

ABSTRACT

Title of Thesis: MODIFYING GREEN ROOF SUBSTRATE
FOR NUTRIENT RETENTION IN URBAN
FARMING SYSTEMS

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Interest in urban agriculture is steadily increasing in the Mid-Atlantic region. The conversion of extensive green roofs to food production is particularly appealing due to space availability. The modification of a relatively unfertile shale-based substrate for increased water and nutrient availability was investigated, adding mushroom and yard-waste composts, but potentially contributing to nutrient runoff from rainfall and irrigation events.

Alumina and biochar were therefore tested as substrate amendments to determine their effect nutrient availability and retention. Fifteen substrate mixes were screened by column leaching tests, and four were further studied over nine-months, with crop and leachate studies. Basil, lettuce and peppers were grown and harvested in succession in replicated 50-liter tubs, with leachate collection systems. Biochar did not reduce nitrogen or phosphorus leaching and did not have an effect on plant

growth. Alumina significantly reduced the amount of phosphorus leached from substrates with little to no effect on plant growth.

MODIFYING GREEN ROOF SUBSTRATE FOR NUTRIENT RETENTION IN
URBAN FARMING SYSTEMS

by

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Chapter 1: Introduction

1.1 Introduction to Green Roofs

Unused roofs represent up to 32% of the area in urban centers (Proksch, 2011). Green roof technology has been steadily transforming urban rooftops into environmental stormwater control systems, which are rapidly being adopted in many cities around the world. Increasingly, green roof technology is also transforming urban rooftops into environmental and ecological resources in many cities (Peck et al. 1999, Getter and Rowe 2006, Oberndorfer et al. 2007). Parallel to this investment in green infrastructure, urban residents have also developed a desire for more local, sustainable, and nutritious food which has fostered development of urban agriculture projects cultivating local, organically grown produce (Proksch, 2011).

Green roofs can be adaptively designed and installed almost anywhere in any climate. They can be designed into buildings from the production of the original drawings or they can be retrofitted to an existing building. Many municipalities are offering stormwater (volume) credits and installation rebates towards building owners that have green roofs installed on their buildings for their positive environmental impacts (Minnesota Pollution Control Agency, 2020). On the other hand, some cities are requiring green roofs to be installed on a minimum square footage of their footprints in new constructions (La Rossa, 2019).

There are two broad classifications of green roof installations, intensive and extensive. Intensive green roof installations are typically planned during the initial design phased of a building because of the required infrastructure to support deeper substrate depths usually greater than 15 cm (~6 inches) and larger plantings.

Intensive green roofs can support a greater variety of plants such as large shrubs and trees along with small, herbaceous plants commonly found on green roofs (Whittinghill et al., 2014). Intensive green roofs can be significantly more expensive than extensive green roofs and require the building's roof to be able to support significant loads.

Extensive green roofs are the most common type of green roof and are installations that are usually retrofitted to existing buildings (Vijayaraghaven et al., 2012). Extensive green roofs are installations that are less than 15 cm (6 inches) in substrate depth. This shallower depth limits the plant selection available to extensive green roofs limiting it to low growing and herbaceous plants such as cool-desert / alpine sedums, native grasses, and perennial agricultural crops (Dvorak, 2010). Extensive green roofs are often a more attractive option as they are significantly cheaper, easier to install, and can be installed onto an existing building.

Due to the shallow depth of many extensive green roofs, specialist substrates are 'engineered' to provide the desired properties for a specific green roof. These substrates must support plant life, retain stormwater to prevent runoff, avoid being a point source of pollution themselves, and be light enough to avoid compromising the structure of the building (Buffam et al., 2015). In contrast, intensive green roof installations can use substrates closer to native soils depending on their depth, although excessive water retention by soils in containers has long been known to affect aeration and foster root diseases (Ownley, 1990). For these reasons, as well as weight, these types of native-like substrates are not typically used in extensive green roof systems. (Rowe et al., 2006, Rowe et al., 2011, Lamond et al., 2016).

Extensive green roof substrates are typically formulated to provide a long-lasting stable medium which can support plant growth, but which also provides approximately 12mm of stormwater retention capacity (Getter et al., 2007). Most green roofs substrates are a mixture of inorganic (e.g. expanded shale, volcanic pumice) and organic components, to provide a blend that has adequate water-holding capacity (WHC), air-filled porosity (AFP) and nutrient retention, (CEC).

There are a wide variety of green roof substrate components and many proprietary blends. Base materials range from native materials to processed and recycled products (Molineux et al., 2009). These base materials are then typically amended with organic matter to ensure that the final substrate performs in the manner that is expected (FLL, 2008). Some examples of common green roof substrates are expanded shale, clay pellets, crushed brick, lava rock, and even carboniferous pellets made from wood or paper. Typical amendments added to these substrates to increase their fertility are compost, sewage sludge, manure, or mixtures of the list (Molineux et al., 2009, Nagase et al., 2011, Hagner et al., 2016, Harper et al., 2015, Ramasahayam et al., 2014, Karczmarczyk et al., 2017). Other amendments such as biochar can be added to increase plant performance for elevating cation exchange capacity (CEC) to increase soil nutrient retention and water holding capacity (Beck et al., 2011, Hagner et al., 2016, Harper et al., 2015, Ramasahayam et al., 2014, Karczmarczyk et al., 2017). The formulation of green roof substrates has a direct impact on the performance of storm water retention and runoff water quality of a green roof.

Due to their design primarily for stormwater mitigation, green roof substrates typically have high porosities. Succulent species such as sedum are typically used for extensive green roof installations, as they are drought resistant and yet respond quickly to rainfall events, yet have low nutrient and maintenance requirements (Beck et al., 2011, Dvorack et al., 2010, Whittinghill et al., 2014, Harper et al., 2015, Hagner et al., 2016).

There are many studies that identify green roofs as being sources instead of sinks for nutrients such as nitrogen (N) and phosphorus (P) (Karczmarczyk et al., 2014, 2017, 2018, Ramasahayam et al., 2014, Rowe et al., 2011, Sagano et al., 2017). This may be due to poor nutrient retention characteristics, combined with high hydraulic conductivities. Nitrate-nitrogen is the most common form in runoff water (typically due to high nitrification rates or volatilization of ammonium) and comes primarily from the decomposition of organic matter in the substrate (USDA NRCS, 2014). Green roof substrates tend to be highly porous allowing more microbial activity (Nagase et al., 2011).

Green roof technology is being adopted in urban areas all over the world to address the problem of stormwater quantity and quality management at the source (Sugano et al., 2017, Karczmarczyk et al., 2018, Malcom et al., 2014). Typically, a green roof's ability to retain stormwater depends on factors such as the intensity and duration of the rain event (Carson et al., 2013), which is also affected by substrate depth, prior (antecedent) substrate moisture content along with the type, health and density of vegetation, and plant transpiration rates (Berndtsson et al., 2009). Runoff from storm events should ideally have low nutrient loads to avoid long term problems

associated with eutrophic pollution. Quality and quantity management of stormwater can be addressed by careful selection of substrate components designed to retain nutrients leached from the organic matter and nutrients either incorporated in or applied to green roofs over time. However, agricultural rooftop farms typically modify green roof substrates with additional organic matter to enhance water and nutrient retention, and crops are also fertilized and irrigated during the growing season, possibly contributing to nutrient runoff issues if not carefully managed.

Urban farmers using vacant lots also face similar issues to commercial rooftop farms, as they typically cannot grow in native soils due to lead and other urban soil contaminants. Raised beds, using sustainable organic substrates with composted material additions are typically used. Correct substrate formulation and nutrient management practices, timely irrigations (even if hand-watered) and the leaching of nutrients from raised beds, are common issues facing urban farmers of all backgrounds. Green roof substrates have been successfully amended to support the higher nutritional requirements of vegetable crops (e.g. by adding organic matter such as spent mushroom compost), but questions as to their ability to hold $\text{NO}_3\text{-N}$ and PO_4^{3-} have not been adequately resolved.

1.2 Nitrogen

This study focused primarily on the plant available form of nitrogen (N); nitrate ($\text{NO}_3\text{-N}$). $\text{NO}_3\text{-N}$ is a highly soluble anion, produced through nitrification by nitrifying bacteria in the soil. This process consumes ammonium (NH_4^+) and ammonia (NH_3) (typically volatilized but can be fixed by bacteria) in the soil and through the process of nitrification, produces nitrate (NO_3^-) (USDA NRCS, 2014). Nitrate

can then either be absorbed into plants, runoff/leach out of the soil profile, or denitrify into N_2O and N_2 where it is lost to the atmosphere. Total Nitrogen (TN, nitrogen in all forms, both inorganic and organic) can be a source of nitrate as soil microbes break down complicated nitrogen-rich organic matter more available forms of nitrogen (USDA NRCS, 2014). Due to nitrate's high solubility, it can leach rapidly from green roofs substrates, out of the root zone. Effective control of N leaching and availability includes appropriate applications of longer term (organic) sources, at appropriate rates.

1.3 Phosphorus

There are many forms of organic and inorganic phosphorus (P) present in agricultural runoff and it is the nutrient that contributes the most (per unit of P mass) to eutrophic pollution (Correl, 1998). Total Phosphorus (TP) describes all forms (fixed and soluble) of phosphorus, in both organic and inorganic forms, present in leachate or the soil profile. This study focused on dissolved phosphorus which includes all soluble forms of organic and inorganic phosphorus (Ca, Fe, Al phosphates). The soluble, plant-available forms of phosphorus are orthophosphates ($H_2PO_4^{1-}$, HPO_4^{2-} , PO_4^{3-}) and are included in the overall category of dissolved phosphorus (USDA, 2014). Testing for dissolved phosphorus allows the detection of all orthophosphates, as well as all other forms of soluble phosphorus that can quickly break down into orthophosphates which become available to organisms and contribute to eutrophic pollution.

Orthophosphates can be mineralized from phosphorus-rich organic matter, weathered from minerals such as apatite, dissolved from precipitated forms (Ca, Fe,

Al phosphates), or through desorption where it leaves phosphate saturated soil particles. Orthophosphates in soil solution can be taken up by the plant, or adsorbed back onto clay particles in the soil and saturating the particles. This adsorption effect is increased with higher concentrations of aluminum and iron oxides present in the soil. As the saturation of phosphorus increases as more orthophosphates are adsorbed onto soil particles, losses of phosphates increase due to leaching and runoff due to lack of bonding sites on clay particles (Prasad, 2019).

1.4 Consequences of Poor Nutrient Management

If eutrophic nutrients in stormwater runoff cannot be adequately controlled, there are several environmental and even human health consequences that can result. In the Mid-Atlantic region, the Chesapeake Bay Watershed encompasses a large proportion of six US states. This area contains streams, rivers, and tributaries that all funnel runoff from 165,000 square kilometers directly into the Chesapeake Bay (Beegle, 2013). When nutrients such as N and especially P run off into the watershed, they can contribute to altering the environment in unsustainable and harmful ways (Correl, 1998).

Eutrophic pollution is most well-known for causing algae blooms which can cause ecological ramifications for the Chesapeake. Eventually, these algae blooms die off and their decomposition rids the water of dissolved oxygen (Boesch, 2001). These “dead zones” kill off fish and other aquatic life which disrupts/destroys the existing ecosystems and disrupts the lives of the people whose livelihoods depend on the Chesapeake Bay.

The greatest single source of nutrient pollution is from agricultural runoff contributing 40% of nitrogen and 50% phosphorus that flows into the Bay. Wastewater and urban runoff contributes the rest of the proportion of nitrogen and phosphorus (Chesapeake Bay Foundation, 2020). This study seeks to lessen the impact of a niche but growing sector of urban agriculture, by reducing the levels of nutrients present in green roof runoff. Through the incorporation of amendments, this study's objective is to evaluate different substrate mixes with these compounds and evaluate their nutrient retention properties and quantify their effects on crop production.

1.5 Novel Green Roof Amendments

1.5.1 Alumina

Alumina (aluminum oxide) is a byproduct of the aluminum smelting industry with a chemical formula Al_2O_3 . It has been the subject of a large amount of interest due to its application in wastewater treatment plants in removing phosphate compounds from polluted waters (Ramasahayam et al., 2014). Alumina exhibits low solubility, but under the proper acidified conditions, aluminum ions can disassociate from the oxide and preferentially bond with other anions in solution, such as phosphate (PO_4^{3-}) forming aluminum phosphate (AlPO_4). Aluminum phosphate is insoluble in water and precipitates out of solution with the captured phosphate. The aluminum phosphate can be recovered and processed to remove the phosphate under basic pH conditions.

There have been several horticultural applications of alumina in container and nursery plant studies. Phosphorus-charged alumina was applied to woody and

flowering herbaceous plants in containers when first potted in commercial grower settings with plants exhibiting fewer symptoms of phosphorous deficiencies later in production than plants which were given a single dose of Osmocote at planting (Yuan-Ji et al., 2002, Lin et al., 1996). These studies indicate that saturated alumina has the ability to slowly desorb phosphorous back into the substrate solution and available to container-grown plants. One of the principle tasks of this research was to reduce the concentration of P in leachates, while still providing enough soluble P in the soil solution to sustain plant growth.

1.5.2 Biochar

Biochar is produced from burning high cellulose material in a low oxygen environment. Biochar has been shown to increase soil cation exchange capacity (CEC) (Liang et al., 2006). At higher temperatures of pyrolysis, biochar can be used as an effective sink for nitrogen through the absorption of ammonia and ammonium ions which starve the nitrification process of valuable ammonium feedstock and inhibits the production of nitrate in the soil profile (Clough, 2013).

When biochar had been added to temperate zone agricultural soils amended with swine manure, the total amounts of N and P decreased in soil leachates compared to soils that were not amended with biochar. This is especially significant as biochar itself contains high amounts of native nitrogen and phosphorous (Laird et al., 2009). In addition to native N and P, the specific nutrient content of biochar is dependent on the source material, which can include hardwood and softwood waste, peanut shells, rice hulls, straw and other crop residues, or sewage (Figueredo et al., 2017). Green roof substrates that were amended with 7% biochar have been shown

to have increased water holding capacity. This amount of biochar was also shown to reduce the amount of nitrogen, phosphorus, and organic carbon leached from amended substrates (Beck et al. 2011). This study also tested the efficacy of biochar added to green roof substrates for increasing soil fertility and managing water quality of the leachates.

1.6 Research Objectives

The main objectives of this study were to develop a component blend using biochar and/or alumina in a green roof substrate that retains $\text{NO}_3\text{-N}$ and dissolved P, demonstrates adequate stormwater retention, and support profitable agricultural production. A key aspect of achieving these goals was the accurate quantification of nutrient content in leachate and harvested plant tissues. This research included two sequential studies, including a column study and a series of crop growth studies. The objective of the first column study was to rapidly screen a number of biochar and alumina amended substrate mixes, comprised of a commercial green roof substrate amended with two sources of organic matter: mushroom and composted yard waste and quantify their nutrient retention properties. The information from this column study was used to choose four substrates to study further in crop growth studies. The objectives of the crop growth studies was to evaluate the four substrates chosen from the column study for long term nutrient leaching performance, quantify the effects of the two amendments on crop growth, and establish a nutrient budget for these substrates for nitrogen and phosphorus.

By accomplishing these research objectives, these studies provided information on a modified green roof substrates' nutrient retention performance

which can be used to inform owner/operators of agricultural green roof operations who are concerned about the release of nutrients into the environment. By using substrates with novel amendments that increase nutrient retention performance, urban farmers can lessen their impact on the environment and contribute to lowering urban areas' nutrient pollution.

Chapter 2: Column Studies

2.1 Introduction

The objective of this preliminary study was to rapidly screen small batches of a number of green roof substrate mixes to understand how different substrate components affected nitrogen (N) and phosphorus (P) leaching and retention. This preliminary data was used to inform decisions on what substrate components and mixes should be chosen for longer-term plant growth studies (Chapter 3).

With a large number of potential substrate mixes that could be chosen, a method to rapidly test and analyze the nutrient retention properties of each substrate was essential. Ideally, each substrate could be studied with crop grown studies, but due to the limited number of replicated facilities to do this, this column study was used to initially quantify the leaching of nitrogen and phosphorus from 15 substrate combinations. The individual components were a commercial green roof expanded shale substrate (M2), SmartLeaf municipal compost, spent mushroom production compost, biochar, and alumina. The hypotheses being tested in the column study were to quantify the native nitrogen from nitrate (NO_3^- -N) and dissolved elemental phosphorus (dissolved-P) in each compost source and quantify the effect of biochar and/or alumina additions on reducing the NO_3^- -N and dissolved-P leached from each of the substrate mixes with successive applications of water to the columns, simulating rainfall effects. The formal hypotheses tested in the column study were:

- H1 Alternate- The presence of biochar in a substrate will reduce the amount of nitrogen from nitrate leached from the substrate profile with sequential leaching.

- H1 Null- The presence of biochar in a substrate will have no effect on the amount of nitrogen from nitrate leached from the substrate profile with sequential leaching.
- H2 Alternate- The presence of alumina in a substrate will reduce the amount of nitrogen from nitrate leached from the substrate profile with sequential leaching.
- H2 Null- The presence of alumina in a substrate will have no effect on the amount of nitrogen from nitrate leached from the substrate profile with sequential leaching.
- H3 Alternate- The presence of biochar in a substrate will reduce the amount of dissolved phosphorus leached from the substrate profile with sequential leaching.
- H3 Null- The presence of biochar in a substrate will have no effect on the amount of dissolved phosphorus leached from the substrate profile with sequential leaching.
- H4 Alternate- The presence of alumina in a substrate will reduce the amount of dissolved phosphorus leached from the substrate profile with sequential leaching.
- H4 Null- The presence of alumina in a substrate will have no effect on the amount of dissolved phosphorus leached from the substrate profile with sequential leaching.

2.2 Materials and Methods:

2.2.1 Column Study Substrate Formulation:

A total of 15 substrate combinations were mixed and prepared for the column studies (Table 2.1), further described below. These substrate components and mixes were chosen based on their availability and frequent use in green roof installations. All substrate materials, except for the biochar, were acquired locally which we defined as sourced within 200 miles of study location (College Park, MD). The primary mineral component of each mix, M2, is a widely available commercial green roof substrate made from expanded shale manufactured by Stancills, Inc. in Perryville, MD. Two types of M2 were combined for use in the substrate mixes: (1) A washed version of M2 which was devoid of most fine substrate particulates and (2) an unwashed version which contained the fine particulates to create a blended M2 mix, hereafter as the “M2 blend.”

SmartLeaf compost is a municipal compost produced by the Public Works Department, (City of College Park, College Park, MD) from leaf collection in the fall. This compost was used as a representative material available in many large mid-Atlantic cities that have their own municipal leaf composting operations. This compost was used in half of the substrate blends. Mushroom compost (Laurel Valley Soils; Avondale, PA) is another highly available component used in green roof media due to its high microbial activity and high nutrient content. Many green roof substrates contain limited (<5% by volume) organic matter, and the high microbial activity along with a high native nutrient content is an attractive organic component. This compost was used in the other half of the substrate mixes.

The two composts contained different amount of nutrients to place differing loads upon the column tests. The mushroom compost contained almost three times as much total nitrogen and almost four times as much phosphate compared the SmartLeaf compost. Mushroom compost was also significantly higher in sodium, aluminum, zinc, sulfur and copper than SmartLeaf compost. The SmartLeaf compost was significantly higher in Iron, calcium, and boron. Significantly more nutrients (some as much as 10 times more) were tested to be available the first year in the mushroom compost versus the SmartLeaf compost. The mushroom compost in these experiments represents an extremely high nutrient risk to give the amendments the best opportunity to produce significant results in nutrient retention. The nutritional analyses for both composts are available in Appendix A.12.

The biochar amendment used was provided by WakeField Biochar (Columbia, MO). This is the only substrate component that was sourced outside the local area due to availability and pricing. The biochar used was a superfine, powdered bagged biochar product designed to be a flowable solid. The primary feedstock of this biochar is pine wood chips sourced from lumber processing and pyrolyzed at 600 degrees Celsius. Alumina, the other amendment used was provided by Phospholutions Inc. (State College, PA). This material is granulated aluminum oxide with a grain size approximately that of coarse sand (0.4-1.0 mm in size). Due to the presence of fine particles, respiratory protection had to be used while working with dry biochar and alumina products.

A small electric cement mixer was used to combine all of the substrate materials for each formulation (see detailed procedure; Appendix A.1). A plastic

3.7L (1 gallon) bucket was used as the measuring standard for the mixing of each substrate over the entire study. Each substrate preparation yielded approximately 43L (approximately 11 gallons) of substrate which were all stored in sealed plastic 19L (5-gallon) buckets in an indoor air-conditioned, unrefrigerated room.

Table 2.1. A list of the fifteen substrate formulations tested. Each formulation is associated with a substrate number. The formulations for each substrate are expressed in percent, by volume. M2 abbreviates the blend of washed and unwashed M2 substrate.

Substrate #	Substrate Composition, by Volume
1	100% M2
2	80% M2 + 20% SmartLeaf Compost
3	80% M2 + 20% Mushroom Compost
4	75% M2 + 20% SmartLeaf + 5% Alumina
5	75% M2 + 20% Mushroom + 5% Alumina
6	70% M2 + 20% Smart Leaf + 10% Alumina
7	70% M2 + 20% Mushroom + 10% Alumina
8	70% M2 + 20% SmartLeaf + 10% Biochar
9	70% M2 + 20% Mushroom + 10% Biochar
10	60% M2 + 20% SmartLeaf + 20% Biochar
11	60% M2 + 20% Mushroom + 20% Biochar
12	65% M2 + 20% SmartLeaf + 10% Biochar + 5% Alumina
13	65% M2 + 20% Mushroom + 10% Biochar + 5% Alumina
14	55% M2 + 20% SmartLeaf + 20% Biochar + 5% Alumina
15	55% M2 + 20% Mushroom + 20% Biochar + 5% Alumina

2.2.2 Column Materials and Construction

The column set up for each substrate consisted of six replicate columns (Fig. 2.1). Each of these columns were constructed from a plastic 130mm Buchner Funnel

(Fisher Scientific, Pittsburg, PA) that had its' height extended by a 200mm section of 127mm (5 inch) diameter clear PVC pipe. This extension allowed the funnels to hold a 145mm depth of each substrate (approximately 5.75 inches) with 55mm of headspace for irrigation. The total volume of substrate within the column was 1500 mL.

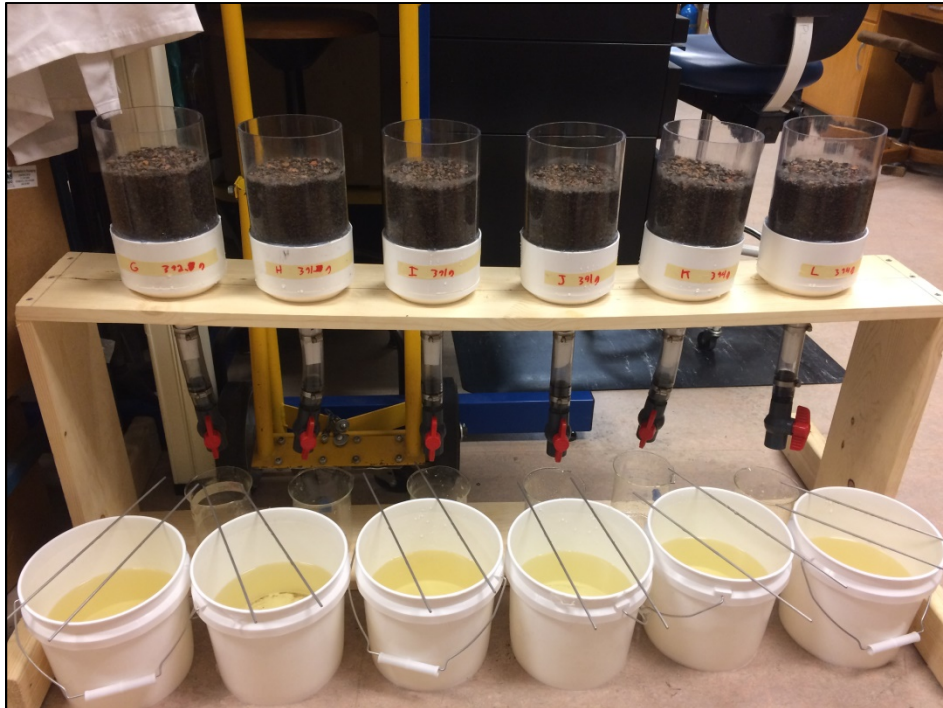


Fig. 2.1 Six replicate columns for each substrate mix to simulate a typical intensive green roof substrate profile for the column study. These columns were filled to a depth of 145mm (1500 mL volume).

A 0.45 micron Whatman glass fiber filter (GE Healthcare, Bensalem, PA) was placed on the bottom of the column on top of the Buchner Funnel perforations to retain the fine particles of substrate from the collected leachate. A clear vinyl tube was attached the bottom outlet of each funnel and a ball valve was attached to the opposite end of the tube. Six columns were mounted on a wooden rack made from 25mm x 200mm (1 inch by 8 inch) common lumber (Fig. 2.1). An even bulk density

of each column was achieved through filling a column with 1500 mL of substrate and taping it on the table 5 times to settle the substrate. Any loss in soil volume was topped up and gently tapped again to 1500 mL.

Six separate 3.8L (1-gallon) buckets were used to initially saturate each column with 2500 mL deionized (DI) water. Six 500mL beakers were used to catch the leachate during each leachate cycle (detailed below). A standard 3.7L (1 gallon) plastic watering can with shower nozzle was used to perform each leaching event, to ensure that the DI water was spread over the entire exposed surface of the substrate in the column, and minimize disturbance of the substrate surface.

2.2.3 Column Testing and Sample Collection

As stated, the six replicate columns were saturated overnight in 2500 mL DI water in buckets, to achieve field capacity of the substrate in the columns. Columns were drained the next day, and the leachate collected in the same bucket. Two, 20mL plastic scintillation vials were used to collect, store, and freeze replicate leachate samples for later analysis. These vials were labeled and stored at -10 degrees Celsius in a Frigidaire upright freezer (Model # FFFH20F2QWE).

After draining from saturation, the columns were placed back onto the funnels in the wooden rack. 290 mL of DI water was applied to the surface of each column with the watering can, equivalent to 25mm of rainfall. The columns were allowed to stand as the water drained out into beakers situated below the columns for at least 30 minutes, or when the columns stopped draining water. The volume of the leachate was recorded and again, two 20mL replicate samples were collected, labeled, and

frozen for later analysis. This procedure was repeated seven times, for a total of eight 25 mm simulated rainfall events.



Fig. 2.2. Funnels with columns removed and draining into the saturation buckets after an overnight soak in DI water. These columns are now ready for simulated rainfall washings.

After these first eight leaching events, 100 mL of $100 \text{ mgL}^{-1} \text{ N}$, $20 \text{ mgL}^{-1} \text{ P}_2\text{O}_5$ fertilizer solution was added to each column and allowed to stand for 24 hours. This fertilizer solution was made from ammonium nitrate, potassium phosphate, and DI water. This solution was prepared in bulk and stored in a 19L (5-gallon) sealed bucket. After this nutrient recharge, another series of eight, 25mm simulated rainfall applications were applied to each column. Each replicate leachate was collected, the volume recorded, labeled, and frozen to await nutrient analysis, as previously described.

2.2.4 Nitrate Analysis:

All nitrogen (NO_3^- -N) analyses were performed on site in the Research Greenhouse Complex Bioremediation Laboratory. Due to its relative stability in leachate water, nitrate-N (NO_3^- -N) was measured to express the N contents (i.e. N concentration x sample volume in mL) in each leachate sample. The machine used for NO_3^- determination was a HANNA Instruments IRIS HI801 spectrophotometer. Determination of NO_3^- -N was performed by colorimetry using a HANNA IRIS HI801 spectrophotometer (HANNA Instruments, Woonsocket, Rhode Island, USA). The absorbance of the sample was analyzed colorimetrically at 410 nm, which was then related to the concentration of nitrate within a sample with a standard curve. Reagents were purchased from Hanna Instruments as a kit of premeasured and premixed reagent vials (Nitrate Kit HI93766-50). Each kit of 50 sample vials/reagents came with a QA certificate indicating that the batch of reagents were accurate to within $1.0 \text{ mgL}^{-1} \text{ NO}_3^-$ -N. A more detailed procedure on the operation and sample preparation using the HANNA Spectrophotometer is provided in Appendices A.2 and A.3

The IRIS HI801 spectrophotometer using the HI93766-50 nitrate kits can measure the concentration of NO_3^- -N from 0.0 mgL^{-1} to 30.0 mgL^{-1} with a resolution of 0.1 mgL^{-1} N. While the machine runs through a self-calibration procedure every time it was turned on using internal filters, a separate calibration curve was established in order to ensure the accuracy of the final data set. This calibration test was performed using a solution of potassium nitrate and DI water to create serial dilutions. The regression curve formula was then applied to determine the exact

value for each sample. The procedure for this calibration curve is detailed in Appendix A.4.

