioretention facilities, would be more effective on a longer term. Careful evaluation of bioretention conditions can allow biochar to be added as an environmentally sound amendment for stormwater remediation.

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