In the event that a sample contained more than 30.0 mgL^{-1} of NO_3^- -N, a serial dilution was performed. A 10x dilution was the most common which increased the range from 0 mgL^{-1} to 300 mgL^{-1} NO_3^- -N. This dilution reduced the accuracy of the spectrophotometer to within 10 mgL^{-1} NO_3^- -N and decreased the resolution to 1 mgL^{-1} NO_3^- -N. In a few extreme cases, an additional 10x dilution had to be performed for samples containing over 300 mgL^{-1} NO_3^- -N using the same materials and procedure.

2.2.5 Dissolved Phosphorus Analysis:

Analysis of dissolved elemental phosphorus (dissolved-P) for the column studies was not performed on-site, due to interference of dissolved organic compounds (such as tannins) present in many of the samples. These organic compounds interfered with the spectrophotometer absorbance. Samples for phosphorus analysis were therefore analyzed by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP) by a commercial laboratory (AgroLab Inc., Harrington, DE). The detailed procedure of sample preparation for AgroLab Inc. is provided in Appendix A.5.

2.2.6 Substrate Chemical and Physical Property Analyses

Several chemical properties of each substrate were measured, relevant to their potential use on green roofs. Active and exchangeable acidity were measured for each substrate, to analyze whether the biochar and alumina amendments affected pH. Changes in pH could possibly have an effect on the ability for phosphates to react

with the amendments in the substrate and affect their availability. In brief, after calibrating a pH meter, five oven-dried 20g samples of each substrate were tested for active acidity. A slurry was made with the substrate, 50 mL DI water and the pH was measured after mixing for 20 minutes. Exchangeable acidity was measured by creating a slurry with each substrate using a 1M KCl solution and measuring the pH after mixing for 20 minutes.

The basic physical properties of each substrate formulation were analyzed, including water holding capacity (WHC), air filled porosity (AFP), total porosity, and bulk density (both wet and dry). These tests were performed using ~500 mL plastic jars with a tight fitting, wide mouth, screw-on lid. Testing also required a pan of adequate volume to hold enough water to cover the plastic jars when submerged fully in water. Foil pans were used to dry the substrate in an oven. The physical property analysis uses simple water displacement to measure WHC, AFP and total porosity. The detailed procedure for these displacement tests is provided in Appendix A.8.

2.3 Column Study Results

2.3.1 Nitrate Results of Column Studies

The average NO_3^- -N leached was significantly higher from two compost sources than from the inorganic (M2 Blend, control) substrate (Fig. 2.3). The NO_3^- -N load was normalized in kilograms of nitrogen per hectare and was calculated from the concentration (mg/L) of NO_3^- -N present in the leachate and the volume of the leachate that was collected from each simulated rainfall event (leaching application = 25 mm), with the exception of the very first leachate event, which was collected from the initial saturation buckets. Leaching applications 1-9 were performed on the native

substrate plus any amendments. Point FA (Fig. 2.3) denotes when 100 mL of 100 ppm nitrogen, 20 ppm P₂O₅ nutrient solution was added to re-charge each replicate column. The second set of leaching applications 1-7 were performed after this simulated fertilization event. These results show that nearly 3 times the amount of NO₃⁻-N was leached from the unamended mushroom compost blend than from the SmartLeaf compost over the first 125 mm simulated rainfall. However, after 175 mm (7 inches rainfall), the amount of NO₃⁻-N began to converge to the low detectable amounts expressed from the unamended SmartLeaf compost substrate.

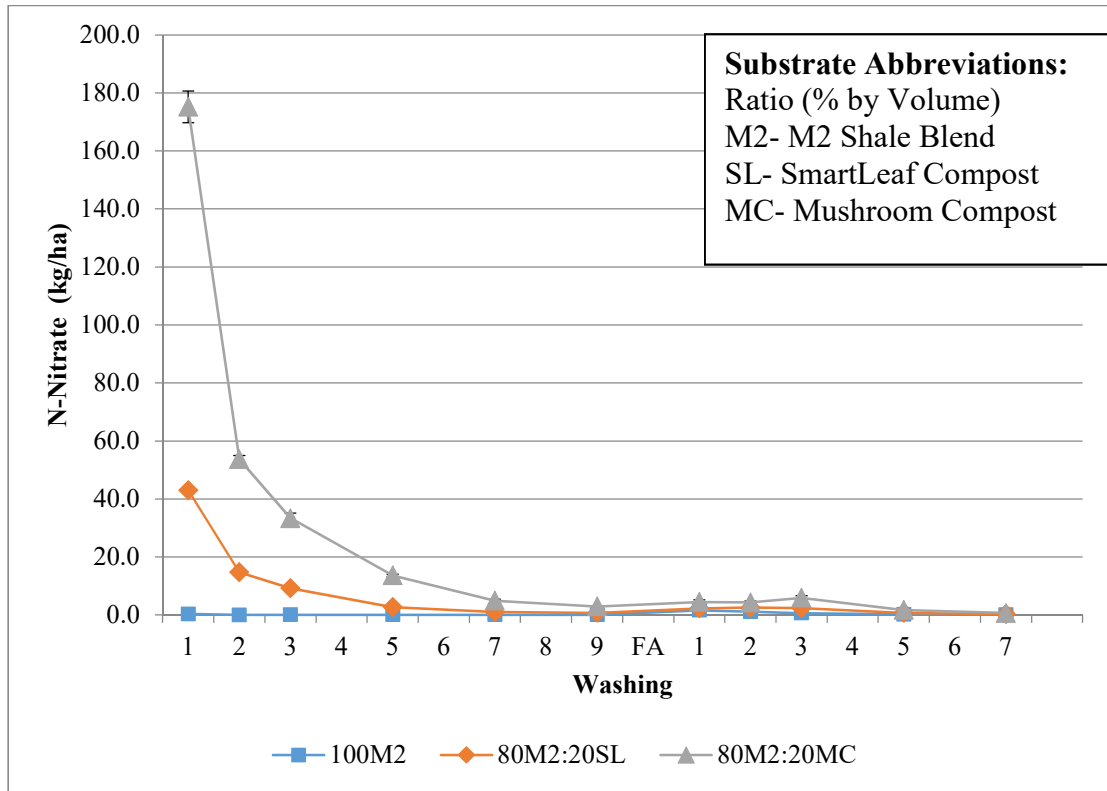


Fig. 2.3. NO₃⁻-N leachate (kilograms per hectare) from the leaching cycles of the column study for each unamended substrate. Leaching applications (LA) 2 through 9 represent 25mm of simulated rainfall applied to substrates. Leaching application 1 represents the sample from the initial saturation event. FA denotes the point where fertilizer was applied, and LA 1 through 7 represent simulated rainfall events 24 hours after fertilization. Error bars show standard error about the means.

Figure 2.4 shows the cumulative NO_3^- -N load from all simulated rainfall events. Substrates containing unamended mushroom compost leached significantly more NO_3^- -N (322 Kg NO_3^- -N / ha) than from the SmartLeaf compost (85 Kg NO_3^- -N / ha), with less than 5 Kg NO_3^- -N / ha being leached from the native M2 substrate.

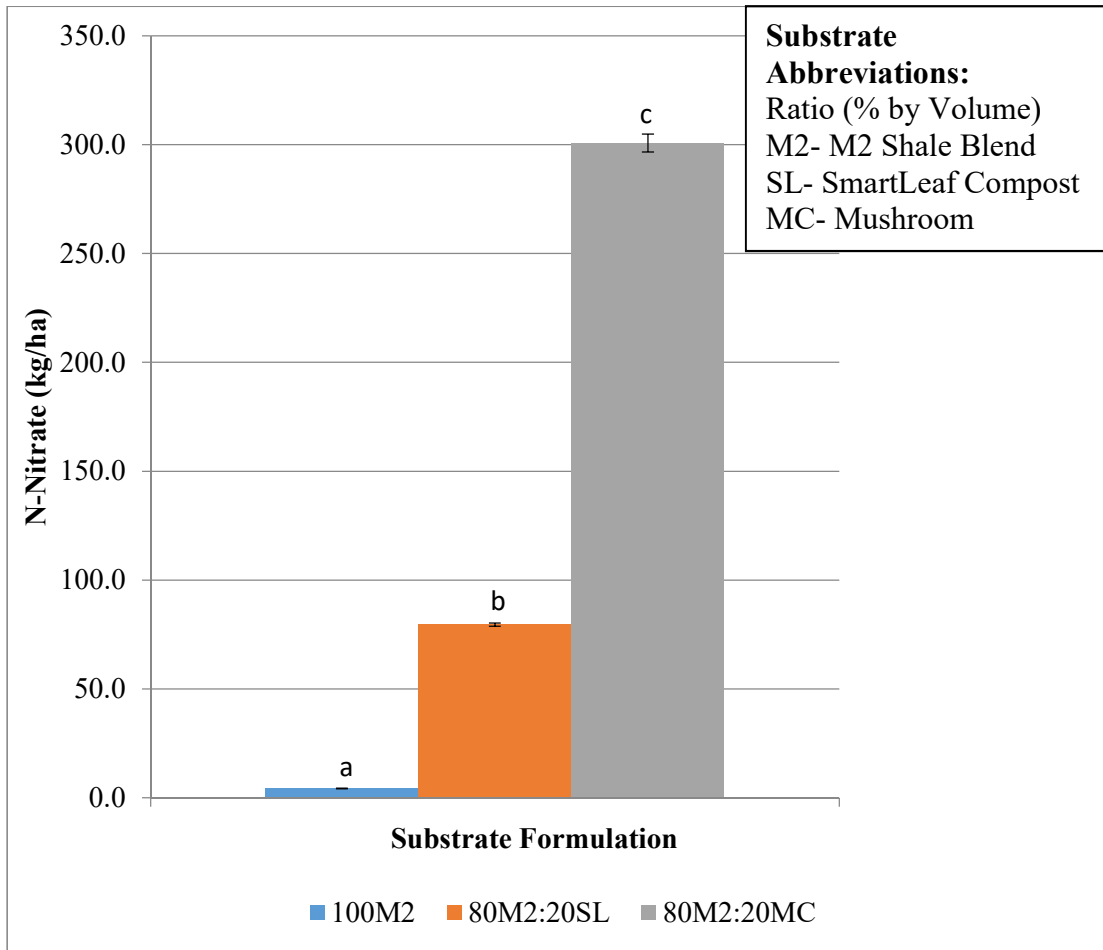


Fig. 2.4. Mean cumulative NO_3^- -N loads in kilograms per hectare over the column study for each unamended substrate. Letters upon bars indicate significance levels (Tukey's HSD $P < 0.05$). Error bars show standard error about the means.

Figures 2.5 and 2.6 illustrates the substrates which were amended with SmartLeaf compost. Similarly to unamended substrates, the NO_3^- -N leached from all SmartLeaf substrates was significantly reduced after 125 – 175mm simulated rainfall (Figure 2.5). The addition of fertilizer caused a small but insignificant increase in NO_3^- -N leached which was subsequently leached after an additional 125 mm

simulated rainfall. Figure 2.6 shows that while statistically significant differences ($p < 0.05$) were seen between SmartLeaf substrates in their cumulative NO_3^- -N loads, it was not consistently attributable to any of the amendments that were added. However, there was a trend to show that the addition of 10% and 20% biochar may have an effect on the amount of NO_3^- -N leached. This reduction was not seen in SmartLeaf substrates that were amended with alumina.

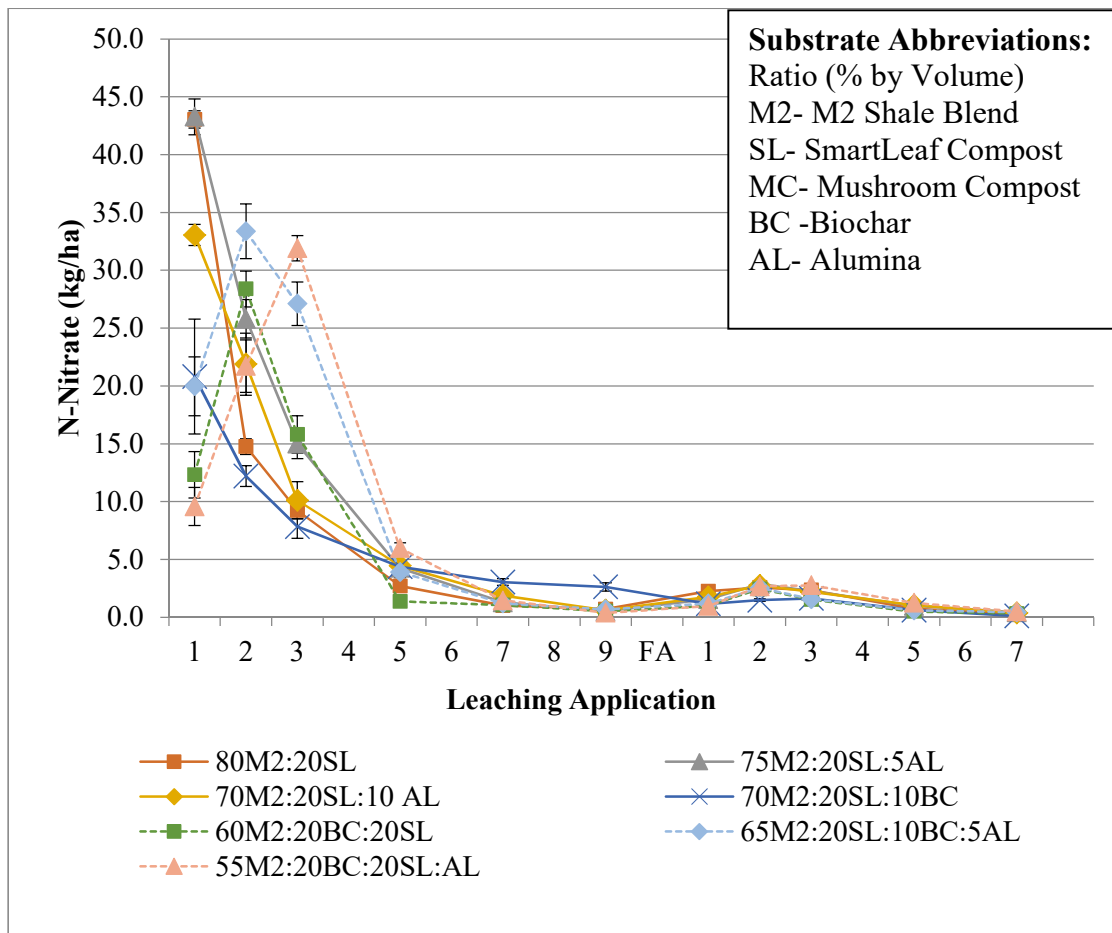


Fig. 2.5. Mean NO_3^- -N leachate (kilograms per hectare) from the leaching cycles of the column study for substrates containing SmartLeaf compost. Leaching applications (LA) 2 through 9 represent 25mm of simulated rainfall applied to substrates. Leaching application represents the sample from the initial saturation event. FA denotes the point where fertilizer was applied, and LA 1 through 7 represent simulated rainfall events 24 hours after fertilization. Error bars show standard error about the means.

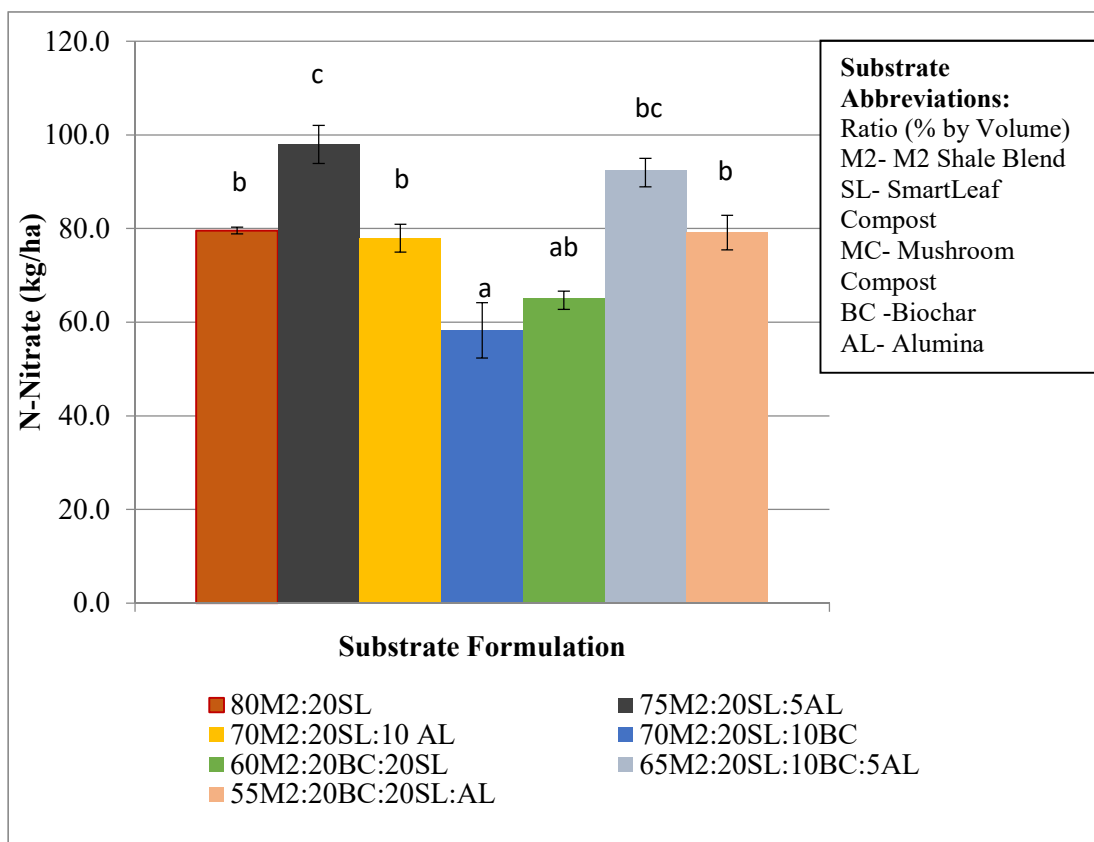


Fig. 2.6. Mean cumulative (total) $\text{NO}_3^- \text{N}$ leachate in kilograms per hectare for each substrate containing SmartLeaf compost. Letters upon bars indicate significance levels (Tukey's HSD $P < 0.05$). Error bars show standard error about the means.

Figures 2.7 and 2.8 illustrate the mean $\text{NO}_3^- \text{N}$ leached from all substrates that contained mushroom compost. Similarly to the results from the unamended and SmartLeaf substrates, the $\text{NO}_3^- \text{N}$ load converged after 5-7 leaching events (Figure 2.7). The addition of nitrogen fertilizer caused a small increase in $\text{NO}_3^- \text{N}$ that was subsequently leached out after 125mm additional simulated rainfall. Figure 2.8 shows that while statistically significant differences ($p < 0.05$) were seen between some of the mushroom compost substrates in cumulative $\text{NO}_3^- \text{N}$ load, it does not seem to be consistently attributable to any of the amendments that were added. However, the addition of alumina may increase the amount of $\text{NO}_3^- \text{N}$ leached, as the two substrates with highest loads were substrates containing alumina. All of the other

mushroom substrates did not show statistically significant differences in NO_3^- -N leached, regardless of amendment.

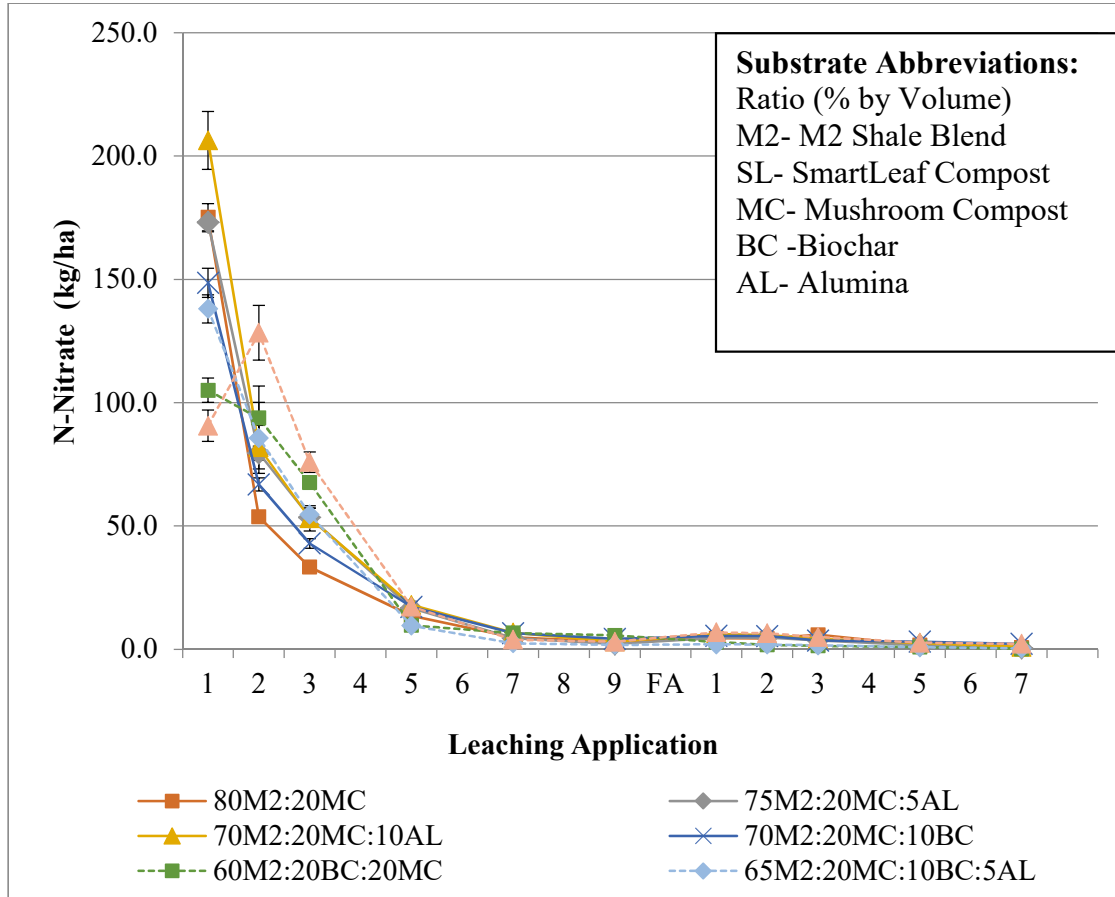


Fig. 2.7. Mean NO_3^- -N leachate (kilograms per hectare) from each leachate for substrates containing mushroom compost. Leaching applications (LA) 2 through 9 represent 25mm of simulated rainfall applied to substrates. Leaching application represents the sample from the initial saturation event. FA denotes the point where fertilizer was applied, and LA 1 through 7 represent simulated rainfall events 24 hours after fertilization. Error bars show standard error about the means.

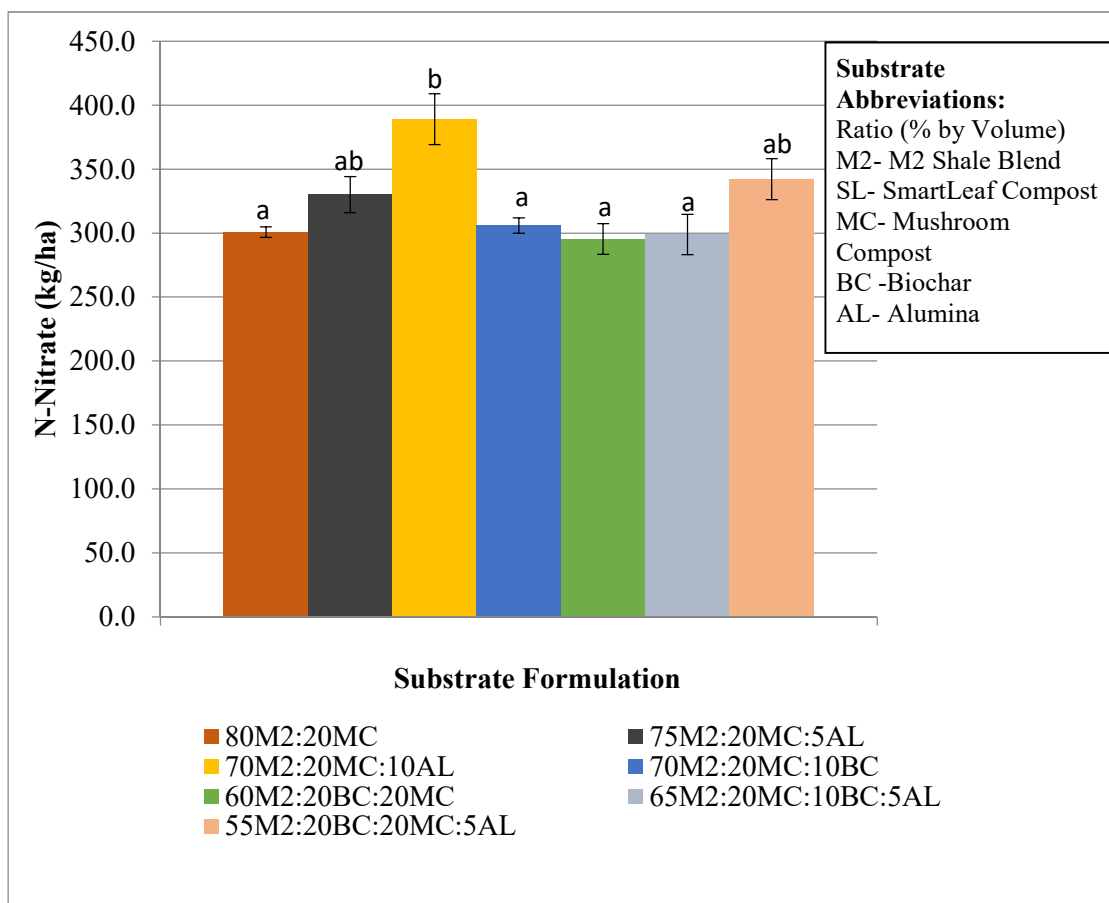


Fig. 2.8. Mean cumulative (total) NO_3^- -N leached in kilograms per hectare over the column study from each substrate containing mushroom compost. Letters upon bars indicate significance levels (Tukey's HSD $P < 0.05$). Error bars show standard errors about the means.

Figures 2.9 and 2.10 show the NO_3^- -N leached from all substrates containing either 10% or 20% biochar. Similar to previous results, the NO_3^- -N leached from all treatments was significantly reduced after 125 – 175mm simulated rainfall (Figure 2.9). The relative significant differences in initial NO_3^- -N leachate between compost source (mushroom vs, SmartLeaf) can be clearly seen, with mushroom compost leaching five to eight times the initial NO_3^- -N load compared to SmartLeaf. The addition of nitrogen fertilizer caused a small increase at point FA, a proportion of which was subsequently leached. Figure 2.10 shows that there were no statistically

significant differences ($p>0.05$) in cumulative NO_3^- -N load between the biochar containing substrates containing SmartLeaf compost, although the compost source clearly dominated overall NO_3^- -N load. Figure 2.10 may suggest that the presence of alumina increases the cumulative amount of NO_3^- -N leached over time.

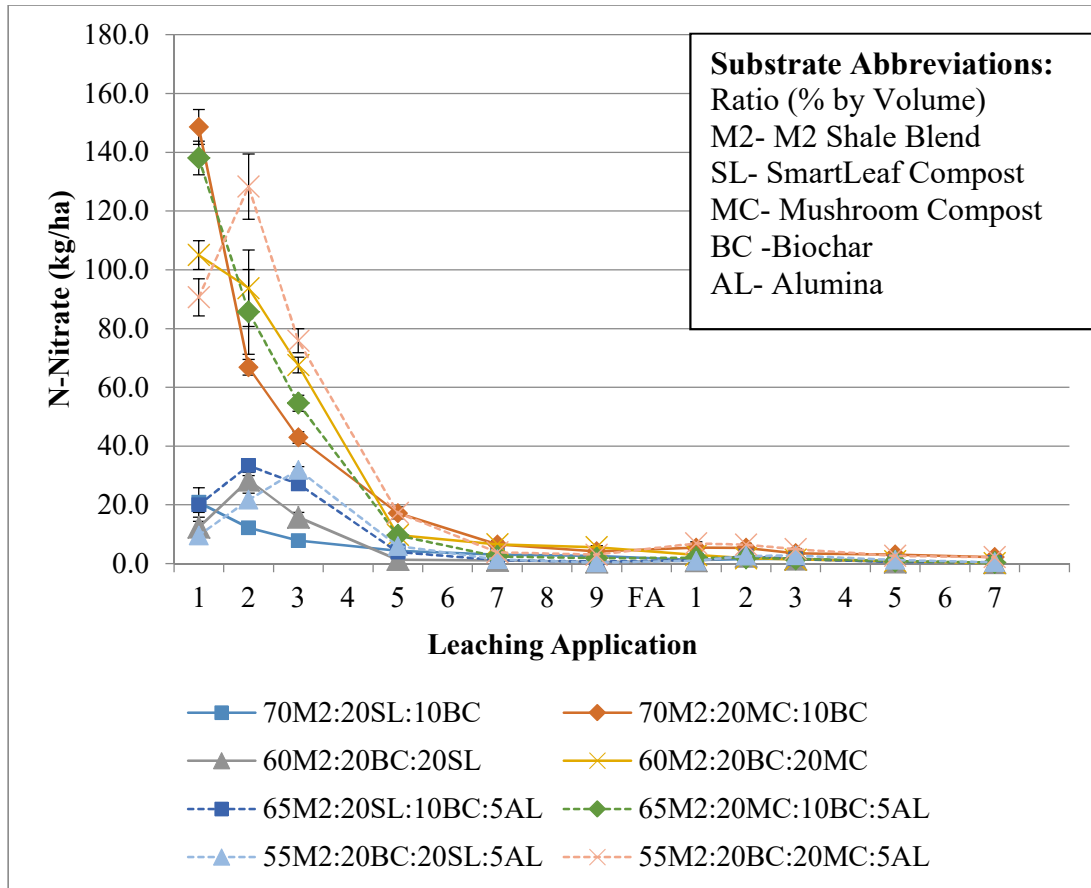


Fig. 2.9. Mean NO_3^- -N leachate (kilograms per hectare) from each leaching event for substrates containing biochar. Leaching applications (LA) 2 through 9 represent 25mm of simulated rainfall applied to substrates. Leaching application represents the sample from the initial saturation event. FA denotes the point where fertilizer was applied, and LA 1 through 7 represent simulated rainfall events 24 hours after fertilization. Error bars show standard errors about the means.

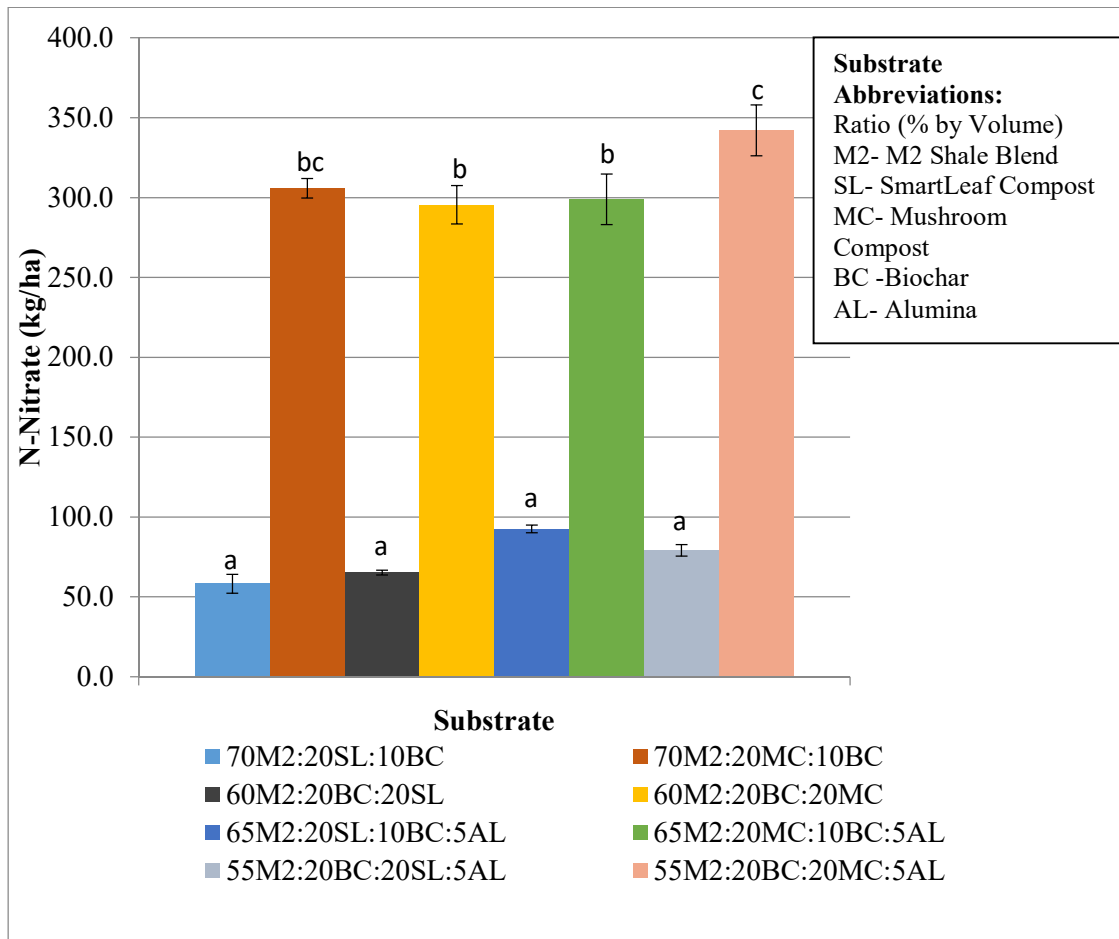


Fig. 2.10. Mean cumulative (total) NO_3^- -N loads in kilograms per hectare over the column study for each substrate containing biochar. Letters upon bars indicate significance levels (Tukey's HSD $P < 0.05$). Error bars show standard errors about the means.

In a similar fashion, Figures 2.11 and 2.12 illustrate how the incorporation of alumina at 5% or 10% initially affected NO_3^- -N leaching with simulated rainfall events. As with prior results, NO_3^- -N leaching was significantly reduced after 125 – 175mm simulated rainfall (Figure 2.11), even though the alumina-containing mushroom compost substrates had a significantly higher initial NO_3^- -N leachate than similar SmartLeaf-containing substrates. The addition of nitrogen fertilizer caused a small increase in NO_3^- -N, which subsequently leached after 75 – 125 mm simulated rainfall. Figure 2.12 shows that there were no statistically significant differences

($p > 0.05$) between the alumina substrates containing SmartLeaf compost. While there were statistically significant differences ($p < 0.05$) between alumina-containing substrates that contain mushroom compost, real differences may not be present. Interestingly, significantly lower NO_3^- -N loading was seen with the 5% compared to the 10% alumina addition in mushroom compost substrates; but this NO_3^- -N loading was not affected by biochar additions (i.e. no interactive effects were seen; Fig. 2.12).

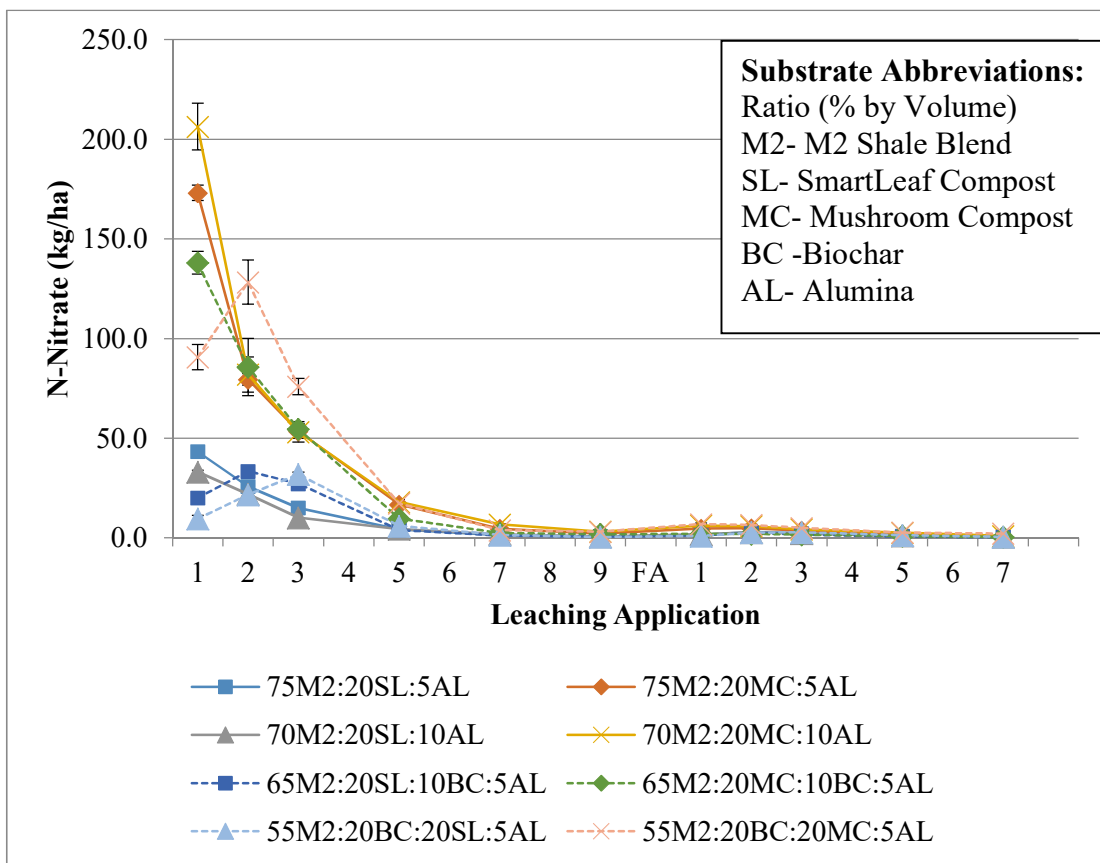


Fig. 2.11. Mean NO_3^- -N leachate (kilograms per hectare) from each leaching event for substrates containing alumina. Leaching applications (LA) 2 through 9 represent 25mm of simulated rainfall applied to substrates. Leaching application represents the sample from the initial saturation event. FA denotes the point where fertilizer was applied, and LA 1 through 7 represent simulated rainfall events 24 hours after fertilization. Error bars show standard errors about the means.

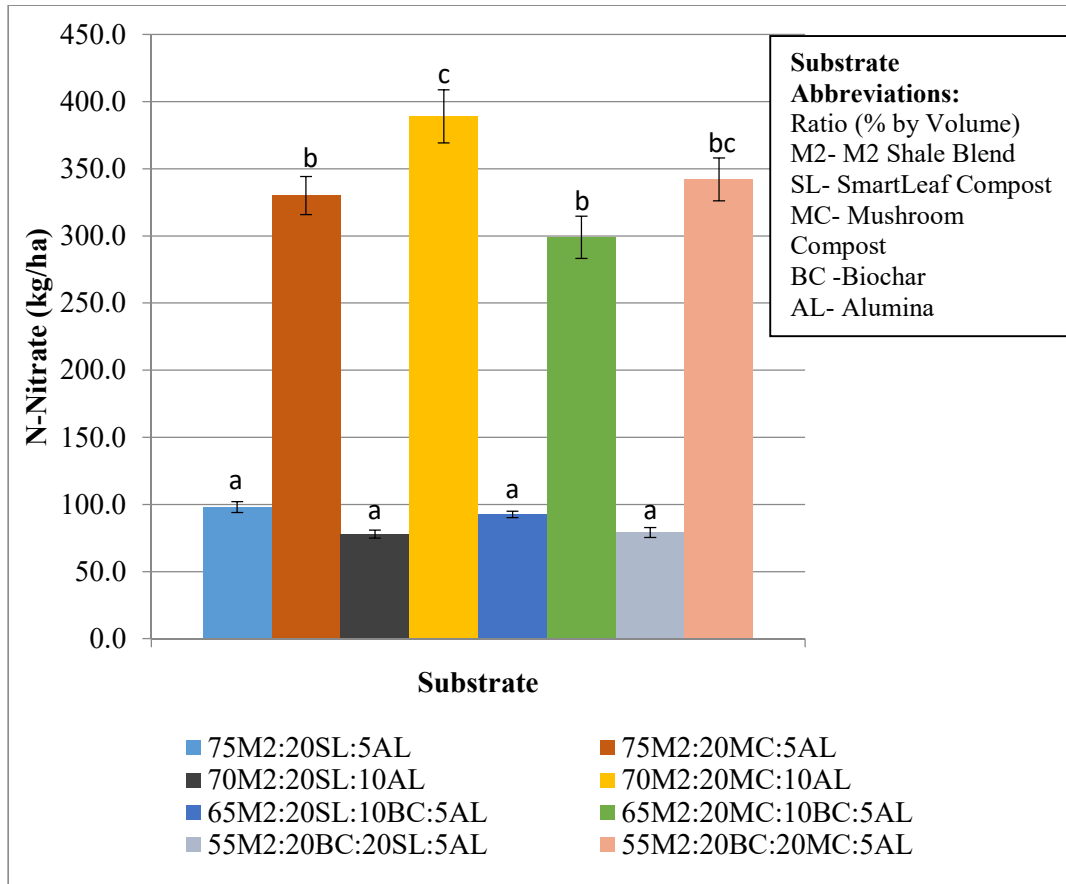


Fig. 2.12. Mean cumulative (total) NO_3^- -N loads in kilograms per hectare for each substrate containing alumina. Letters upon bars indicate significance levels (Tukey's HSD $P < 0.05$). Error bars show standard errors about the means.

2.3.2 Dissolved Phosphorus Results for Column Study

The results for dissolved phosphorus (dissolved-P) are again expressed in kilograms of dissolved-P per hectare, calculated from the concentration of dissolved elemental phosphorus (mg-P/L) multiplied by the volume of leachate collected for each sample and replicate. Figure 2.13 shows the unamended substrates leach significantly different amounts of dissolved-P leachate, and that these differences can be accounted for by the organic matter source. The M2 blend contained virtually no dissolved phosphorus.

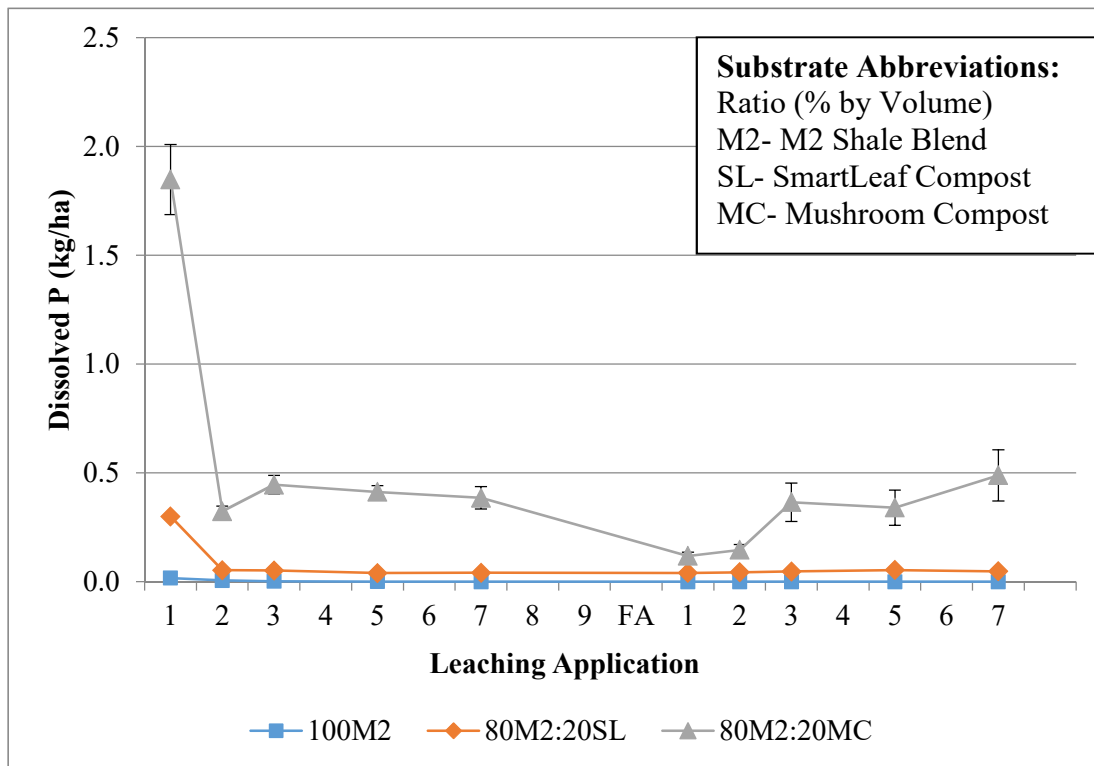


Fig. 2.13. Mean dissolved phosphorus (dissolved-P) leachate (kilograms per hectare) from unamended substrates from each leaching event during the column study. Leaching applications (LA) 2 through 9 represent 25mm of simulated rainfall applied to substrates. Leaching application represents the sample from the initial saturation event. FA denotes the point where fertilizer was applied, and LA 1 through 7 represent simulated rainfall events 24 hours after fertilization. Error bars show standard errors about the means.

Unlike the NO_3^- -N leachate results, the dissolved-P mushroom compost and SmartLeaf leachate P values did not converge over time. The mushroom compost substrate leached dissolved-P at a significantly slower and consistent rate, compared to its desorption of nitrate. Recharge with the fertilizer solution did increase dissolved-P leaching from the mushroom compost, but dissolved-P leachate levels remained almost entirely constant for the SmartLeaf substrate and were not affected by the fertilizer application. Figure 2.14 illustrate total dissolved-P loading results for each unamended substrate, with significant differences ($p < 0.05$) between organic

matter source, with mushroom compost leaching a total of 4.876 Kg P / Ha, compared to 0.72 and 0.02 Kg P / Ha for SmartLeaf and M2 blends, respectively.

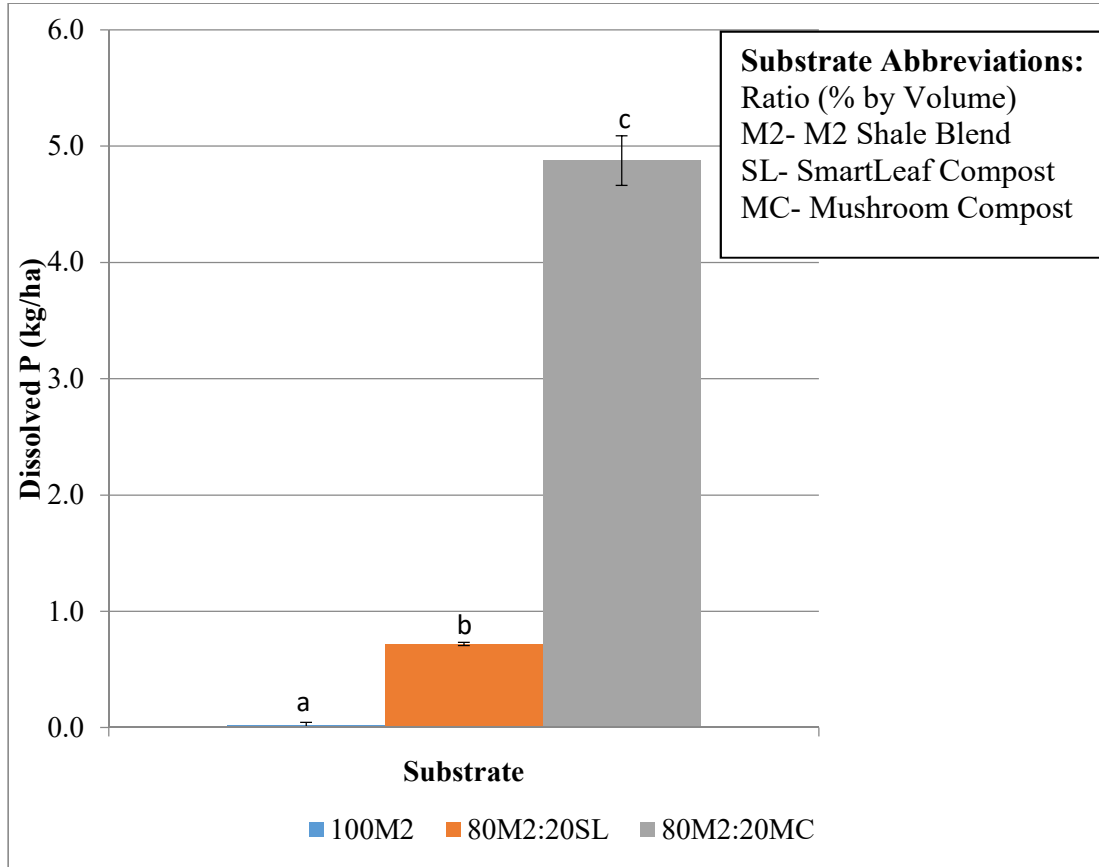


Fig. 2.14. Mean cumulative (total) dissolved phosphorus (dissolved-P) loads in kilograms per hectare over the column study for each unamended substrate. Letters upon bars indicate significance levels (Tukey’s HSD $P < 0.05$). Error bars show standard errors about the means.

Figures 2.15 and 2.16 shows all substrates containing SmartLeaf compost as the organic matter component. The highest leachate of dissolved-P came from substrates containing biochar only or were not amended (Fig. 2.15). The P leachates from SmartLeaf substrates with alumina were lower than from non-alumina amended substrates. These P loading amounts did not converge on one another and dissolved-P leachate amounts remain relatively constant throughout. There were instances

where the dissolved-P content in the leachate rose after fertilization in the SmartLeaf substrates that did not contain alumina. The amount of dissolved-P trended downwards for SmartLeaf substrates containing alumina after fertilization, reaching nearly undetectable levels towards the end of the second cycle of rainfall simulations.

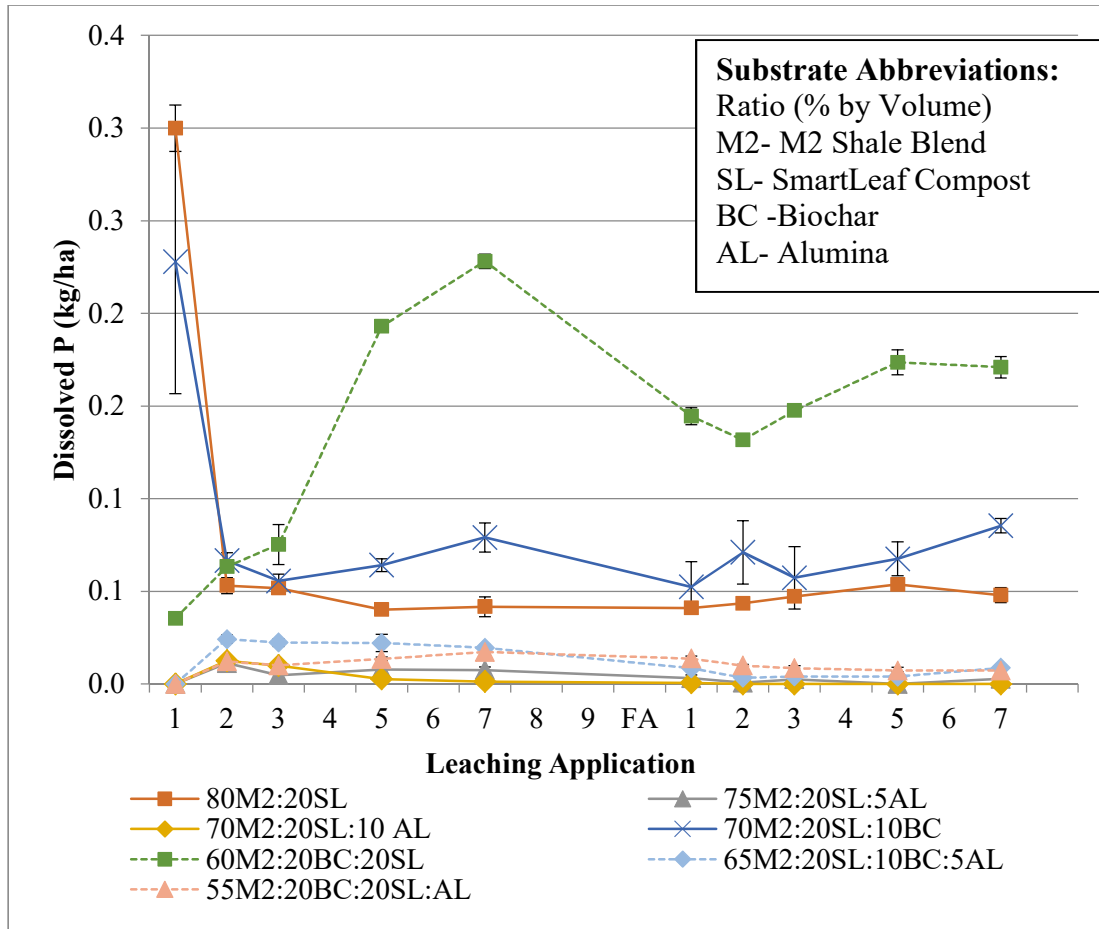


Fig. 2.15. Mean dissolved phosphorus (dissolved-P) leachate (kilograms per hectare) from each leaching event during the column study for substrates containing SmartLeaf compost. Leaching applications (LA) 2 through 9 represent 25mm of simulated rainfall applied to substrates. Leaching application represents the sample from the initial saturation event. FA denotes the point where fertilizer was applied, and LA 1 through 7 represent simulated rainfall events 24 hours after fertilization. Error bars show standard errors about the means.

Figure 2.16 shows the accumulated dissolved-P for each SmartLeaf compost containing substrate. There was a significant ($p < 0.05$) reduction in dissolved-P from all SmartLeaf substrates containing alumina relative to SmartLeaf substrates that did not contain alumina. There was no significant difference between the unamended SmartLeaf substrate and the SmartLeaf substrate containing only 10% biochar. There was a significant ($p < 0.05$) increase in dissolved-P in the SmartLeaf substrate that contained only 20% biochar.

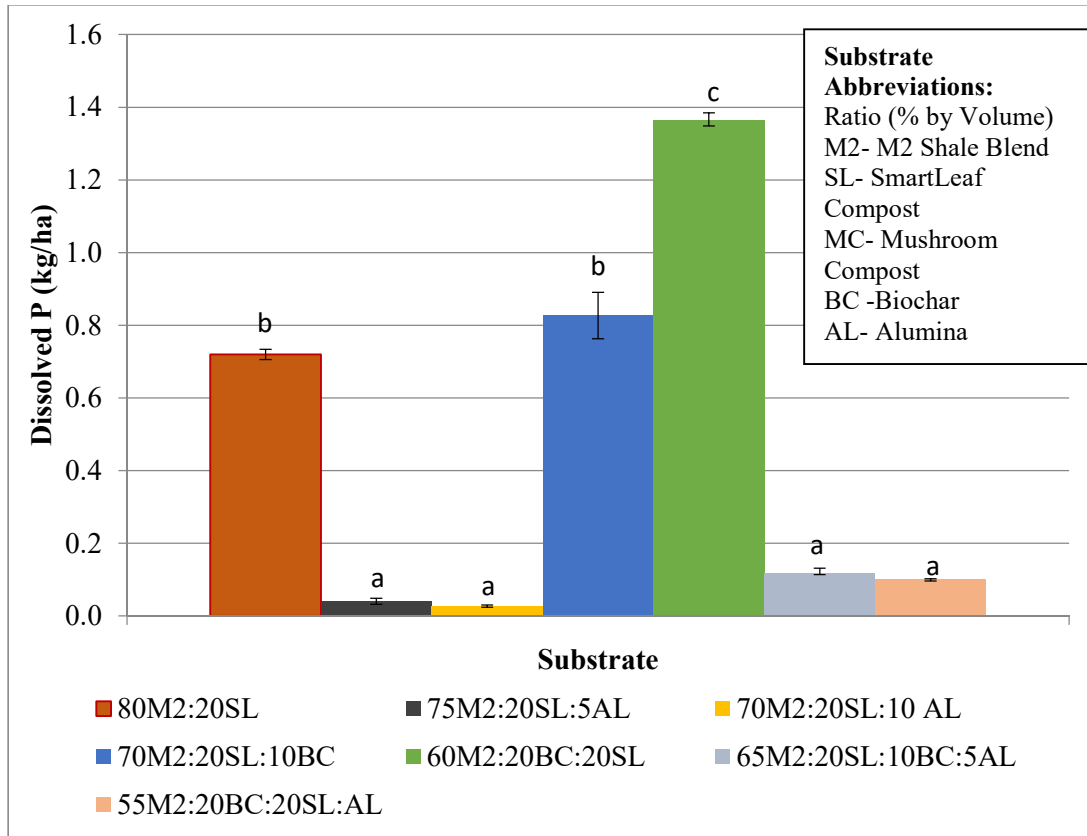


Fig. 2.16. Mean cumulative (total) dissolved phosphorus (dissolved-P) loads in kilograms per hectare over the column study for each substrate containing SmartLeaf compost. Letters upon bars indicate significance levels (Tukey's HSD $P < 0.05$). Error bars show standard errors about the means.

Figures 2.17 and 2.18 show all of the substrates with mushroom compost. In Figure 2.17 the amount of dissolved-P leached was from substrates containing only

biochar or were not amended. P leached from substrates containing alumina were lower than from substrates that did not contain alumina. Leachate totals did not converge on one another and dissolved-P leachate amounts remain relatively constant throughout the study. Dissolved-P content in the leachate rose after fertilization from substrates that did not contain alumina.

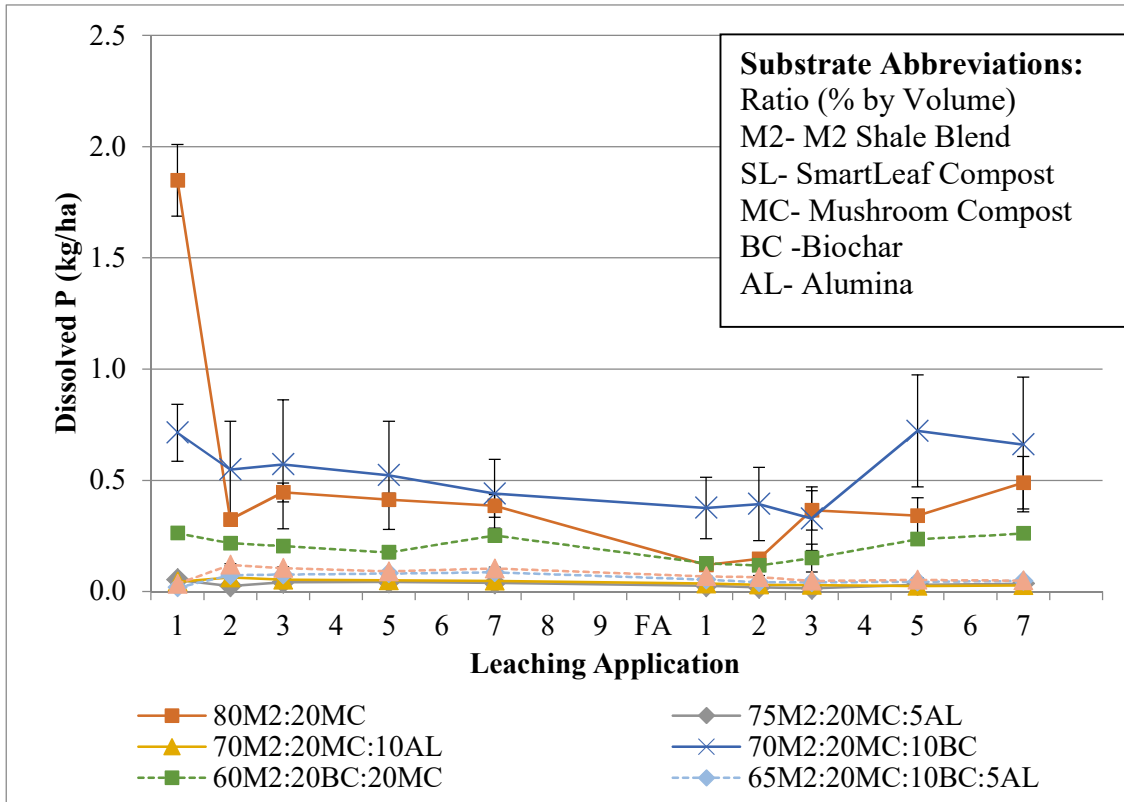


Fig. 2.17. Mean dissolved phosphorus (dissolved-P) leachate (kilograms per hectare) from each leaching event during the column study for substrates containing mushroom compost. Leaching applications (LA) 2 through 9 represent 25mm of simulated rainfall applied to substrates. Leaching application represents the sample from the initial saturation event. FA denotes the point where fertilizer was applied, and LA 1 through 7 represent simulated rainfall events 24 hours after fertilization. Error bars show standard error.

Figure 2.18 shows the accumulated dissolved-P for each mushroom compost substrate. There was a significant ($p < 0.05$) reduction in phosphorus from all

substrates containing alumina relative to those without alumina. The substrates with the highest dissolved-P contents were from substrate without alumina; the unamended mushroom substrate showed the highest phosphorus leaching. There were significant reductions ($p < 0.05$) in dissolved-P between the unamended mushroom compost substrate and the mushroom substrates containing biochar.

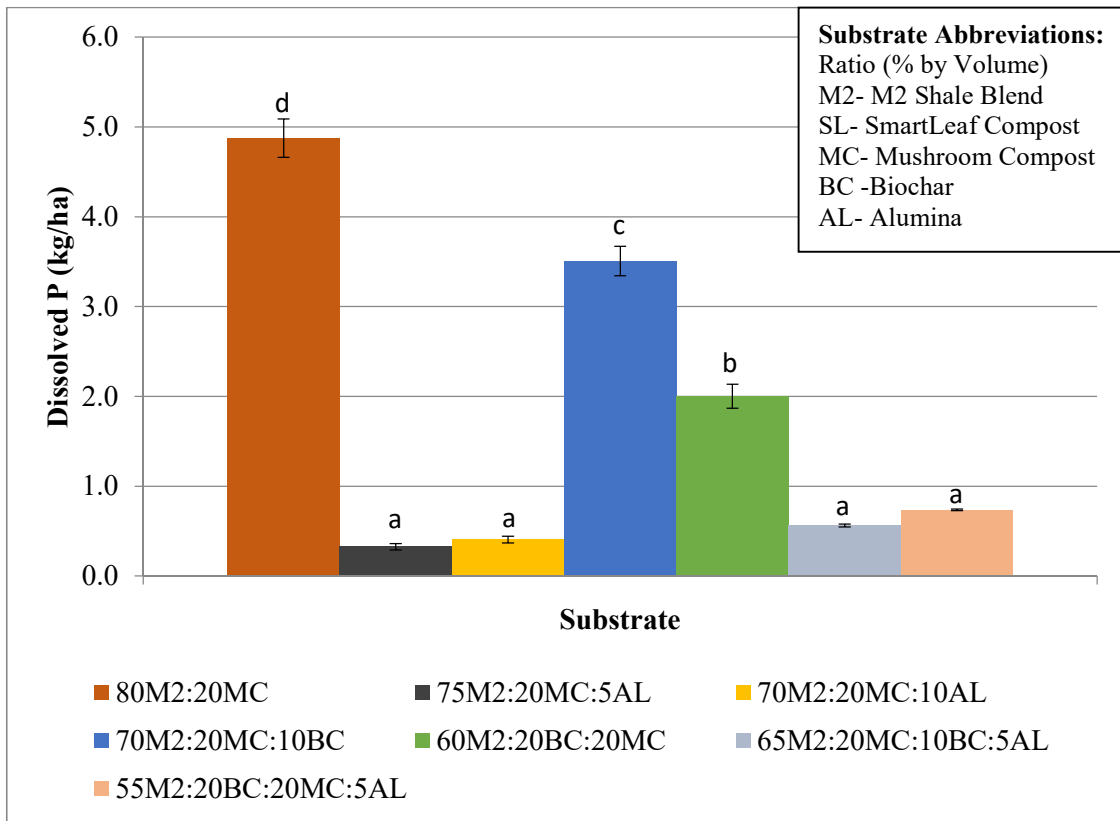


Fig. 2.18. Mean cumulative dissolved phosphorus (dissolved-P) loads (kilograms per hectare) over the column study for each substrate containing mushroom compost. Letters upon bars indicate significance levels (Tukey’s HSD $P < 0.05$). Error bars show standard errors about the means.

Figures 2.19 and 2.20 show all of the substrates containing biochar. In Figure 2.19 the amount of dissolved-P leached varied between each substrate with the highest leaching from biochar-only mushroom compost mixes. The P loading from biochar substrates containing alumina were lower than from those not containing

alumina. These leachate amounts did not converge and dissolved-P loads remained relatively constant throughout the study. Phosphorus content in the leachate rose after fertilization from biochar substrates that did not contain alumina. The amount of phosphorus trended slightly upward for biochar substrates containing alumina after fertilization.

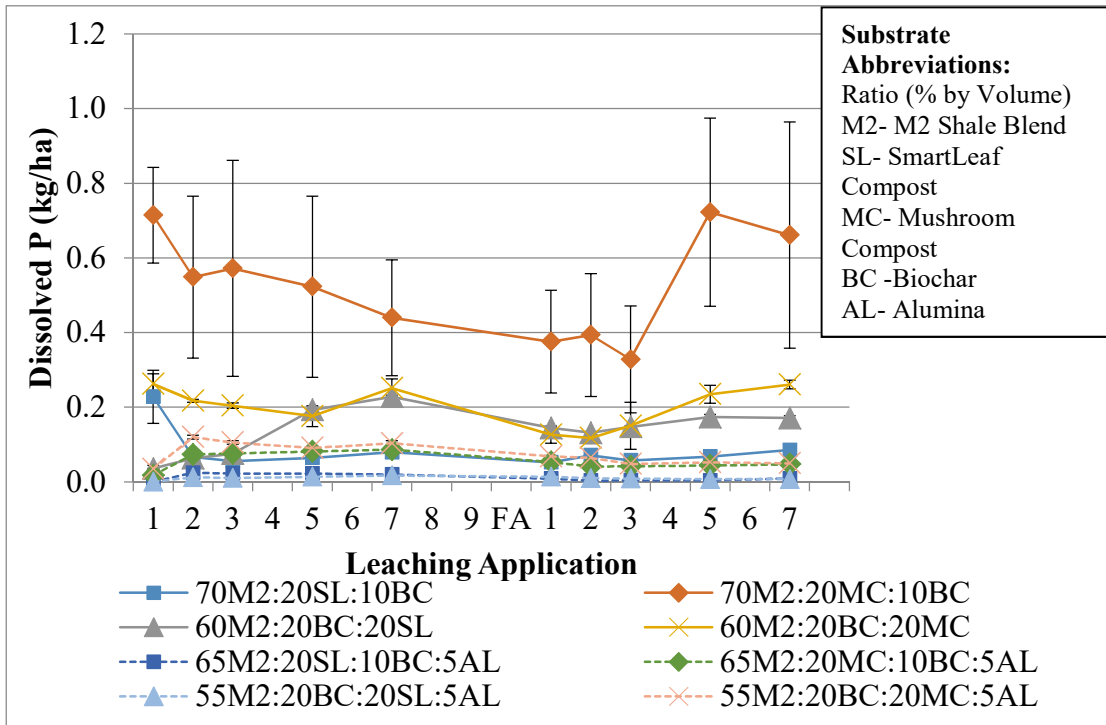


Fig. 2.19. Mean dissolved phosphorus (dissolved-P) leachate (kilograms per hectare) from each leaching event during the column study for substrates containing biochar. Leaching applications (LA) 2 through 9 represent 25mm of simulated rainfall applied to substrates. Leaching application represents the sample from the initial saturation event. FA denotes the point where fertilizer was applied, and LA 1 through 7 represent simulated rainfall events 24 hours after fertilization. Error bars show standard errors about the means.

Figure 2.20 shows the accumulated dissolved-P for each biochar substrate.

There was a significant reduction ($p < 0.05$) in dissolved-P from all biochar substrates containing alumina relative to substrates that did not contain alumina. This

significant reduction occurred regardless of the organic matter source of each biochar substrate. The substrates with the highest dissolved-P content in leachate were from any mushroom substrate without alumina. There were significant differences between the biochar substrates in phosphorus loads that contain alumina that was dependent on the organic compost source.

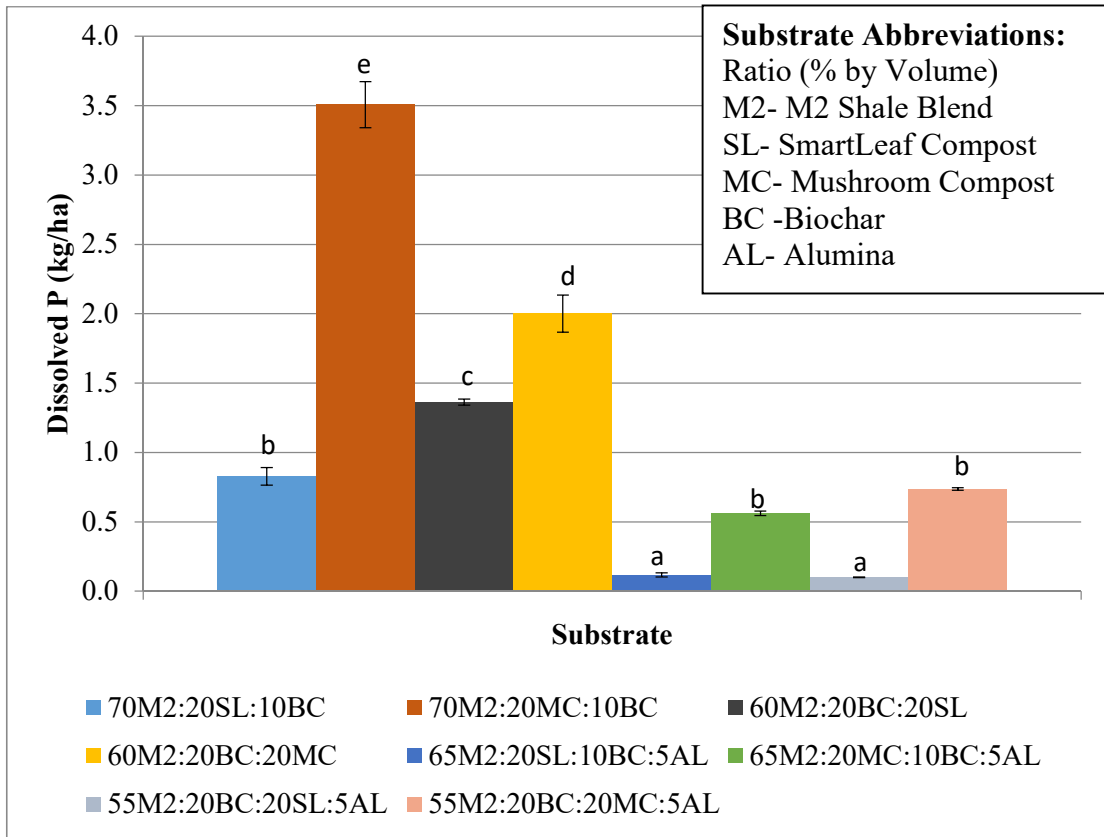


Fig. 2.20. Mean cumulative phosphorus (dissolved-P) loads in kilograms per hectare over the column study for each substrate containing biochar. Letters upon bars indicate significance levels (Tukey's HSD $P < 0.05$). Error bars show standard errors about the means.

Figures 2.21 and 2.22 show all of the substrates whose common characteristic between them was the presence of alumina in each substrate. In Figure 2.21 the amount of dissolved-P leached varies between each substrate with the highest leaching belonging to the substrates containing mushroom compost substrates. The P

loading from alumina substrates containing SmartLeaf compost were lower than from substrates that contained mushroom compost. These leachate totals did not converge on one another and dissolved-P amounts remained relatively constant throughout the study. Dissolved-P content in the leachate rises after fertilization in biochar substrates that did not contain alumina. The amount of dissolved-P stayed relatively constant for substrates containing mushroom compost and trends slightly downward for alumina substrates containing SmartLeaf after fertilization.

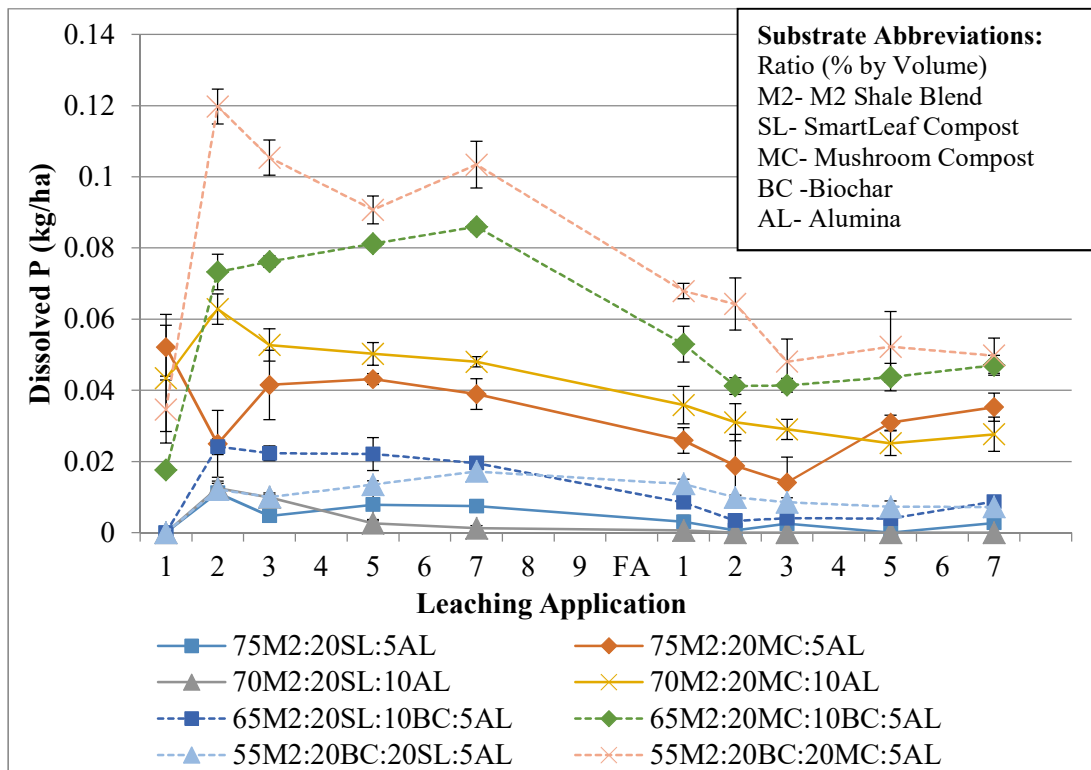


Fig. 2.21. Mean dissolved phosphorus leachate (kilograms per hectare) from each leaching event during the column study for substrates containing alumina. Leaching applications (LA) 2 through 9 represent 25mm of simulated rainfall applied to substrates. Leaching application represents the sample from the initial saturation event. FA denotes the point where fertilizer was applied, and LA 1 through 7 represent simulated rainfall events 24 hours after fertilization. Error bars show standard errors about the means.

Figure 2.22 shows the accumulated dissolved-P for each alumina containing substrate. There were significant differences ($p < 0.05$) between the accumulated dissolved-P in these substrates, based on their organic matter source. There were significant reductions in dissolved-P in all alumina substrates containing SmartLeaf relative to alumina substrates that contain mushroom compost. The substrates with the highest dissolved-P content in leachate were any mushroom substrate with biochar present. There were no significant differences ($p > 0.05$) between the alumina substrates containing SmartLeaf compost.

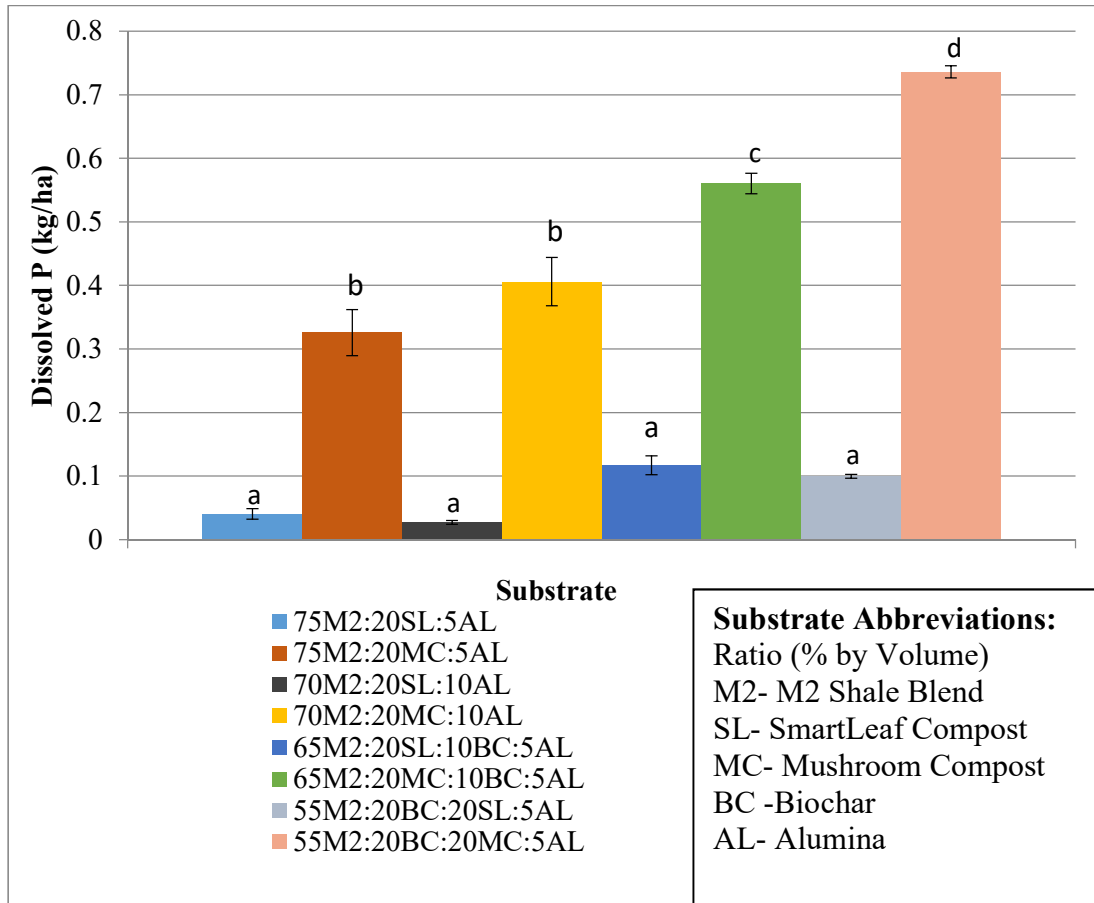


Fig. 2.22. Mean cumulative dissolved phosphorus loads in kilograms per hectare over the column study for each substrate containing alumina. Letters upon bars indicate significance levels (Tukey's HSD $P < 0.05$). Error bars show standard errors about the means.

2.3.3 Physical Properties

Both active and exchangeable acidity tended to decrease with the increase in amendment content as the M2 blend itself tested at pH 4.56. Most amended substrates tested in the upper 7s (pH) for active acidity and most substrates tested in the lower 7s (pH) for exchangeable acidity. There was no consistent amendment effect on either active or exchangeable acidity. The data for active and exchangeable acidity is available in Appendix A.8. Water holding capacity increased with increasing amounts of amendments added to unamended substrates. Some substrates containing only alumina had less water holder holding capacity than substrates amended with biochar but amendment effects were inconsistent and were mostly insignificant. The data for water holding capacity of all substrates are available in Appendix A.8.

2.4 Column Study Discussion

The purpose of the column study was to rapidly screen a large number of potential combinations of organic matter source and biochar / alumina amendments. This study provided a broad understanding of NO_3^- -N and dissolved-P leaching dynamics from native nutrient contents and a single fertilization event. The results showed that mushroom compost had the highest NO_3^- -N and dissolved-P contents, and which showed significant leaching (load) over a relatively short period of time, with simulated rainfall events.

SmartLeaf compost may be more available in Mid-Atlantic urban areas due to the relative availability and sustainability of leaf litter collected by the urban municipalities during the fall season. This would make leaf litter compost a more

reliable source of organic matter for green roof substrates if local sourcing was a priority. More importantly, the results provide good information on potential N and P loading from this source material, which was significantly lower than that from mushroom compost.

Mushroom compost substrates released significantly more NO_3^- -N and dissolved-P, irrespective of biochar and alumina amendments. These high levels of native nutrient content suggest that if maximizing soil fertility is the primary goal of the formulation of a particular substrate, the use of mushroom compost as the primary organic matter component would most likely provide superior performance in aspects of crop production tied to soil fertility than a substrate containing SmartLeaf compost. However, one of the consequences of this increased soil fertility is the significant increase in potential nutrient runoff into the environment. If amendments that retain N and P are not available, mushroom compost would not be recommended due to the high nutrient leaching potential.

NO_3^- -N leaching was not affected by the presence of or specific amount of alumina or biochar in the substrate. There were some significant differences regarding the effect of biochar on NO_3^- -N leaching, but it is questionable whether those statistical differences translate into real differences. This was especially true with substrates containing SmartLeaf compost. There were instances of substrates containing biochar reducing the amount of NO_3^- -N leached, but its' nutrient retention performance was not consistent enough to support the conclusion that biochar can retain NO_3^- -N. There were also instances of substrates containing alumina increasing the amount of NO_3^- -N leached. The substrates with the lowest nitrogen retention all

contained alumina, including substrates containing biochar and alumina. This could support the possibility of an antagonistic mechanism that decreases the substrate's ability to retain NO_3^- -N. While this effect was present in the data, the performance of alumina containing substrates was not consistent enough to conclusively support the idea of such a mechanism.

The leaching of dissolved-P was also greatly affected by the organic matter source. Substrates containing mushroom compost produced significantly higher concentrations of leached dissolved-P than from SmartLeaf-containing substrates. Biochar did not seem to significantly affect the amount of leachate dissolved-P. The presence of alumina in substrates produced significantly lower concentrations of dissolved-P in the leachates with successive simulated rainfall events. This dissolved-P retention effect was seen regardless of the organic matter source. There was some evidence to support that adding additional alumina will slightly increase a substrate's retention of dissolved-P, particularly with SmartLeaf compost.

Some anecdotal observations were made while conducting the column studies; this includes the stability of biochar in the soil profile and its effect on irrigation practices while in the columns. Due to the fine particle size of the biochar product used in this study, biochar that was near the surface of the media frequently floated out of the substrate and redeposited on the surface when all of the wash water drained below the surface of the substrate. Additionally, the biochar at the bottom of the columns had a tendency to clog up the glass fiber filters, keeping the substrate in each column causing the rate of leaching to slow down. This movement of the biochar in the substrate profile appeared to be dependent on whether or not the biochar wetted

properly when the leaching applications were made. Biochar that was already wetted stayed relatively stable in the profile, while un-wetted biochar appeared more mobile.

The goal of this screening study was to produce enough information to be able to understand the nutrient and leaching dynamics from a large number of potential compost / amendment mixes. The intent of this study was to allow for the selection of a limited number of substrates for crop production, where longer-term irrigation, fertilization and crop yield dynamics could be evaluated.

To this end, four mushroom compost substrates were selected to provide “worst-case” nutrient runoff, combined with “best-case” amendment potential. The 10% biochar amendment was selected to avoid any potential hydrophobicity and irrigation problems with 20% biochar. While biochar was not shown to increase nutrient retention for NO_3^- -N and dissolved-P, it was thought that biochar could increase longer-term soil fertility by increasing cation exchange capacity (CEC) and water-holding capacity. While the addition of greater amounts of alumina in the substrate (from 5% to 10%) did increase retention of dissolved-P in most cases, we had some concerns that higher alumina ratios could induce P and perhaps other micronutrient deficiencies. Since substrate blends with 5% alumina significantly increased dissolved-P retention, this incorporation rate was chosen as a compromise for the crop growth studies documented in Chapter 3.

2.5 Column Study Conclusions

Biochar and alumina did not produce any significant, nor consistent effect on reducing NO_3^- -N leached from any substrate mix tested. Additionally Biochar did not produce any significant short-term effect on reducing dissolved-P leached from any

substrate mix. Alumina, however, did produce a significant and consistent reduction in the amount of dissolved-P leached from each substrate. Based on the results of these short-term column studies, the following substrates were selected for crop growth studies: 80% M2 Blend+20% Mushroom Compost, 70% M2 Blend+10% Biochar+20% Mushroom Compost, 75% M2 Blend+5% Alumina+20% Mushroom Compost, and 65% M2 Blend+10% Biochar+5% Alumina+20% Mushroom Compost.

Chapter 3: Crop Growth Studies

3.1 Introduction

Open agricultural operations tend to be point sources for eutrophic pollution (Chesapeake Bay Foundation, 2020). In order to modify a commercial green roof substrate into a medium that can support crop production, large amounts of extra organic matter need to be added in order to maintain fertility. These additions of organic matter, coupled with poor nutrient retention properties typical of green roof substrates, shallow substrate profiles, and the need to apply fertilizers to crops result in high levels of nutrient leachate/runoff into the environment (Karczmarczyk et al., 2014, 2017, 2018, Ramasahayam et al., 2014, Rowe et al., 2011, Sagano et al., 2017). While a short-term understanding of the nutrient retention performance of additions of biochar and alumina was achieved from the column studies (Chapter 2), their interactions with agricultural crops in a green roof operation are unknown. The objectives of the crop growth study were to establish the long-term leaching performance of N and P in a cultivated setting and to quantify the effects of these amendments on plant growth.

Information learned from the rapid screening of substrates from the column study allows us to make informed decisions about the substrates to use in the crop growth study. Due to financial, space, and time constraints, all 15 substrates could not be feasibly tested in the Research Greenhouse Complex on the University of Maryland College Park campus. Using the leachate data from the column study as well as some anecdotal experience from working with each substrate, four substrates were chosen based on “worst-case” scenario conditions regarding nutrient content in

the leachates, and a “best-case” scenario regarding nutrient retention potential. The substrates that were chosen from the column studies were the unamended 80% M2 Blend and 20% mushroom compost; the 10% biochar, 70% M2 Blend, and 20% mushroom compost; the 5% alumina, 75% M2 blend, and 20% mushroom compost; and the 5% alumina, 10% biochar 65% M2 Blend, and 20% mushroom compost.

The hypotheses being tested in the crop growth study were whether the additions of biochar and/or alumina would reduce the amount of nitrogen from nitrate (NO_3^- -N) and dissolved elemental phosphorus (dissolved-P) leached from each of the replicated green roof tubs. Further hypotheses were whether the effects of nutrient retention due to the addition of alumina and/or biochar (if present) will effect crop growth. The formal hypotheses were as follows:

- H1 Alternate- The presence of biochar in a substrate will reduce the amount of nitrogen from nitrate (NO_3^- -N) present in crop leachate from simulated rainfall.
- H1 Null- The presence of biochar in a substrate will have no effect on the amount of NO_3^- -N present in crop leachate from simulated rainfall.
- H2 Alternate- The presence of alumina in a substrate will reduce the amount of NO_3^- -N present in crop leachate from simulated rainfall.
- H2 Null- The presence of alumina in a substrate will have no effect on the NO_3^- -N present in crop leachate from simulated rainfall.
- H3 Alternate- The presence of biochar in a substrate will reduce the amount of dissolved elemental phosphorus (dissolved-P) present in crop leachate from simulated rainfall.

- H3 Null- The presence of biochar in a substrate will have no effect on the amount of dissolved-P present in crop leachate from simulated rainfall.
- H4 Alternate- The presence of alumina in a substrate will reduce the amount of dissolved-P present in crop leachate from simulated rainfall.
- H4 Null- The presence of alumina in a substrate will have no effect on the amount of dissolved-P present in crop leachate from simulated rainfall.
- H5 Alternative- The presence of biochar in a substrate will reduce the amount of plant growth/yield from crops, over time.
- H5 Null- The presence of biochar in a substrate will have no effect on the amount of plant growth/yield from crops, over time.
- H6 Alternative- The presence of alumina in a substrate will reduce the amount of plant growth/yield from crops, over time.
- H6 Null- The presence of alumina in a substrate will have no effect on the amount of plant growth/yield from crops raised, over time.

3.2 Crop Growth Studies Materials and Methods

A series of three plant growth studies were performed to test the four selected substrate mixes from the column studies (Chapter 2) according to the objectives and hypotheses outlined above. The crop growth study was performed in the Research Greenhouse Complex (RGC) on the University of Maryland College Park, MD campus. 16 replicate tubs (72.4cm x43.8cm x 14.6 cm, 46.3 L) were constructed and situated in a greenhouse range (Fig 3.1). All tubs were constructed and mounted on an 8 m by 2 m metal lath table. Each replicate tub was constructed out of two nested Sterilite 60 quart plastic containers, lined with a 6mm green roof substrate filter

material (Conservation Technology, Baltimore, MD). The bottom nested tub required a 12mm uniseal to couple the tub to a 12mm CPVC pipe for leachate collection. This pipe led to the first flush collection system, mounted underneath the bench where the tubs were installed (Fig.3.2). Excess leachate was collected in a 19L (5 gallon) bucket below each tub.

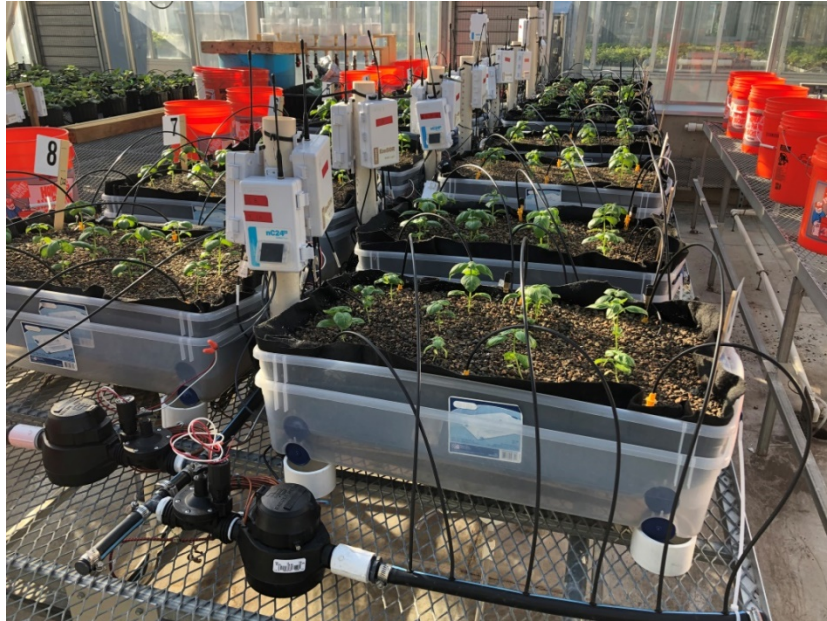


Fig. 3.1. All 16 tubs set up with newly transplanted basil (growth study 1). Each replicate tub was equipped with an independent flow meter, solenoid valve and irrigation system, monitoring node, first flush (runoff monitoring) system, and overflow collection system.



Fig. 3.2. First flush leachate collection systems attached to the drains of each tub under the bench. Each first flush collection system overflowed into a separate 19L (5 gallon) overflow bucket, to catch all runoff generated from each tub from each leaching event.

3.2.1 Leachate Capture

The first flush collection system (Figure 3.3) was used to collect the first 320 mL of leachate and separate this volume from the rest of the leachate. This first flush collection system was designed to catch the initial large spike of nutrients when leachate first begins to exit the substrate profile. The first flush samples were separately analyzed for N and P for each tub. The first flush collectors were made out of 25mm PVC, 50mm Clear PVC (collection chamber), and 12mm CPVC (overflow). A float was installed in the collection chamber and would seal the collection chamber from the rest of the leachate flow once the collection chamber filled. After leaching a tub, two 20 mL samples were collected and stored from each first flush collector. A Meter-Group EC-20 temperature and electrical conductivity sensor was installed into each of the collection systems to provide electrical conductivity data from leachate.

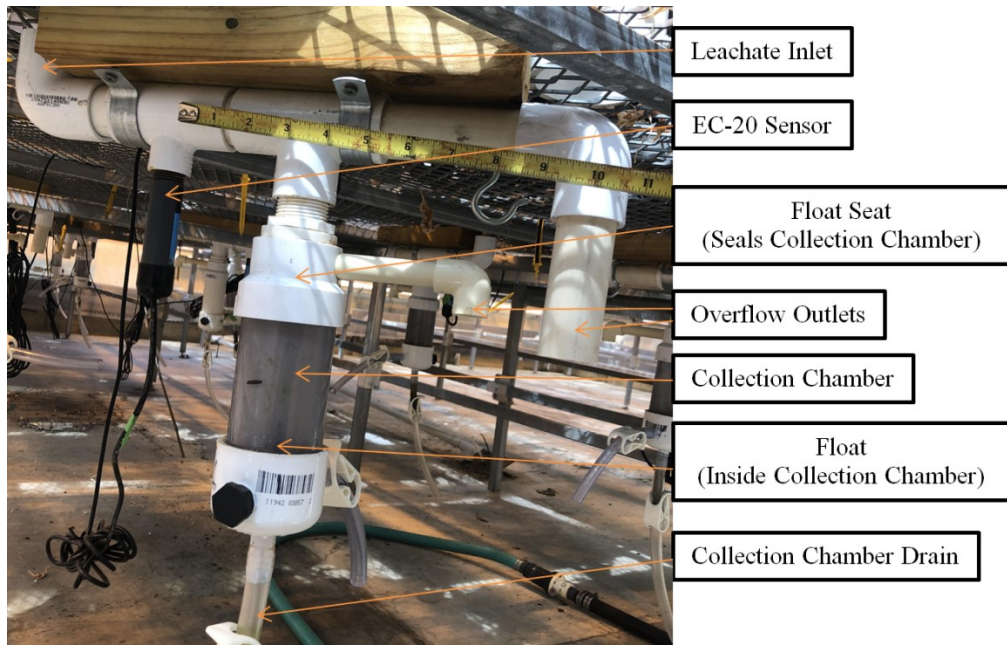


Fig. 3.3. A diagram of the construction of each first flush collection system.

3.2.2 Irrigation System

An 18mm ($\frac{3}{4}$ inch) lateral polypipe line supplied water from the main spigot to each replicate tub. To ensure consistent flow and pressure, a 172 kPa (25 PSI) in-line pressure regulator was installed between the hose spigot and the lateral to the tubs. Each replicate tub was connected to the main lateral, with a sub-lateral with in-line solenoid valve in series with a gallon-resolution flow meter (Model# 34554-011; Badger Corp). The sub-lateral continued along the side of the tub from which six Netafim 300 mL/minute micro irrigation spray stakes were attached.

3.2.3 Tub Construction

The replicate tubs were arranged in a modified Latin square configuration (Fig. 3.4), with each substrate treatment assigned to one of the four replicates. This randomized arrangement was to account for the effect of any environmental variance

on the growth of the plants. Each of the four substrates was formulated as described in Chapter 2 (see Appendix A.1 for detail). The volume of substrate in each replicate tub was approximately 43L. The flow meter data for each replicate tub measured the volume of water applied via supplemental irrigation (See below).

Greenhouse Exhaust Fan	
Tub 1 70M2:20MC:10BC	Tub 16 75M2:20MC:5AL
Tub 2 65M2: 20MC:10BC:5AL	Tub 15 80M2:20MC
Tub 3 75M2:20MC:5AL	Tub 14 70M2:20MC:10BC
Tub 4 80M2:20MC	Tub 13 65M2: 20MC:10BC:5AL
Tub 5 70M2:20MC:10BC	Tub 12 75M2:20MC:5AL
Tub 6 65M2: 20MC:10BC:5AL	Tub 11 80M2:20MC
Tub 7 75M2:20MC:5AL	Tub 10 70M2:20MC:10BC
Tub 8 80M2:20MC	Tub 9 65M2: 20MC:10BC:5AL

Substrate Abbreviations:
 Number- % by Volume
 M2- M2 Shale Blend
 SL- SmartLeaf Compost
 MC- Mushroom Compost
 BC -Biochar
 AL- Alumina

Fig. 3.4. Tub arrangement and substrate assignments for the crop growth study relative to relevant greenhouse appliances.

Each tub was monitored for VWC by using two GS1 (VWC) sensor placed in the substrate profile within each tub at two locations at an 8-cm depth, connected to an EM50R radio data logger (Meter-Group, Inc., Pullman, WA). A 5TM (VWC, temperature) sensor was placed in the middle of the tub, 8 cm deep for substrate

temperature measurement. A gateway and base station were set up to receive the data transmissions from each EM50R that were uploaded into Sensorweb software (Mayim, LLC; Pittsburg, PA) for remote access and data analysis.

3.2.4 Crop Selection

Three crops were grown in the tubs over a 31-week growing season. The three crops chosen for this study are typically grown in urban farms due to their performance, turnover and profit margin. Each crop was started from seed (Jonny Seed Co.; Fairfield, ME) in 96-cell trays in the misting room of the RGC. Crops were *Genovese* Basil (final plant density of 12 plants per tub), *Newham* Leaf Lettuce (15 plants per tub), and *Lunchbox* Peppers (5 plants per tub). All seeds were planted in LC-1 peat-based potting medium and were transplanted into the tubs a few weeks after germinating.

Table 3.1. Date for planting, transplanting, and harvesting of the three crops for the crop growth studies.

Crop	Planted	Transplanted	Harvested
<i>Genovese</i> Basil	11/16/2018	12/15/2018	2/13/2019
<i>Newham</i> Lettuce	2/11/2019	2/25/2019	4/23/2019
<i>Lunchbox</i> Peppers	3/11/2019	4/30/2019	8/14/2019

During the 8-week period, the Basil branches were not pinched or pruned. Flower buds were pinched from basil plants just as they appeared and did not significantly contribute to removal of biomass (>0.2g dry mass per tub). Lettuce was grown in high density and was harvested as heads began to form and stems just began to elongate. Peppers were hand pollinated with a generic electric toothbrush and cotton swab once they reached flowering and peppers were harvested once fruits lost their green color (changed to red, orange, or yellow).

3.2.5 Crop Harvests

At the end of each cropping cycle, all plant matter was harvested. Fresh mass were taken of the leaves, stem, root, and if applicable, fruit tissue. Leaf area was measured using a LI-COR leaf area meter. All separated plant material was dried in an oven at 60 degrees C for 7-10 days and weighed to provide the dry mass of each tissue.

3.2.6 Irrigation and Simulated Rainfall (Leaching) Events

Simulated rain events were performed each week using a 3.7L watering can with a shower style nozzle and 19 liter buckets to collect leachate (Fig. 3.2). Every week, 12.9L (25mm rainfall depth, based on the tub surface area) of DI water was applied evenly over the entire area of each replicate tub, irrespective of VWC status. The tubs were allowed to drain for 1 hour into the collection containers (first flush system and the overflow bucket). Two 20mL scintillation vials were used to collect and store each leachate sample every week from the first flush collection chamber and overflow bucket (four samples per replicate tub / week). Samples were stored at -10 degrees C for long term storage (as described in Chapter 2) until analyzed.

Supplemental irrigation was applied to the tubs when the VWC from the two GS1 sensors fell below the threshold set point for each substrate (Table 3.2). The tubs were checked on a daily basis and a 30-second irrigation pulse applied 900mL of water to the tub if the VWC was lower than the set point for that substrate. The supplemental irrigation never produced any leachate.

3.2.7 Leachate Nutrient Analysis

Leachate samples were analyzed on campus for nitrogen from nitrate ($\text{NO}_3\text{-N}$) and dissolved elemental phosphorus (dissolved-P). $\text{NO}_3\text{-N}$ was analyzed using the HANNA Spectrophotometer via colorimetry. A detailed procedure for the $\text{NO}_3\text{-N}$ analysis is available in Appendix A.2 and A.3. Dissolved-P was analyzed with a Shimadzu ICPE-9000 (ICP) for the first six weeks. Due to cost considerations, week 7 through 31 were analyzed for phosphorus with an AQ300 SEAL Analytical Discrete Analyzer spectrophotometer (SEAL). Samples from the ICP were also tested by the SEAL and a regression curve was established to convert between the two, to normalize the two data sets. The procedure for this regression and the curve itself are provided in Appendix A.11.

Dissolved-P testing requires that leachate samples be filtered with a 0.45 micron filter before testing to eliminate undissolved phosphorus as per EPA recommendations. After filtration, these samples contain a solution of phosphates and other forms of organic and inorganic phosphorus suspended in solution. The filters used were Pall Corporation 25mm mixed cellulose ester (MCE) based filter membranes (GN-6 Metrical) that are primarily compatible with syringe filters. After filtration, samples were tested for dissolved elemental phosphorus (mg-P/L).

The ICP procedure requires no chemical digestion as the heat of the plasma accomplishes that step. The ICP requires a minimum of 10 mL per leachate sample. The only chemical reagents required for ICP analysis were the creation of serial diluted standards for calibration at the beginning of each run of the ICP. These standards were made with a certified stock solution of 1000 mg-P/L. Once a

calibration run was completed, the leachate samples were analyzed by the ICP for dissolved-P concentration. A more detailed procedure on the use and preparation and use of the ICP is available in Appendix A.7.

The AQ300 SEAL is a colorimetric spectrophotometer that analyzes for dissolved P at 660nm and 880nm. In order for the SEAL to be able to analyze for dissolved P, each sample must be filtered and chemically digested. The SEAL then analyzes the filtered and digested sample for dissolved-P concentration using colorimetry. This chemical digestion requires a 0.45 micron MCE filter, potassium persulfate, sulfuric acid, phenolphthalein, and sodium hydroxide. Filtered samples were digested by boiling samples with 5M hydrochloric acid and potassium persulfate. The digestion chemicals were neutralized with sodium hydroxide using phenolphthalein as an indicator. A more detailed procedure of the sample digestion procedure is provided in Appendix A.8. The SEAL machine mixes its own serial dilutions for calibration but a digested sample of standard solutions (1.0 mg-P/L and 0.5 mg-P/L) must be provided.

In order for the SEAL to analyze for dissolved-P, coloring reagents and other reactants must be prepared. The digested calibration solution must be used to set up the machine. The coloring agent was made of a solution containing ammonium molybdate, sulfuric acid, potassium antimonate tartrate, and DI water. The SEAL also requires a solution of ascorbic acid in order to process dissolved-P. A more detailed procedure for reagent preparation is provided in Appendix A.9. After sample digestion and reagent preparation, 2 mL of each sample was analyzed. The data

received from the analysis expressed as ppm dissolved-P (mg-P/L). A more detailed procedure for sample analysis using the SEAL is provided in Appendix A.10.

3.2.8 Hyprop Substrate Analysis

A UMS Hyprop (UMS, Munich, Germany) was used to measure the water potential curve of each of the four substrates used in the tub study to ensure that plants would not be exposed different amounts of maximum water stress in different substrates. In order to compare pF and kPa, the conversion formula $pF = \log(-hPa)$ was used (UMS, 2015). Three simultaneous replicate measurements of pF and %VWC were performed on each soil sample and a van Genuchten curve was fitted to the data. A van Genuchten curve is a model that describes the water retention and hydraulic conductivity of a given substrate using data from the tensiometers in the Hyprop. This model provides a way to relate soil water potential to %VWC, as matric potential measurements (UMS, 2015). %VWC is often easier to measure in the field and utilized available equipment in the lab.

3.3 Crop Growth Study Results

3.3.1 Hyprop Media Analysis

A water potential of -35kpa (2.54 pF) was selected for all substrates, to (1) normalize the WWC readings at a non-stressful matric potential, with adequate water availability (between 0 and -35 kPa) and (2) to avoid any water stress between the different substrates. As an example, combining the three replicate curves for the unamended (80 M2: 20MC) substrate, an average of 15.5%VWC was found to correlate with -35kPa (2.54 pF). This was then used as the VWC set point for all

supplemental irrigations for this substrate (see Irrigation Application section). Figure 3.5 shows a sample chart of the hyprop analysis of the unamended control substrate containing 80% M2 blend and 20% mushroom compost.

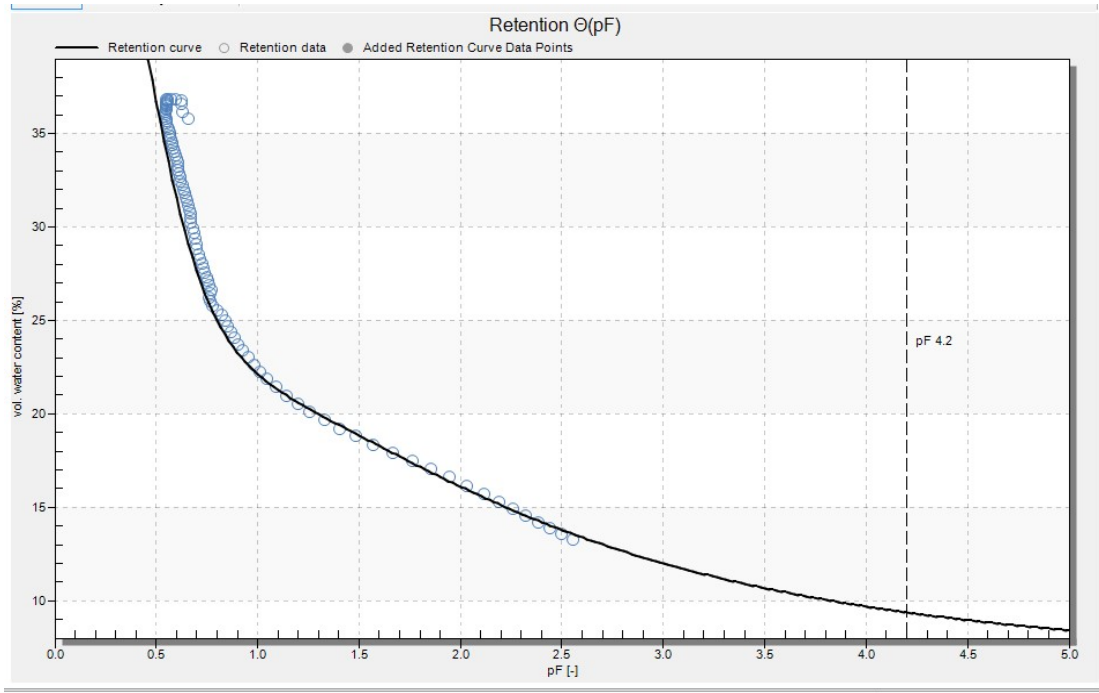


Fig. 3.5. One replicate of a Hyprop graph relating soil water potential (pF) to percent volumetric water content (%VWC) for the unamended control substrate used in the tub study containing 80% M2 blend and 20% mushroom compost. The curve is used to determine the %VWC at a particular soil water potential. The tub study uses 2.54 pF (-35kPa) as the minimum set point to begin supplemental irrigation of the tubs to prevent water stress. The line at pF 4.2 (-1500kPa) which denotes the permanent wilting point where plants can no longer physically uptake water.

Similar Hyprop procedures were performed for the other three substrates, with the VWC set-point thresholds noted in Table 3.2. There were no significant differences between the %VWC levels that correlated with 2.54 pF (-35kPa); (data not shown).

Table 3.2. Table of irrigation set points (%VWC) for each substrate generated via Hyprop procedure as per the manual (UMS, 2015).

Substrate Mix	VWC Setpoint @ -35kPa (mol/mol; %)
80 M2: 20 MC	15.5
75 M2: 20 MC: 5A1	19.8
70 M2: 20 MC: 10B	17.2
65 M2: 20 MC: 10B: 5A1	18.8

3.3.2 Soil Moisture Irrigation

In each tub, two GS1 sensors measured and recorded the %VWC every 15 minutes (Figure 3.6). The reading from these two sensors were averaged together to calculate the average %VWC in the tub. This measured VWC value was compared to the %VWC values for the substrate in the tub (Table 3.2). Figure 3.6 illustrates %VWC data from a tub containing 65M2B:20MU:10BC:5AL over the entire study as an example. Each major spike represents the time when the 25 mm of simulated rainfall were applied. During the basil and lettuce cropping cycles, no supplemental irrigation was necessary. The large drops between the crops were times where the tubs went unirrigated while harvests were being processed. During the latter half of the pepper season, the %VWC dropped below the irrigation set point and irrigation had to be applied, raising the %VWC.

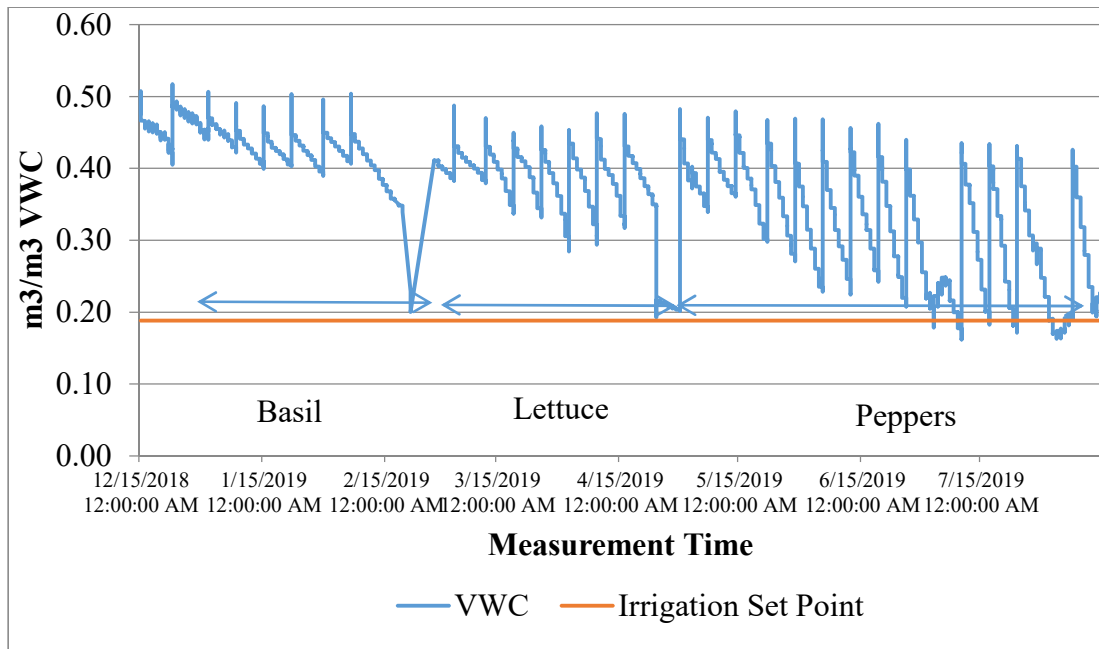


Fig. 3.6. %VWC of a tub containing 65M2B:20MC:10BC:5AL over all cropping cycles. The red line indicates the irrigation set point at 0.188 (18.8%) VWC and indicates when irrigation was to be applied.

3.3.3 Crop Growth Study Harvest Results

Figures 3.7, 3.8, and 3.9 show the dry mass for the destructive harvest of root, stem, leaves and total mass for each crop raised in the tubs for each substrate. Figure 3.7 provides dry mass data for the basil crop (study 1). At the end of the growth period, the 12 basil plants from each replicate tub were harvested, separated into roots, stems, and leaves. The separate tissues from all plants from each replicate were pooled and dried for dry mass analysis. Average total dry mass (TDM) was calculated by summing the dry mass for roots, stems, and leaf and dividing by the number of replicates (n=4).

The biochar-amended substrate (70M2:20 MC:10BC) produced the highest average TDM (45.9 g), as well as the highest leaf and stem dry mass, although this was not significantly different to the unamended (80M2:20MC) substrate.

Reductions in basil yield were seen in both substrates containing alumina; however the dry mass of all tissues from the substrate amended with only alumina were not significantly different from the 80M2:20MC or 70M2:20MC:10BC treatments. The 65M2:20MC:10BC:5AL substrate had the lowest total plant dry mass (19.8g), but the two alumina amended substrates did not show any significant differences between any plant tissue dry mass. Interestingly, the greatest reduction in yield was seen between the substrate amended with biochar and the substrate containing both alumina and biochar; a 50% reduction in yield was noted across all tissues between these treatments.

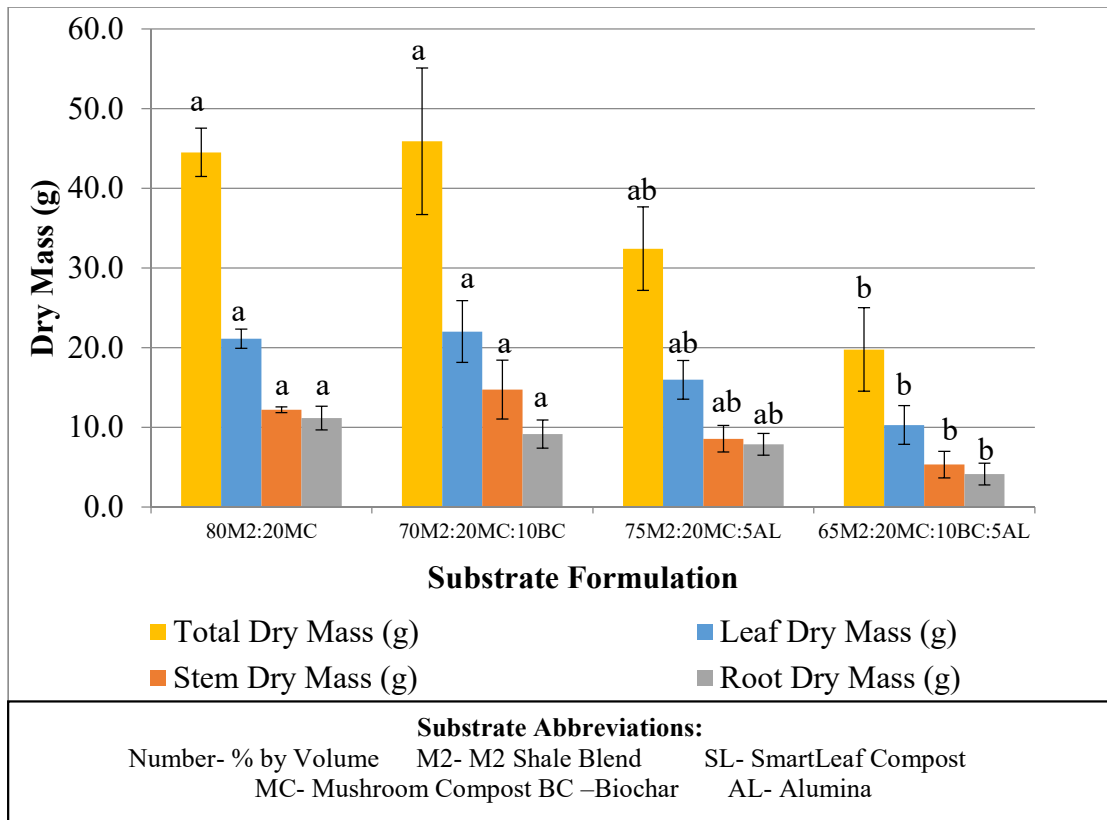


Fig. 3.7. Average dry mass in grams from destructive harvest of roots, stems, and leaves of basil plants from the first cropping cycle for each substrate. Letters denote significance levels (Tukey’s HSD $P < 0.05$). Error bars show standard errors about the means.

Figure 3.8 shows the average root, stem, leaf and TDM of the lettuce (crop 2) for each substrate at the end of the crop production cycle. Each replicate was plated with 15 lettuce plants; tissues were separated and pooled from each replicate, dried, and fresh mass measured (data not shown).

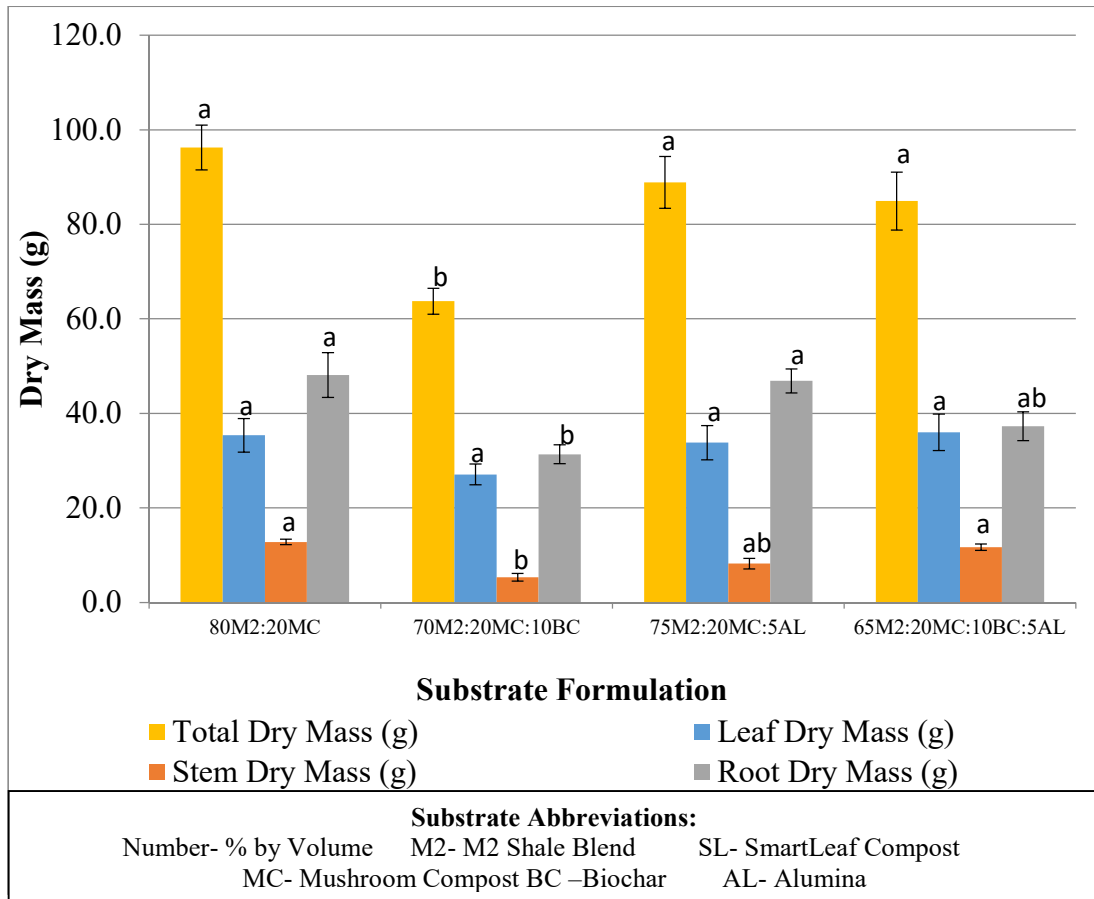


Fig. 3.8. Average dry masses in grams from destructive harvest of roots, stems, and leaves of lettuce plants from the second cropping cycle for each substrate. Letters denote significance levels (Tukey’s HSD $P < 0.05$). Error bars show standard errors about the means.

The unamended substrate containing 80M2:20MC produced the highest average TDM (96.2g). In contrast to the basil crop, the substrate that produced significantly ($p < 0.05$) lower TDM to all other treatments was the 70M2:20 MC:10BC (63.7g). Most notable was that leaf dry mass (the edible portion of the crop) was not

significantly affected by any substrate, except that there was a 22% reduction in leaf dry mass with the 70M2:20MC:10BC treatment, compared to the 80M2:20MC substrate. There were no significant differences between the other three substrates in total dry mass, stem tissue, or root tissue.

Figure 3.9 shows the average root, stem, leaf and TDM of the pepper (crop 3) for each substrate at the end of the crop production cycle. Each replicate contained 5 pepper plants; tissues were separated and pooled from each replicate, dried, and fresh mass measured (data not shown). Similarly to the basil (crop 1), the 70M2:20MC:10BC substrate produced the highest average TDM (252.4g).

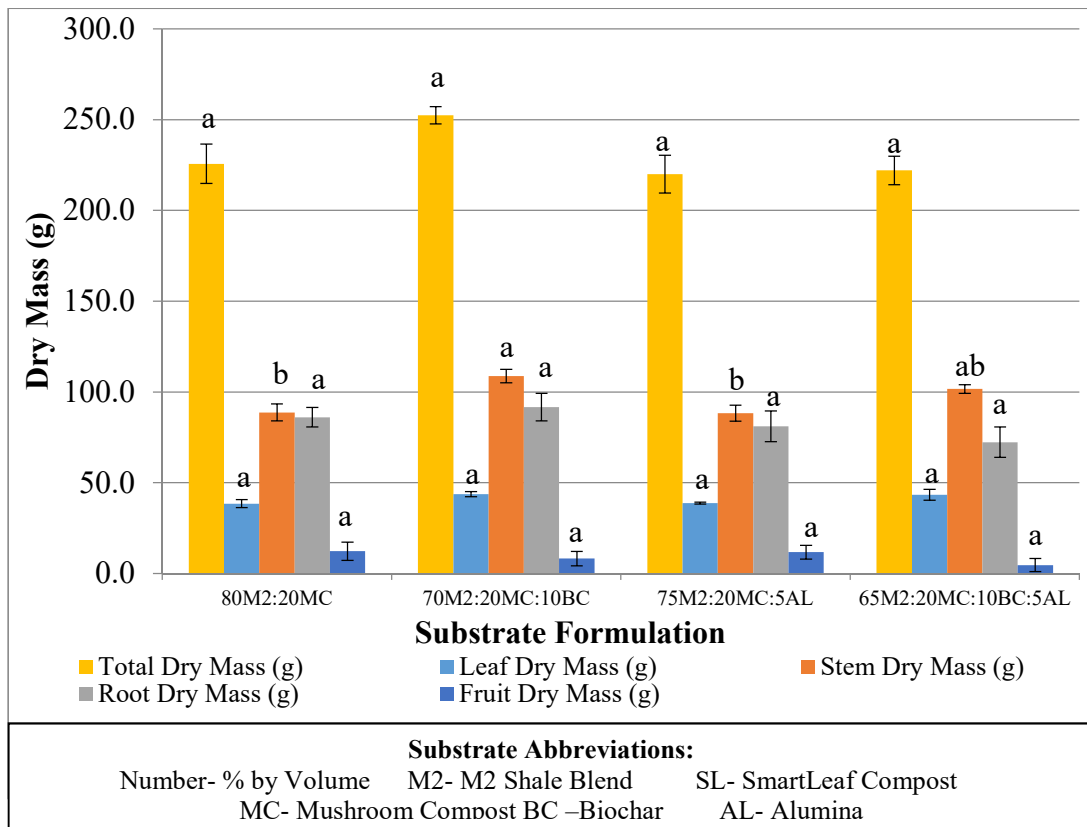


Fig. 3.9. Average dry masses in grams from destructive harvest of roots, stems, and leaves of pepper plants from the third cropping cycle for each substrate. Letters denote significance levels (Tukey's HSD $P < 0.05$). Error bars show standard errors about the means.

The 75M2:20MC:5AL substrate produced the lowest TDM (220.0g), although no significant differences were noted in TDM between any substrate. There were no significant differences between substrates in dry mass for leaves, fruits, and roots. Some significant but small differences were noted in stem dry mass between treatments.

3.3.4 Nitrate-Nitrogen Leachate Results

Figures 3.10, 3.11, and 3.12 provide nitrate-nitrogen ($\text{NO}_3\text{-N}$) leachate data from each substrate over the course of the 3 cropping cycles (31 weeks). Figure 3.10 provides weekly average $\text{NO}_3\text{-N}$ load (kg/ha) from each substrate, presented on a linear scale. Each point represents the average and SE from four replicates of each substrate. Each of the data points was calculated from the concentration of $\text{NO}_3\text{-N}$ in mg L^{-1} and multiplied by the volume of leachate collected from each tub every week and a unit coefficient to convert from mg/tub (0.32 m^2) to kg/ha. The graphs are divided into 3 labeled sections (per crop); the gaps between cropping cycles indicate time periods between harvests. During these gaps, leaching procedures were suspended until the next crop was planted. The thin vertical lines indicate four separate dates where 1.0 L of 100ppm nitrogen (made with potassium nitrate) fertilizer was applied to each replicate tub. No additional phosphorus fertilizer was ever added to the treatments over the three cropping cycles (31 weeks).

As can be seen from Figure 3.10, the four weekly leachates after the first leachate (from 12/15/18 - 01/15/19) produced the highest amount of $\text{NO}_3\text{-N}$ from all four substrates, regardless of the amendments. Many tubs produced zero leachate on the first day due to the substrate being relatively dry at the start of the basil crop. The

highest single load of NO₃-N was produced by the 75M2:20MC:5AL substrate (152.9 kg/ha) from the 25mm simulated rainfall event on 12/23/2018. The substrate with

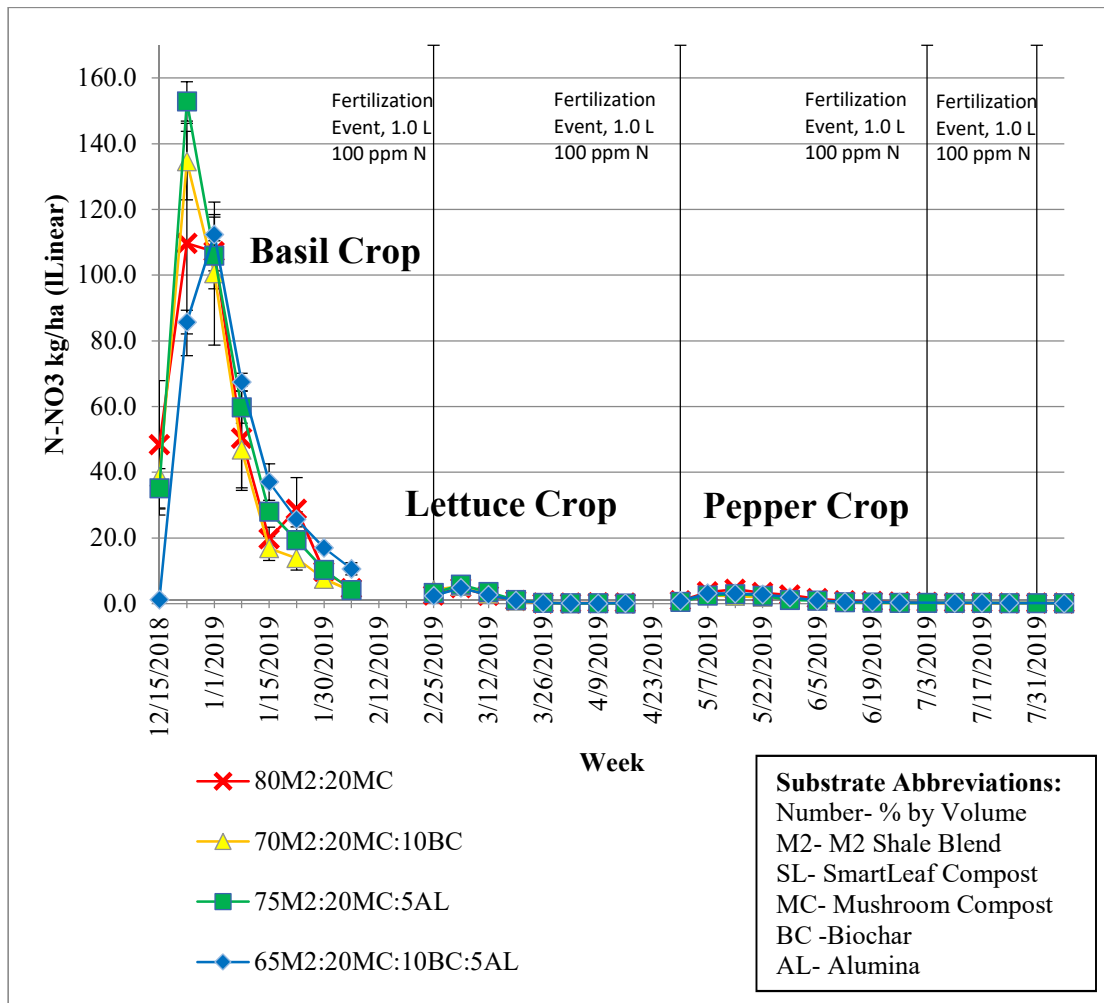


Fig. 3.10. A visual representation of the NO₃-N loads present in the leachate of each 25mm simulated rainfall event. Each line represents one substrate and each point represents the average load of four replicate tubs. The NO₃-N loads are presented on a linear scale and demonstrate the massive spike and depletion of nitrogen from nitrate in the leachate for each substrate tested in the tub study. Error bars show standard errors about the means.

the lowest loading during this period was the unamended (80M2:20MC) substrate blend (at 109.6 kg/ha). The amended 65M2:20MC:10BC:5AL substrate leached 112.4 kg/ha NO₃-N, compared to the 70M2:20MC:10BC which leached 134.5 kg/ha NO₃-N from this single simulated rainfall event. By the last week of the basil crop

(on 02/6/2019), all NO₃-N leachates from all substrates were less than 11 kg/ha NO₃-N or less.

Nitrate loading was low at the start of the lettuce crop, possibly due to substrate drying out. NO₃-N loading increased briefly after fertilization on 2/25/2019 but was very low again after a further two weeks. NO₃-N loading continued to stay low for the remainder of the lettuce crop after the peak from the first fertilization event on 2/25/2019 took place. A fertilization event also occurred during the first week of the peppers being transplanted into the tubs on 4/30/2019. A similar small increase in NO₃-N leaching was seen and lasted for a few weeks after fertilization. Two more fertilization events occurred during the pepper crop on 7/3/2019 and 7/31/2019. These fertilization events did not produce a noticeable increase in the NO₃-N leached during subsequent weekly simulated rain events.

Overall, there did not appear to be a significant effect of amendments on the rate of NO₃-N lost through leaching. Figure 3.11 shows the exact same data as in Figure 3.10 except it was plotted on a logarithmic scale for NO₃-N load. Figure 3.11 provides increased resolution of differences between treatments during the lettuce and pepper crop cycles. During the lettuce crop, and particularly during the pepper crop, there seemed to be a significant reduction in NO₃-N leaching in both biochar-amended substrates. However, towards the end of the pepper crop, these differences became non-significant, even with two additional fertilization events on 7/3/2019 and 7/31/2019; neither of these fertilizations affected the amount of NO₃-N leached, presumably because the pepper crop was taking up significant amounts of N during fruiting.

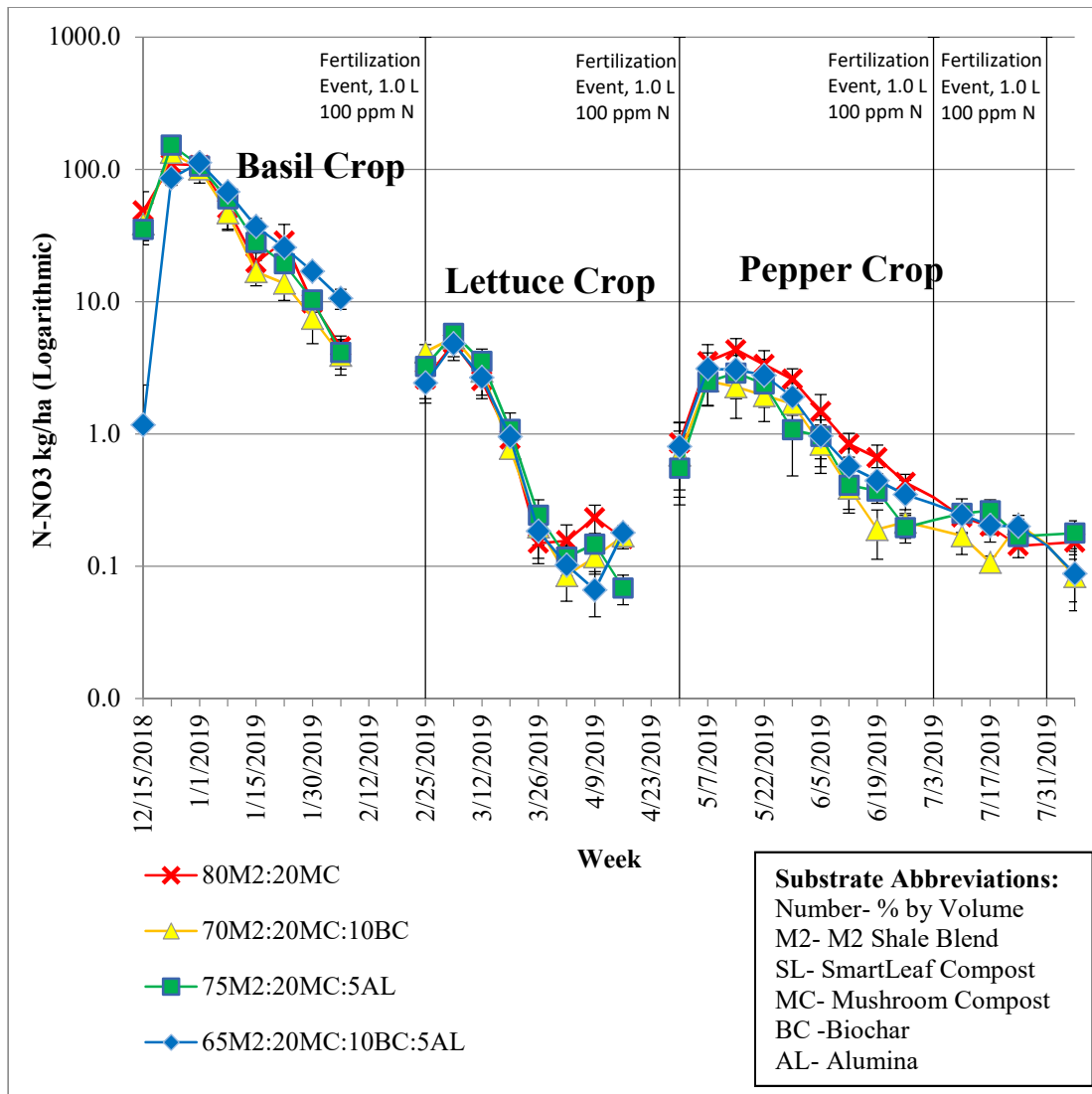


Fig. 3.11. A visual representation of the NO₃-N loads present in the leachate of each 25mm simulated rainfall event. Each line represents one substrate and each point represents the average load of four replicate tubs. The NO₃-N loads are presented on a logarithmic scale and allow for greater exploration of the lettuce and pepper crop seasons. Error bars show standard errors about the means.

Figure 3.12 shows the average cumulative NO₃-N load from each substrate, summing all NO₃-N loads over the 3 cropping cycles (31 weeks). The highest cumulative load was for 75M2:20MC:5AL with an average cumulative load of 441.7 kg/ha NO₃-N leached. The lowest cumulative load was for the 70M2:20MC:10BC

substrate with an average cumulative load of 383.0 kg/ha NO₃-N. There were no significant differences in NO₃-N leaching between the four substrates, regardless of the amendments added.

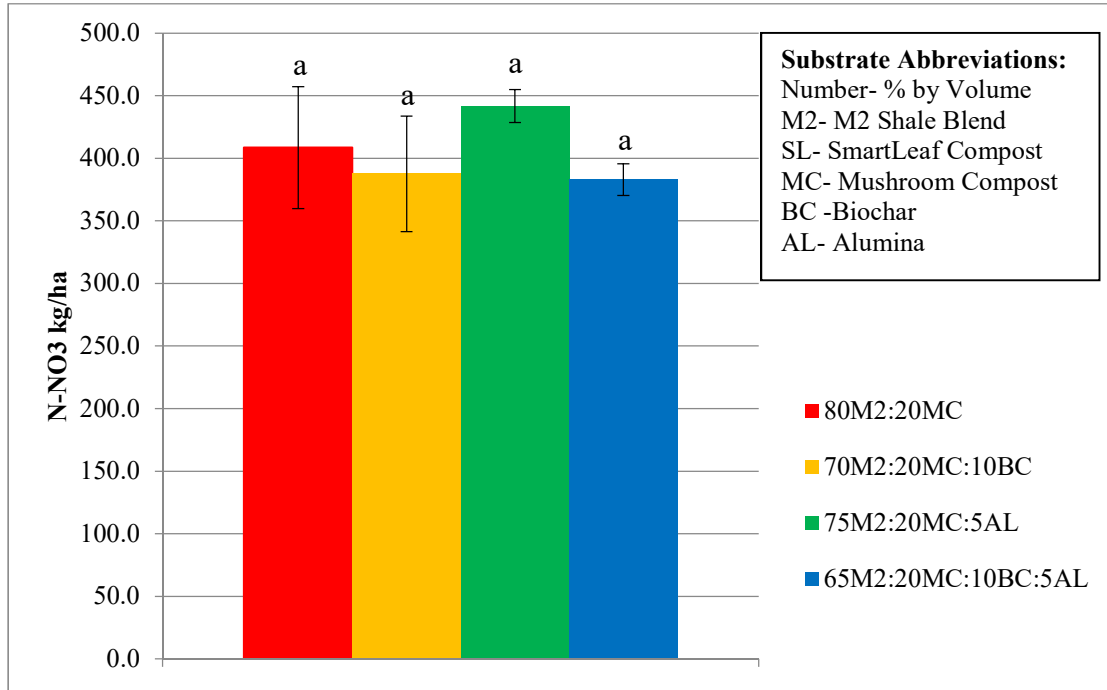


Fig. 3.12. Mean cumulative NO₃-N loads in kilograms per hectare over the entire 31 week tub study for each substrate. Letters upon bars indicate significance levels (Tukey’s HSD, P<0.05). Error bars show standard errors about the means.

3.3.5 Crop Growth Study Dissolved Phosphorus Results

Figure 3.13 provides the dissolved elemental phosphorus (P in kg/ha) loading data from each substrate over the course of the 3 cropping cycles (31 weeks). Each point represents the average and SE from four replicates of each substrate. Each of the data points were calculated from the concentration of dissolved P in mg L⁻¹ and multiplied by the volume of leachate collected from each tub every week. The graphs are divided into 3 labeled sections (per crop); the gaps between cropping cycles indicate time periods between harvests. During these gaps, leaching procedures were

suspended until the next crop was planted. The thin vertical lines indicate four separate dates where 1.0 L of 100ppm nitrogen (made with potassium nitrate) fertilizer was applied to each replicate tub. No additional phosphorus fertilizer was ever added to the tubs over the three cropping cycles (31 weeks). However, due to the use of municipal water supply as supplemental irrigation, a small amount of orthophosphorus were added to the tubs due to water treatment. The load produced by this phosphorus addition via the municipal water supply was insignificant and was quantified and accounted for in Chapter 4.

A total of four fertilization events occurred over the course of the tub study and are noted on the graph at the time they were applied. The dissolved-P loads for the first six weeks were measured using ICP. Due to financial constraints, the remaining weeks 7 through 31 had to be analyzed with the SEAL spectrophotometer. Conversion was required between the ICP and SEAL readings and the procedure for the regression curve generation and use are detailed in the Appendix A.11.

Figure 3.13 shows that overall, the substrates which contained alumina reduced the amount of dissolved-P present in the leachate over the entire 31-week study period. The dissolved-P readings were low the first week on 12/15/18 due to several tubs not producing any leachate or produced only small amounts of leachate. The highest dissolved-P loads originated from the unamended 80M2:20MC and the amended 70M2:20MC:10BC substrates. The 80M2:20MC substrate leached the highest levels of dissolved-P leachate throughout the entire study period, although they were not significantly different to the 70M2:20MC:10BC substrate.

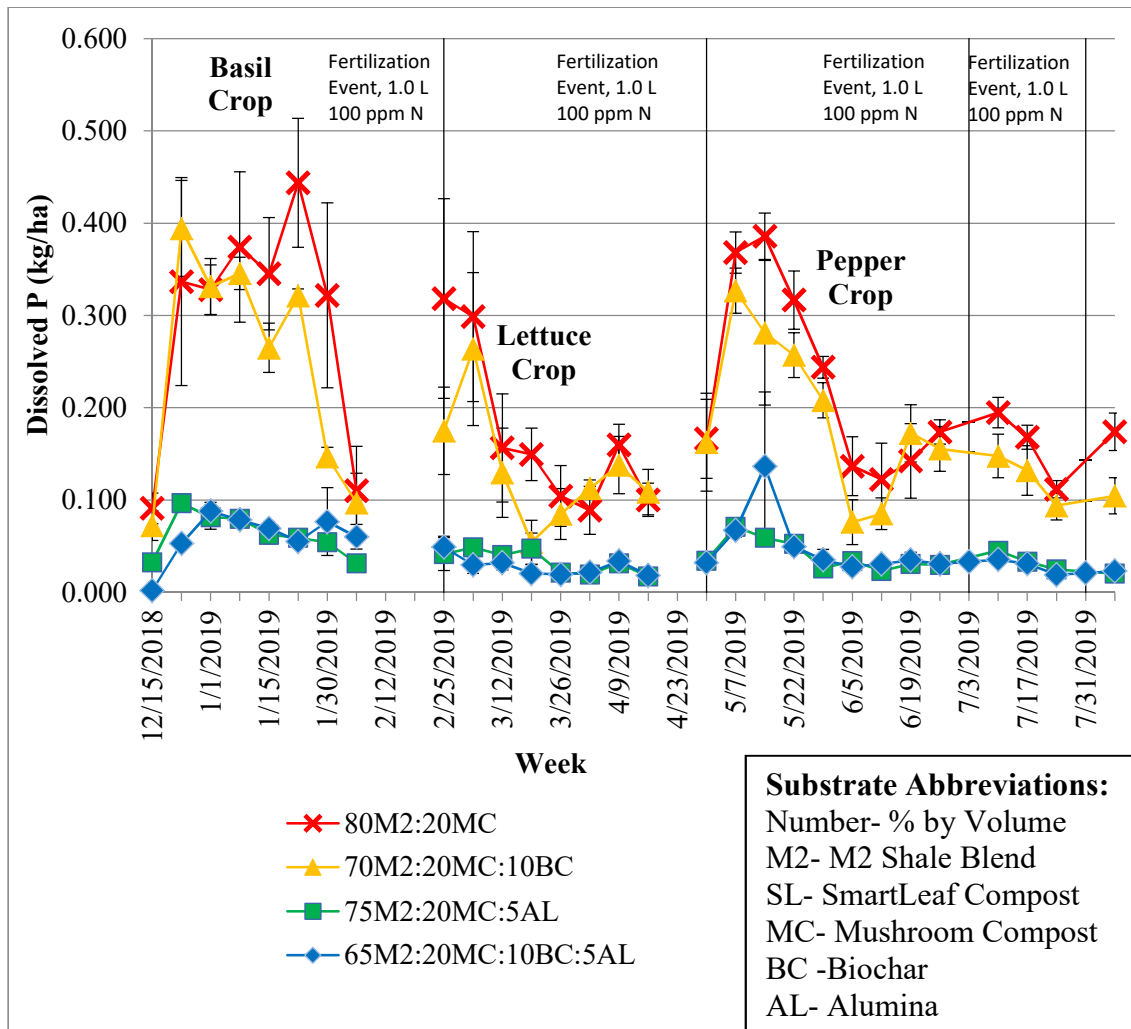


Fig.3.13. A visual representation of the dissolved-P loads present in the leachate of each 25mm simulated rainfall event. Each line represents one substrate and each point represents the average load of four replicate tubs. Error bars show standard errors about the means.

The largest single peak for a single week’s leachate was 0.444 kg/ha dissolved-P produced by the unamended substrate. Both the unamended and biochar amended substrates experienced a sharp decline in dissolved-P leachate towards the end of the cropping cycle. The substrates containing alumina and alumina plus biochar had significantly lower amounts of dissolved-P in leachates than substrates containing no alumina. Differences between leachate dissolved-P from the two alumina amended substrates were not significantly different at any time during the

three cropping cycles (over 31 weeks), except briefly after fertilization events where slightly more dissolved-P leached from the biochar-amended substrate. .

Figure 3.14 shows the cumulative dissolved phosphorus loads for the total 31-week cropping cycle. Total dissolved-P loads were summed from each week that produced leachate during the weekly simulated rainfall applications. The highest cumulative dissolved phosphorus load was generated by the unamended substrate containing 80% M2 blend and 20% mushroom compost producing 6.4 kg of dissolved phosphorus /Ha, followed by a significant reduction in P leaching from the 70M2:20MC:10 BC substrate (5.2 kg / Ha). The two substrates amended with alumina both produced significantly reduced cumulative dissolved-P loads of 1.3 kg per hectare of dissolved-P. There were no significant differences present between the two alumina amended substrates.

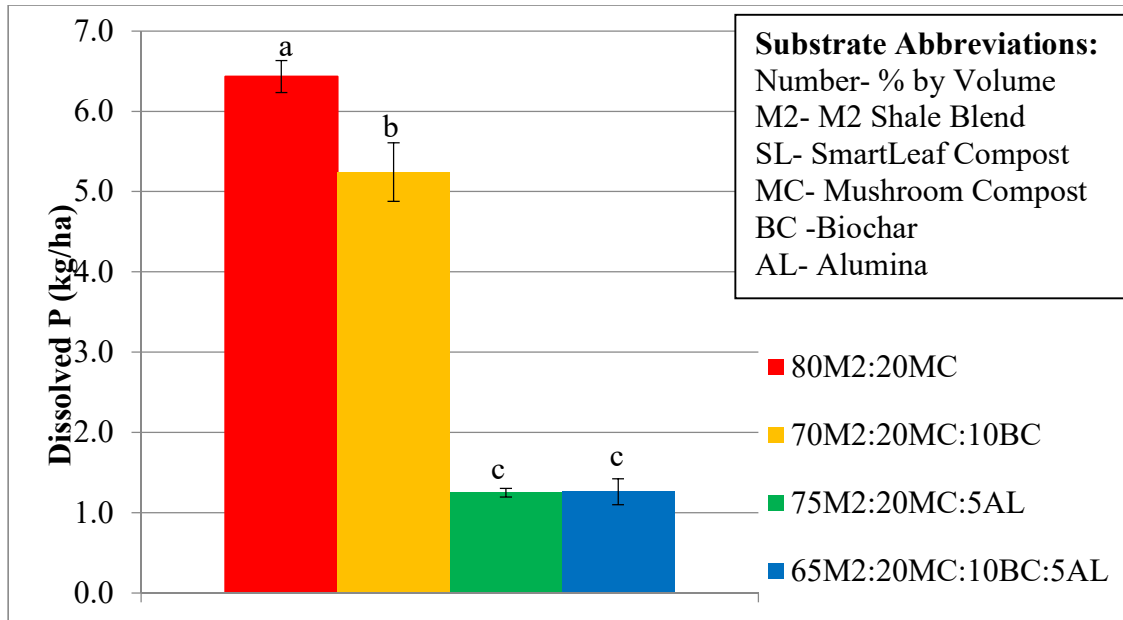


Fig. 3.14. Cumulative dissolved-P loads in kilograms per hectare over the entire 31 week tub study for each substrate. Letters upon bars indicate significance levels (Tukey’s HSD, P<0.05). Error bars show standard error about the means.

3.3.6 Soil Water Retention

Figure 3.15 provides the total amount of water applied to each substrate, the average water volume retained by each substrate during the tub study, the average cumulative leachate volume from each substrate, and the amount of supplemental irrigation that was applied to each substrate to prevent water stress during the pepper cropping cycle. During the basil and lettuce cropping cycles, no supplemental irrigation was required (Figure 3.5) as the irrigation set point was not reached within 7 days of the simulated rainfall application that occurred every week. The 80M2:20MC substrate required the most irrigation (71mm), compared to the 75M2:20MC:5AL substrate which had the least irrigation requirement (54mm) over 16 weeks. There were no significant differences in supplemental irrigation between any substrate.

Figure 3.15 also shows the average water retained by each substrate during the tub study. Twenty-nine, 25mm simulated rainfall events were applied to each tub over the 31 weeks for a total of 725 mm. The volume of leachate was measured from each tub each week and the amount retained was calculated. The substrates containing biochar retained the greatest amount of water (373 vs 378 mm for 70M2:20MC:10BC and 65M2:20MC:10BC:5AL, respectively). There were significant differences ($p < 0.05$) seen between the alumina plus biochar amended substrate and the unamended (80M2:20 MC) substrate. No other significant differences were present between the stormwater retention performances of the substrates.

Figure 3.15 also shows the average cumulative leachate volume from each substrate. The unamended (80M2:20 MC) produced the most leachate of all the substrates at (393 mm; 54% of total) compared to the 65M2:20MC:10BC:5AL substrate which leached 347 mm (48% of total applied). These treatments were significantly different from each other, but all other treatments were not different, statistically ($p < 0.05$).

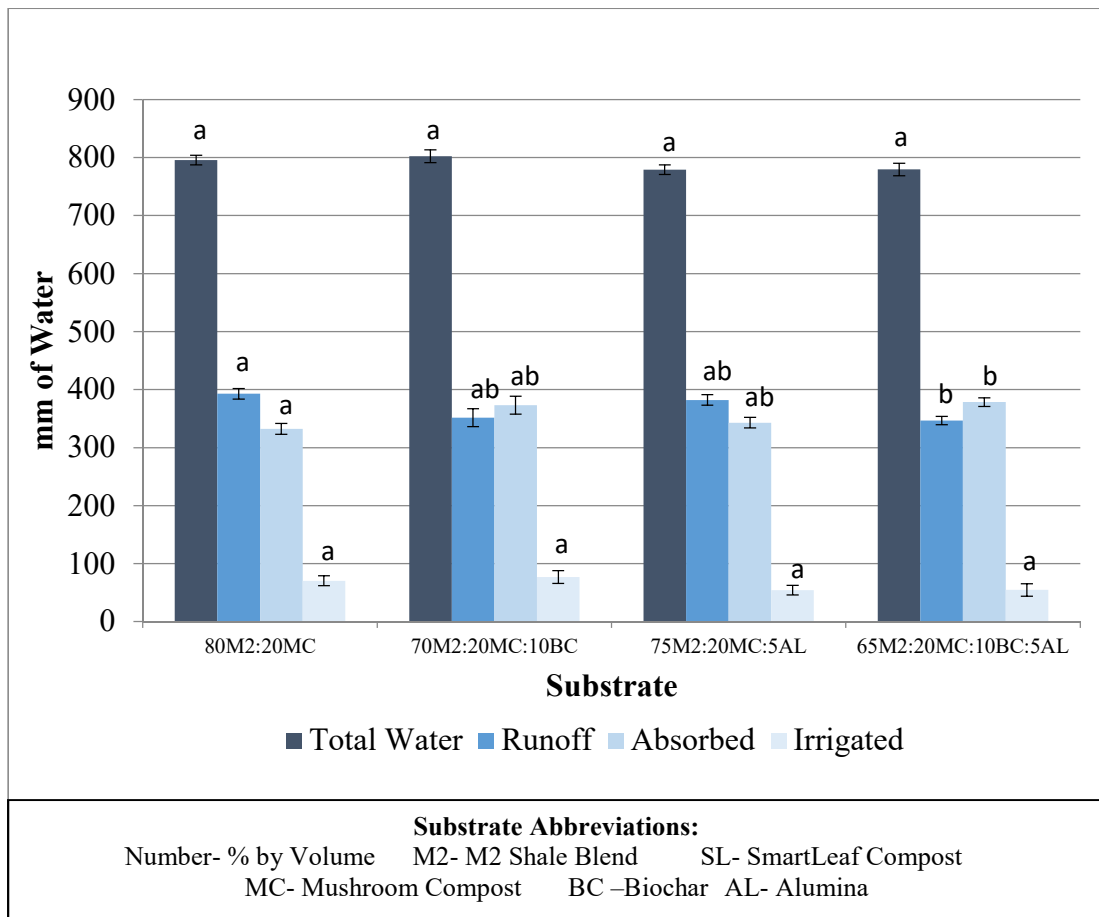


Fig. 3.15. Histogram of average total volume of water applied, runoff volume, absorbed volume, and water added through supplemental irrigation for each substrate. Letters upon bars indicate significance levels (Tukey's HSD, $P < 0.05$). Error bars show standard error about the means.

3.4 Crop Growth Discussion

The main objective of the crop growth studies was to study the long-term dynamics between crop performance in four substrates selected from the column study (Chapter 2) simulating conditions that could be typical on a green roof modified for agricultural crop production. While short-term leaching dynamics were demonstrated in Chapter 3, it was necessary to understand the longer-term effects of these substrate formulations on nutrient availability, leaching and crop growth. The greatest concern with the alumina amendment was the possibility of reducing plant available phosphorus, resulting in crop deficiencies. This was the main reason that the 5% alumina level was used, rather than 10%.

Over the course of the crop growth studies, longer-term $\text{NO}_3\text{-N}$ leaching dynamics were very similar to the short-term dynamics seen in Chapter 2. Early season simulated rainfall produced very large spikes in $\text{NO}_3\text{-N}$ leaching when the basil crop started with newly mixed substrate. This large initial spike in $\text{NO}_3\text{-N}$ was significantly reduced within 5-7 weeks of planting. New compost was used to make the substrate and had not experienced any planting or leaching events. However, due to the low anion-exchange capacity of these substrates and the high solubility of $\text{NO}_3\text{-N}$, once a leaching event occurs, there was little to prevent $\text{NO}_3\text{-N}$ from leaching.

This leaching was observed in all substrates, as none of the amendments appeared to have any significant effect on the amount of $\text{NO}_3\text{-N}$ retained. Increases in $\text{NO}_3\text{-N}$ present in leachates were also observed following the fertilizer applications applied at the beginning of the lettuce and during the pepper crop cycles. During the first basil cropping cycle, much of the $\text{NO}_3\text{-N}$ could not be utilized due to the

transplant's relatively small size and lack of roots in the substrate profile. Notably, the final two fertilizer applications occurred during the latter half of the pepper cropping cycle once the plants were mature and had no significant effects on the weekly leaching dynamics. This was most likely due to the roots' exploration of the substrate at this time and that the larger, more mature plants could more readily take up any nutrient applications before the next simulated rainfall event occurred. While a decrease was seen in the amount of cumulative $\text{NO}_3\text{-N}$ leached from substrates containing biochar over the entire study, this small decrease could be explained by these substrates having higher water-holding capacities that reduced the volume of leachate expressed. However, while significant differences in the total amount of leachate produced were seen, the differences in cumulative $\text{NO}_3\text{-N}$ in the leachate were not significantly different. Thus it does not appear that the leaching of $\text{NO}_3\text{-N}$ was affected by the presence of biochar or alumina.

Extremely high levels of $\text{NO}_3\text{-N}$ (instances of over 1000 ppm $\text{NO}_3\text{-N}$) were seen at the beginning of the growth studies. This was due to the excessive amounts of available nutrients present in the mushroom compost, but it also may be an effect of low saturation time. Full saturation time was less than 1 minutes due to the highly porous substrates. With this low saturation time, $\text{NO}_3\text{-N}$ appears to leach out of compost amended soil profiles faster than if given longer saturation times (Hurley, 2017).

The leaching dynamics of dissolved-P from the various substrates highlighted the importance of performing longer-term studies. Leached dissolved-P levels were initially relatively high, but decreased over the cropping cycles, especially in

substrates that were not amended with alumina. The first week of the basil study produced low amounts of dissolved-P due to the substrate starting off dry when the first simulated rainfall was applied. For substrates that were not amended with alumina, a decrease in dissolved-P present in the leachate occurred a few weeks after plants were transplanted into these substrates, most likely as a function of increased root density and increased crop P uptake. There seemed to be few significant differences in dissolved P leaching between substrates not amended with alumina.

The two substrates containing alumina, however, leached significantly lower amounts of dissolved-P. This reduction was an approximately 80% average decrease in the amount of dissolved-P leached from the tubs over the whole study compared to the unamended substrate. Interestingly, it seemed that dissolved-P availability was still maintained over the course of all three cropping cycles, even in Al-amended substrates (see Chapter 4).

The addition of biochar to the alumina amended substrate produced almost no detectable effects on the retention performance of dissolved-P. Unsurprisingly, the substrate that produced the most dissolved-P leachate was the unamended 80M2:20MC substrate. There was a significant difference between this substrate and the 70M2:20MC:10BC amended substrate which averaged 17% less cumulative dissolved-P over the 31 weeks. Of course, this difference could be due to the fact that the 70M2:20MC:10BC substrate produced 10% less leachate volume over this time compared to the 80M2:20MC substrate. It is also possible that the biochar may have increased the plant's ability to scavenge for dissolved-P throughout the substrate.

Plant growth is the most important factor to consider when evaluating a substrate combination for suitability. One of the main objectives of the crop growth study was to establish if the nutrient retention properties of the amendments would still allow for healthy plant growth without inducing any nutrient deficiencies. The component tissue (leaf, stem, root, and fruit) dry mass were analyzed to allow for detecting certain nutrient deficiencies and toxicities. As an example, aluminum toxicity is most often observed as reduction in the root mass of a plant.

With basil, there were statistically significant reductions in plant dry mass in all tissues in the 70M2:20MC:10BC:5AL substrate, compared to the other three substrates. The reduction in total plant dry mass from basil plants grown in this substrate was over 50%. While there was a reduction in basil plant dry mass in the 75M2:20MC:5AL substrate, it was not statistically significant from the unamended and biochar-only amended substrate. While it may be easy to conclude that alumina was detrimental to basil yield, the mechanisms behind this reduction in plant yield may be more complicated. It was possible that the alumina-amended substrates could have induced an unseen nutrient deficiency. Also, basil is known to perform poorly in high nutrient environments with excess nitrogen and phosphorus availability actually decreasing the overall health and yield of the plant (Nurzynska, 2012). But while soil nitrate levels were extremely high at the time of transplanting the basil, the low nitrogen retention resulted in most of the nitrogen being leached from all substrates, likely before affecting the young transplants.

Considering the effects of the biochar and alumina amendments on yield, the 70M2:20MC:10BC substrate did not see any significant reductions in plant dry mass,

but the substrate containing both biochar and alumina exhibited significant reductions in plant dry mass, perhaps because of unseen P deficiency. Another cropping of basil after the substrates have been leached for a season (a 4th cropping cycle) would be required to firmly establish any detrimental effects alumina may have on basil yields.

With the second crop of lettuce, there was a significant reduction in total plant dry mass observed in the 70M2:20MC:10BC substrate, when compared to the other three substrates. However, this reduction in total dry mass was primarily the result of reduced root and stem dry mass as statistical differences between the other substrates were also evident for these particular tissues. Both substrates that contained biochar saw a decrease in root mass, though only the biochar only amended substrate produced a root dry mass reduction that was statistically significant from the other substrates. While leaf dry mass was reduced for the biochar-only amended substrate, there were no statistically significant differences between any of the substrates with leaf dry mass. Unlike the basil, there did not seem to be any significant effect on tissue dry mass from the presence of alumina in the substrate. The reduced root masses seen in both biochar-amended substrates could be explained by the slight increase in water retention due to the presence of biochar.

For the third (pepper) crop, there were no significant differences in TDM or fruit dry mass for any of the substrates. Fruit yields were very low for all treatments, as there was a significant issue with floral abscission from the pepper plants once they began reaching reproductive maturity. This increase in flower abortion seen in all substrates was most likely caused by incomplete pollination or pollination with non-viable pollen as the flowers would begin to set fruit and then die off. Pollination was

performed daily by hand with a generic electric toothbrush in an effort to overcome this. However persistent elevated temperatures may have affected the viability of the pollen. Hand pollination occurred in the later afternoon, when optimal pollination times are typically in the mornings. The presence of adequate pollinators, such as bumblebees if conducted in a greenhouse, or conducting the experiment outside with native pollinators would most likely have solved the pollination problem and improved fruit set.

3.5 Crop Growth Study Conclusions

Biochar and alumina did not produce any significant effects on reducing NO_3^- -N leaching from any substrate during the three cropping cycles (over 31 weeks). Biochar did produce a significant reduction in dissolved-P leaching in the biochar-only amended substrate. Biochar did not have any additional significant interactive effect in reducing dissolved-P, due to the significant effects of alumina on P adsorption in those substrates.

The presence of biochar in the substrate reduced lettuce and basil total dry mass, but biochar had no effect on the total plant dry mass or fruit yield of peppers. The presence of alumina in the substrate reduced leaf dry mass of basil, but these effects were not seen in lettuce or pepper.

Chapter 4: Crop Growth Study Tissue and Substrate Nutrient Analysis

4.1 Introduction

One consequence of the successful retention of N and/or P by the base substrate or amendment material is the possibility that the retention effect is so high that it can induce nutrient deficiencies. Nutrient deficiencies can decrease yield, reduce overall plant health, and produce a negative impact on the profitability of any agricultural production system. The hypotheses tested in this chapter were whether the additions of biochar and alumina have an effect on the content of elemental N, P, or elemental aluminum (Al) present in plant tissues. Additional hypotheses were whether the biochar and alumina amendments affect any change in the fates of N and P from the initial mixing of the substrates to the end of the crop growth studies. The formal hypotheses were the following:

- H1 Alternate: The addition of biochar will significantly affect the amount of total nitrogen taken up by crops from the substrate.
- H1 Null: The addition of biochar will not significantly affect the amount of total nitrogen taken up by crops from the substrate.
- H2 Alternate: The addition of alumina will significantly affect the amount of total nitrogen taken up by crops from the substrate.
- H2 Null: The addition of alumina will not significantly affect the amount of total nitrogen taken up by crops from the substrate.
- H3 Alternate: The addition of biochar will significantly affect the amount of total phosphorus taken up by crops from the substrate.

- H3 Null: The addition of biochar will not significantly affect the amount of total phosphorus taken up by crops from the substrate.
- H4 Alternate: The addition of alumina will significantly affect the amount of total phosphorus taken up by crops from the substrate.
- H4 Null: The addition of alumina will not significantly affect the amount of total phosphorus taken up by crops from the substrate.
- H5 Alternate: The addition of biochar will significantly affect the amount of total aluminum taken up by crops from the substrate.
- H5 Null: The addition of biochar will not significantly affect the amount of total aluminum taken up by crops from the substrate.
- H6 Alternate: The addition of alumina will significantly affect the amount of total aluminum taken up by crops from the substrate.
- H6 Null: The addition of alumina will not significantly affect the amount of total aluminum taken up by crops from the substrate.

4.2 Tissue Analysis Materials and Methods

All dried stem, leaf, and root tissue from each crop grown (as detailed in Chapter 3) was dried at 60 degrees C for at least 7 days after harvesting. Once dried, all plant tissues were milled with a benchtop impeller driven grinder for the analysis of various elements present in the plant tissue (AgroLab Inc., Harrington, DE). Each type of plant tissue from each treatment was carefully segregated during milling to fill a 20 mL scintillation vial. The only tissue not analyzed was the pepper fruit, due to limited dry mass, and were consequently not included in the nutrient analysis. Total N was analyzed using LECO combustion; all other elements were analyzed using

chemical digestion (with nitric acid and hydrogen peroxide) which were then measured for various concentrations of elements via ICP.

Substrate samples were also sent to AgroLabs Inc. for nutrient analysis. A 300 mL sample of the substrate from each replicate treatment was collected at the end of each crop, once all plant material had been harvested. A ‘pooled’ sample was taken from each replicate, mixing three samples from different locations around each tub at a ~50mm depth. Additionally, samples of freshly mixed, unplanted substrate were similarly prepared and analyzed by AgroLabs, Inc. The samples were analyzed using combustion analysis for total nitrogen and acid digestion/ICP analysis for total phosphorus.

4.3 Nutrient Analysis Results

4.3.1 Crop Nitrogen Uptake

Basil grown in the unamended (80M2:20MC) and biochar-amended (70M2:20MC:10BC) substrates took up significantly more N than alumina-amended substrates. There were significant differences ($p < 0.05$) in total plant N, leaf N, and stem N between the alumina plus biochar substrate (65M2:20MC:10BC:5AL) and all other substrates, although the substrate with only biochar (70M2:20MC:10BC) had a reduced total N uptake (1214 mg N) (Figure 4.1). There were also significant differences ($p < 0.05$) between this biochar-only substrate and the substrate with alumina which had the lowest plant N uptake at 538 mg N.

In the second (lettuce) crop, plant grown in the unamended (80M2:20MC) substrate had the highest total plant N uptake (1448 mg N; Fig. 4.2). Curiously, the only significant differences among plant N uptake among any substrate was in the

biochar-amended (70MS:20MC:10BC) substrate, where total plant N uptake was lower because of significantly lower root and leaf N.

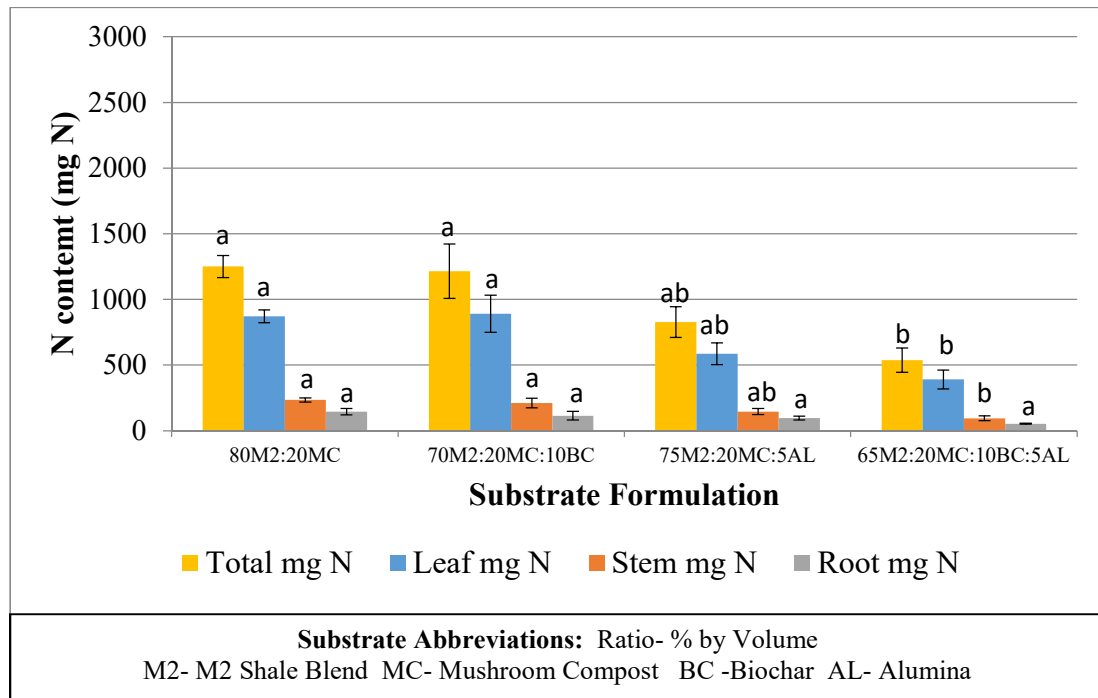


Fig. 4.1. Basil plant nitrogen (N) content, by tissue type from each substrate formulation. Mean separation in N content between substrates (letters) denotes $P < 0.05$ level of significance, based on Tukey's HSD test. Error bars denote standard error about the mean.

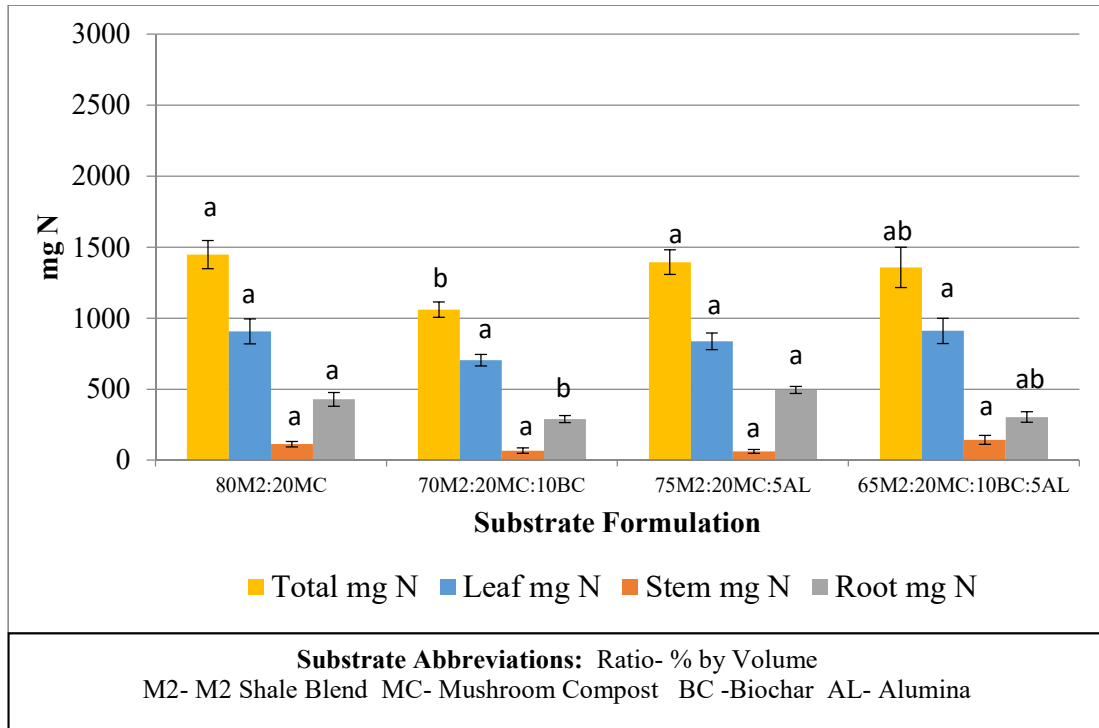


Fig 4.2. Lettuce plant nitrogen (N) content, by tissue type from each substrate formulation. Mean separation in N content between substrates (letters) denotes $P < 0.05$ level of significance, based on Tukey's HSD test. Error bars denote standard error about the mean.

Figure 4.3 illustrates the N uptake, by tissue, by the third (pepper) crop in all substrates. In contrast to the lettuce crop, peppers grown in the biochar-amended ((70MS:20MC:10BC) substrate had the highest total N uptake (2933 mg N). Notably however, there were no significant differences in N uptake among any plant tissue type or substrate treatment.

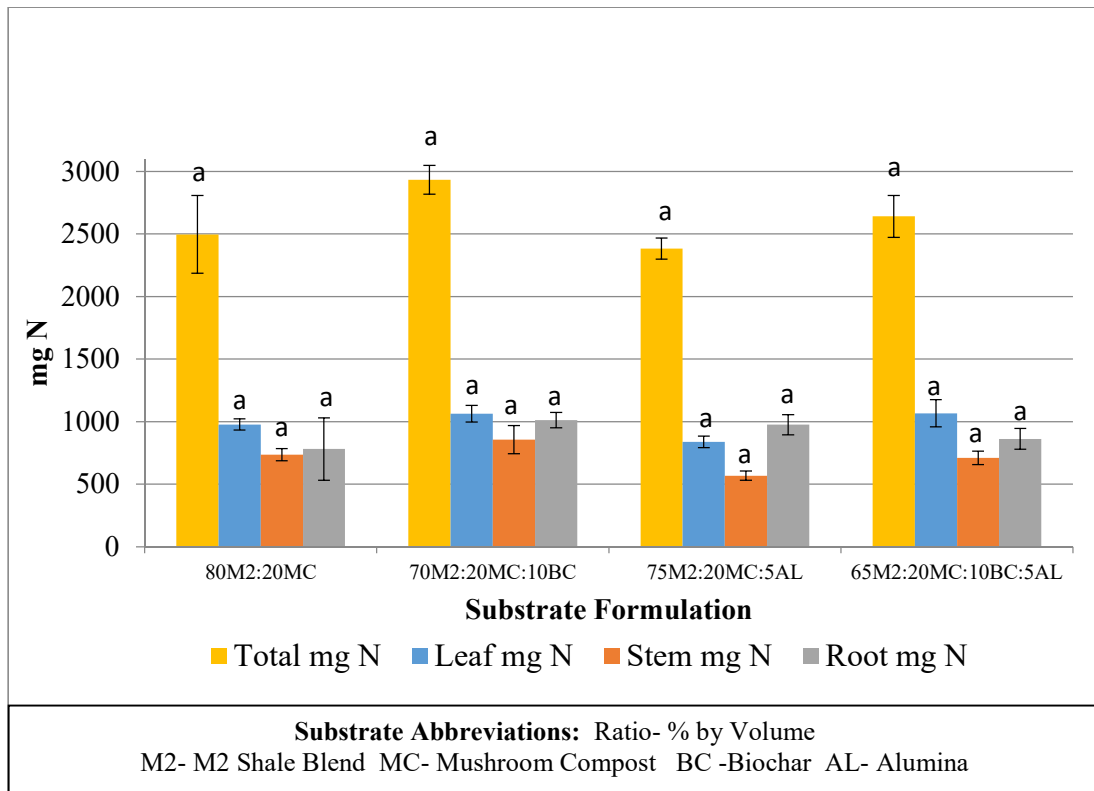


Fig. 4.3. Pepper plant nitrogen (N) content, by tissue type from each substrate formulation. Mean separation in N content between substrates (letters) denotes $P < 0.05$ level of significance, based on Tukey's HSD test. Error bars denote standard error about the mean.

4.3.2 Crop Phosphorus Uptake

Basil had the greatest P uptake from the unamended (80M2:20MC) substrate (170 mg P), but there were no significant differences in plant P uptake this and the biochar-amended (70M2:20MS:10BC) substrate (Fig. 4.4). There were however significant differences ($p < 0.05$) in the total and most tissue P uptake between these unamended substrates and the two substrates amended with alumina. The biochar plus alumina substrate (75M2:20MS:10BC:5AL) had the lowest total P uptake at 42 mg P (Fig 4.4).

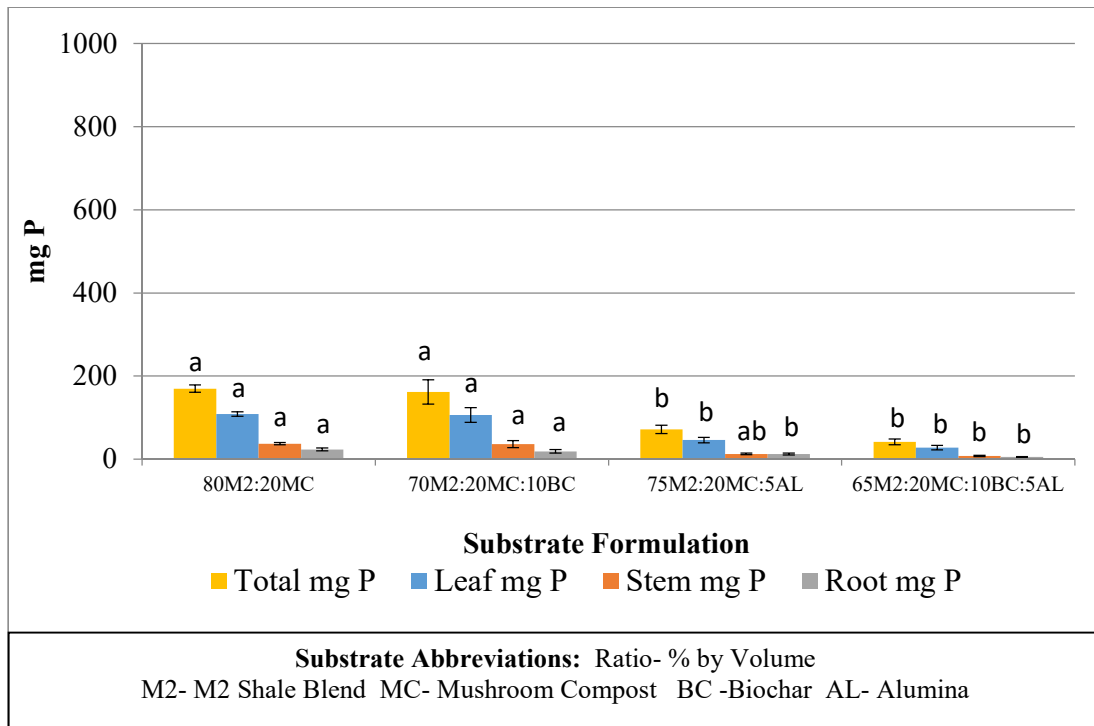


Fig. 4.4. Basil plant phosphorus (P) content, by tissue type from each substrate formulation. Mean separation in P content between substrates (letters) denotes $P < 0.05$ level of significance, based on Tukey's HSD test. Error bars denote standard error about the mean.

During the second cropping phase, the highest P uptake by lettuce again occurred in the unamended (80:M2:20MS) substrate, but which again was not significantly different from the P uptake by the plants in the biochar-only (70M2:20MS:10BC) amended substrate (Fig 4.5) There were no significant differences in plant tissue uptake between these two substrates. Similar to basil however, there was significantly less ($p < 0.05$) total P taken by lettuce plants in the two alumina-amended substrates. Notably however, there were no significant differences in leaf or stem P uptake between any treatment; the only significantly different uptake by tissue was by the roots in the biochar and alumina-based (75M2:20MS:10BC:5AL) substrate. Overall, lettuce took up more between two and three times the amount of P, compared to basil (Figs. 4.4 and 4.5).

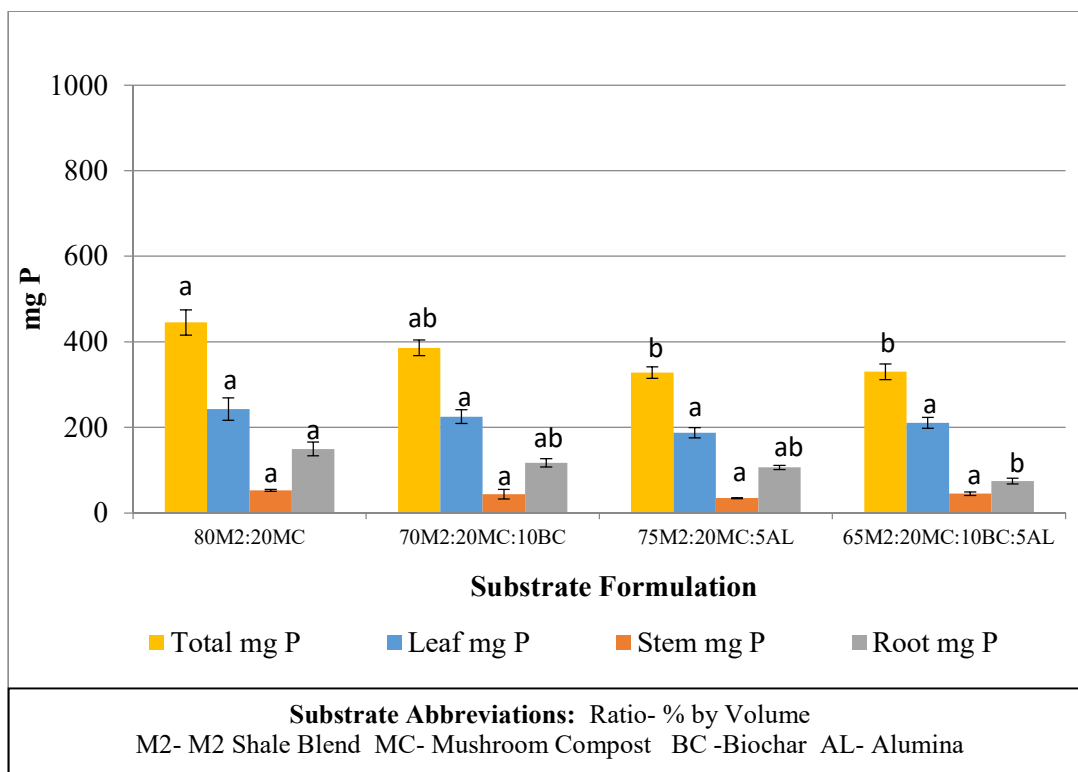


Fig. 4.5. Lettuce plant phosphorus (P) content, by tissue type from each substrate formulation. Mean separation in P content between substrates (letters) denotes $P < 0.05$ level of significance, based on Tukey's HSD test. Error bars denote standard error about the mean.

Despite the relatively low availability of P in all substrates during the third cropping (pepper) cycle (see Chapter 3; Fig. 3.13), the pepper crop took up between 417 and 755 mg P over this crop cycle (Fig. 4.6). Similar P uptake dynamics were seen in peppers as in the two previous (basil and lettuce) crops. Plants in alumina-amended substrates took up significantly less P than unamended substrates (Fig. 4.6), although the only significantly ($P < 0.05$) lower P uptake was seen in stem and leaf tissue in the (75M2:20MS:10BC:5AL) substrate. No other significant differences were seen among tissues, and no P-deficiency symptoms were noted at any time during the cropping cycle, even in the alumina-amended substrates as there was no significant reduction in TDM or root dry mass (see Chapter 3).

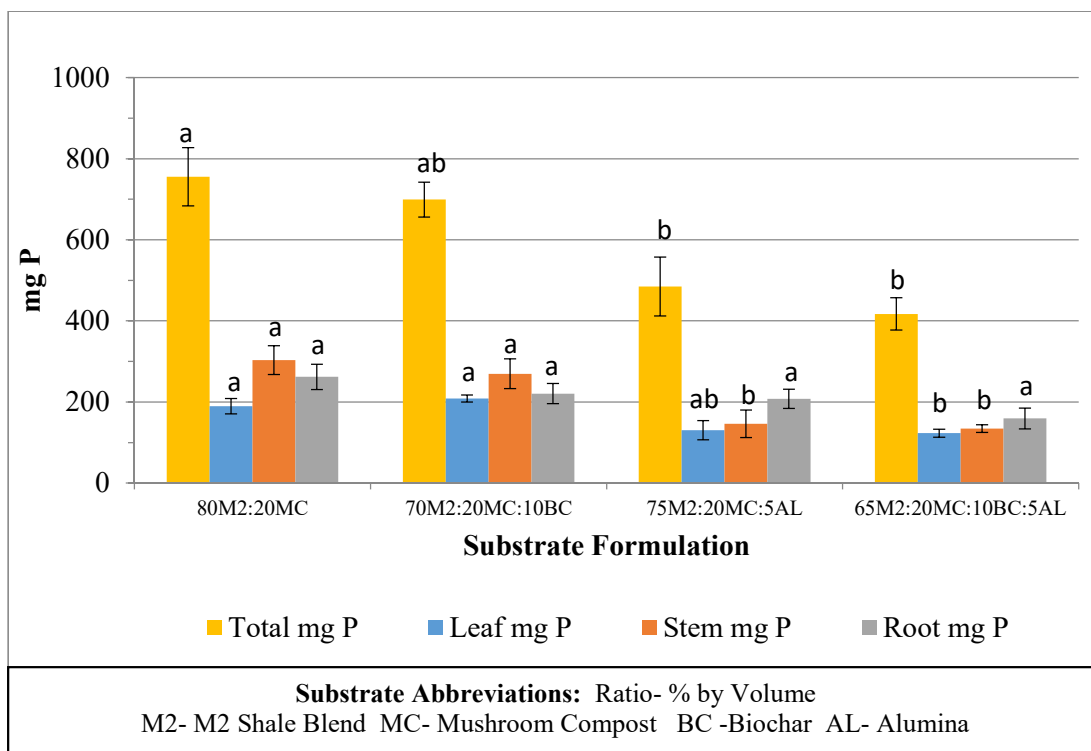


Fig. 4.6. Pepper plant phosphorus (P) content, by tissue type from each substrate formulation. Mean separation in P content between substrates (letters) denotes $P < 0.05$ level of significance, based on Tukey's HSD test. Error bars denote standard error about the mean.

4.3.3 Crop Aluminum Uptake

Figures 4.7 through 4.9 provide the aluminum (Al) uptake data, by crop and tissue type. Most notably, Al uptake was sequestered almost entirely in root tissue by all three crops. Al uptake by basil and lettuce crops was relatively low compared to pepper, even for the two alumina-amended substrates, although significantly higher than for the unamended (80M2:20MC and 70M2:20MC:10BC) substrates (Figs. 4.8). Interestingly, there seemed to be some suppression of Al uptake in biochar-amended (70M2:20MC:10BC and 65M2:20MC:10BC:5AL) substrates, in both basil and lettuce crops (Figs. 4.7 and 4.8).

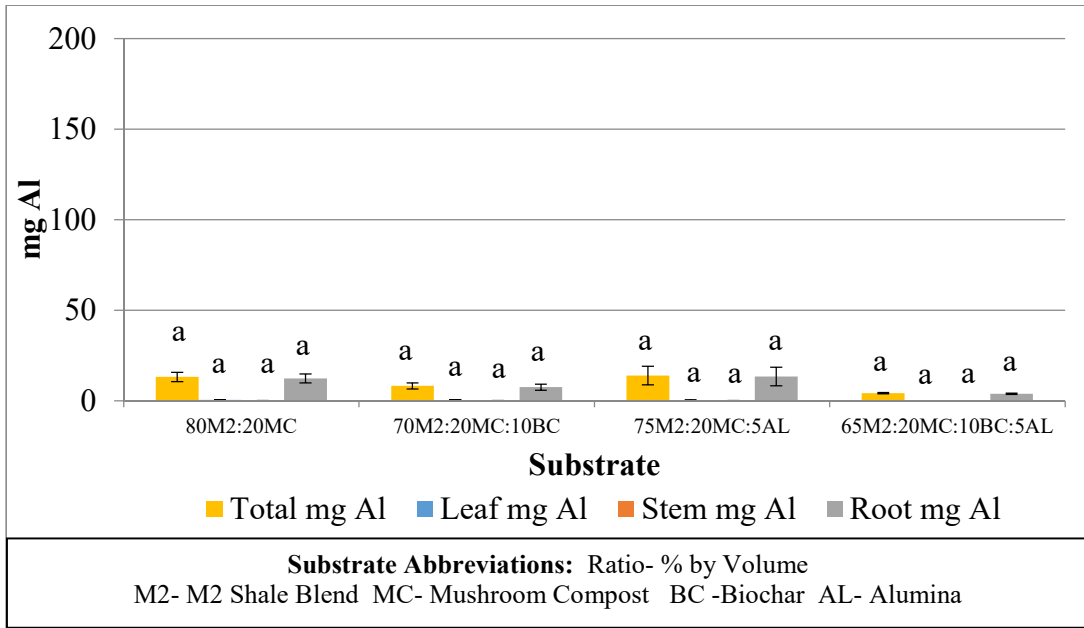


Fig. 4.7. Basil plant Aluminum (Al) content, by tissue type from each substrate formulation. Mean separation in P content between substrates (letters) denotes $P < 0.05$ level of significance, based on Tukey's HSD test. Error bars denote standard error about the mean.

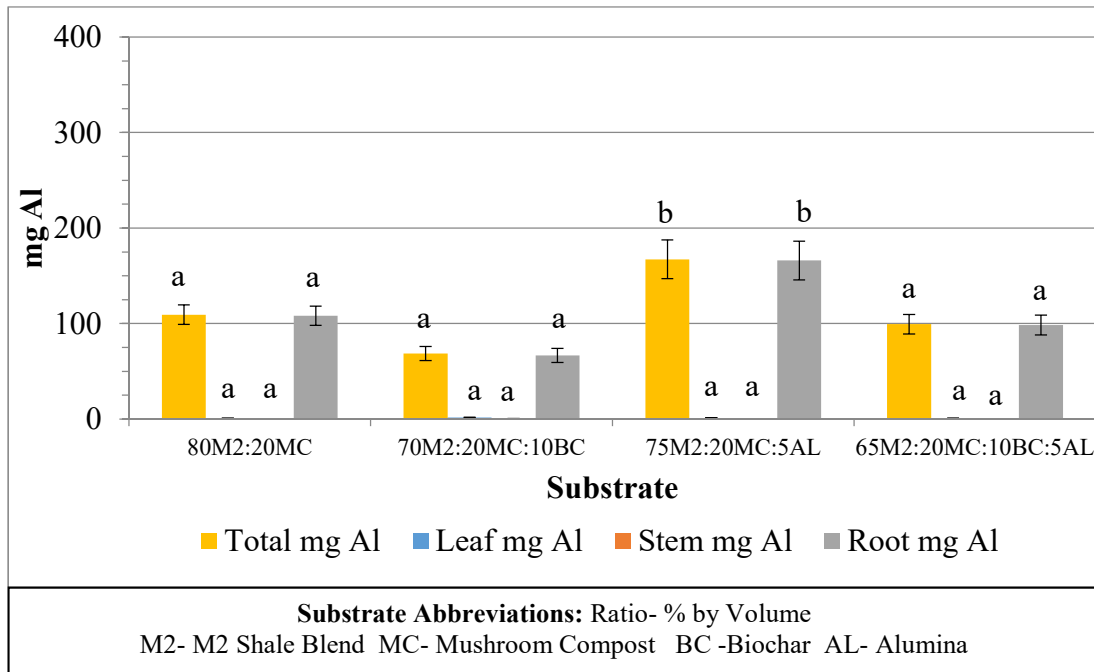


Fig. 4.8. Lettuce plant Aluminum (Al) content, by tissue type from each substrate formulation. Mean separation in P content between substrates (letters) denotes $P < 0.05$ level of significance, based on Tukey's HSD test. Error bars denote standard error about the mean.

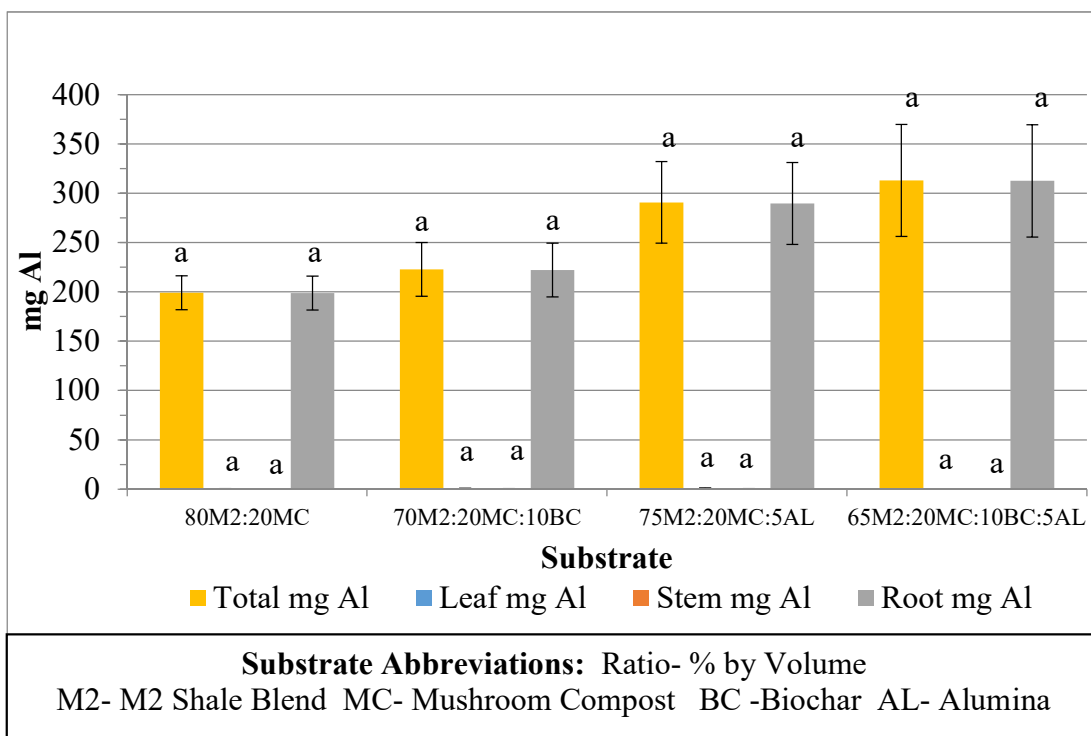


Fig. 4.9. Pepper plant Aluminum (Al) content, by tissue type from each substrate formulation. Mean separation in P content between substrates (letters) denotes $P < 0.05$ level of significance, based on Tukey's HSD test. Error bars denote standard error about the mean.

Aluminum uptake by the pepper crop (Fig. 4.9) was almost double that taken up by the basil crop. Interestingly, more than 200 mg Al was taken up by plants in the non-alumina amended substrates, indicating that there must have been an unaccounted source of Al in all substrates, either from the M2 or perhaps the MC base components. There was also no apparent suppression of this Al uptake by pepper plants in the biochar-amended substrates (Fig. 4.9), as was seen in the two prior crops. There were no statistically significant differences in any of the tissues between substrates in basil and peppers in Al uptake. 75M2:20MC:5AL was significantly higher in root and total mg Al in lettuce.

4.3.4 Nutrient Mass Balances

Table 4.1 shows the N mass balance for all the components for, each substrate. There were no significant differences among substrates in the initial total substrate nitrogen (N), although the analyzed N in the 65M2:20MC:10BC:5AL substrate was substantially less (2700 – 3400kg) than the other three substrates (Table 4.1). Interestingly, there was substantially more N remaining in this particular substrate at the end of the three growth cycles (6780 kg vs. 3357 – 4736kg in the other treatments), although none of these differences were significant, due to the high SEs (low replication). There were no significant differences between the substrates in the amount of N (NO₃-N) that was cumulatively lost in the leachate over the entire tub study. A total of 12.5 kg/ha of N was added via fertilization to each tub as plant need arose (as described in Chapter 3). The unaccounted N totals the initial and final substrate N, less N leached and taken up by the plants over the course of the three crop growth cycles. The seeming positive N balance for the 65M2:20MC:10BC:5AL substrate was an artifact of the apparent initial sequestration of N by this substrate before planting.

Table 4.1. Average nitrogen (N) mass balance for each substrate with standard errors about the mean (SE). Numbers with (-) sign denote plant N uptake or total leachate N over the three crop growth cycles; all other numbers denote N inputs. Letters denote significance differences (P<0.05) between substrates (within columns).

Substrate Formulation	Initial Substrate N (kg/ha)	Final Substrate N (kg/ha)	Total Nitrate Leached (kg/ha)	Plant Nitrate Uptake (kg/ha)	N Fertilization (kg/ha)	Unaccounted N (kg/ha)
80M2:20MC	9246 (a)	3357 (a)	-424 (a)	-162 (a)	12.5	-5295 (a)
80M2:20MC SE	1414.4	479.4	49.8	9.6	0.0	1806.3
70M2:20M2:10BC	8690 (a)	4864 (a)	-385 (a)	-162 (a)	12.5	-3232 (a)
70M2:20M2:10BC SE	1378.1	882.0	51.0	8.3	0.0	823.9
75M2:20MC:5AL	8561 (a)	4736 (a)	-463 (a)	-143 (a)	12.5	-3256 (a)
75M2:20MC:5AL SE	869.8	1207.3	14.2	4.2	0.0	1953.4
65M2:20MC:10BC:5AL	5828 (a)	6780 (a)	-393 (a)	-141 (a)	12.5	+1512 (b)
65M2:20MC:10BC:5AL SE	1215.2	1024.4	9.4	12.3	0.0	1664.1

Table 4.2 shows the P mass balance for all the components, for each substrate. There were significant differences ($p < 0.05$) in total P present in the substrate amended with biochar and those substrates that did not contain biochar, prior to planting. Although there were no significant differences in P between substrates at the end of the study (due to high SE's), there was again a net increase in the P in the 65M2:20MC:10BC:5AL substrate (2060kg/ha vs. 1930kg/ha). The 70M2:20MC:10BC substrate also showed a similar response with 295kg/ha P being available at the end vs. 2117kg/ha P being seemingly only available at the beginning of the study. Both of the positive P balances for these substrates (Table 4.2) were again likely an artifact of the apparent initial sequestration of P by the amendments to these substrates before planting.

Table 4.2. Average phosphorus (P) (Dissolved Phosphorus = DP) mass balance for each substrate with standard errors about the mean (SE). Numbers with (-) sign denote plant P uptake or total leachate P over the three crop growth cycles; all other numbers denote P inputs. Letters denote significance differences ($P < 0.05$) between substrates (within columns).

Substrate Formulation	Initial Substrate P (kg/ha)	Final Substrate P (kg/ha)	Total DP Leached (kg/ha)	Plant P Uptake (kg/ha)	P Fertilization (kg/ha)	Unaccounted P (kg/ha)
80M2:20MC	3612 (b)	2290 (a)	-6.4 (c)	-42.8 (a)	0.5 (a)	-1273 (a)
80M2:20MC SE	358.8	636.5	0.2	3.3	0.1	775.8
70M2:20M2:10BC	2177 (a)	2953 (a)	-5.2 (a)	-39.0 (a)	0.5 (a)	+819 (a)
70M2:20M2:10BC SE	248.0	408.6	0.4	1.7	0.1	216.8
75M2:20MC:5AL	3074 (b)	2780 (a)	-1.3 (b)	-27.6 (b)	0.4 (a)	-265 (a)
75M2:20MC:5AL SE	139.5	588.9	0.1	2.0	0.1	509.8
65M2:20MC:10BC:5AL	1930 (a)	2060 (a)	-1.3 (b)	-24.7 (b)	0.4 (a)	+155 (a)
65M2:20MC:10BC:5AL SE	274.9	214.9	0.2	1.7	0.1	406.4

There were significant differences ($p < 0.05$) in P lost to leachate as well as the amount of P taken up by plants between the two substrates that contained alumina, compared to substrates that had none. Virtually no P was leached from these substrates over the 31 weeks of the three crop studies (Table 4.2). Although no P was applied with fertilizations, the small amount of P gained came from the use of tap water in supplemental irrigation for the peppers, as it contains low levels of phosphate as part of local water treatment (DCWASA, 2004). There were no significant differences between unaccounted P from each substrate, although a substantial amount of the P budget (35% = 1273/3612) was not recovered from the unamended (80M2:20MC) substrate.

4.4 Nutrient Analysis Discussion

The main objective of these mass balance calculations was to quantify and understand the amendment effects on N, P availability and plant uptake. Plant tissue analyses allow us to see the amount of a particular nutrient portioned by the each crop, to explain any “hidden” nutrient deficiencies which could explain differences in yield (dry mass), documented in Chapter 3. The initial and final substrate analyses allow us to better understand the magnitude of N and P leaching losses and uptake in each crop, and see if any substrate amendment effects impacted these dynamics. Substrate analysis and crop tissue analysis also allow us to understand the N and P crop needs, and better budget for particular crops/growing seasons, to influence future incorporation rates for both the compost source and amendment rates. These dynamics then can help determine the risk for compost additions, nutrient leaching and the overall nutrient requirements for cropping these green roof substrates.

Neither biochar nor the alumina amendments had any significant effect on the uptake of N by any of these crops, over the 31 weeks. This was consistent with the non-significance of amendments on N seen in the short-term leaching studies in Chapter 2 and from the dry mass (yield) analyses in Chapter 3 and from the N tissue contents seen in Figs. 4.1, 4.2 and 4.3 for basil, lettuce and pepper crops, respectively. Approximately 5% of the total N was leached from these substrates over 31 weeks, with less than 2% being taken up by the three crops (Table 4.1).

The mass balance for N demonstrates that uptake efficiency for N was very low with the majority of N being unaccounted for; include the leaching of N in forms other than $\text{NO}_3\text{-N}$, loss of fine compost particles in the leachate, denitrification, and unanalyzed fruit tissue. In constructed wetlands for stormwater management nitrate is often seen as one of the lower constituents of total N runoff. The majority of nitrogen based runoff appears to be contained in organic particulate and ammonium ions and should be included in further study (Magnum et al., 2020). Similar unaccounted for losses have been noted in N mass balance studies (Lea-Cox et al., 1996; Ristvey et al., 2007). With the 65M2:20MC:10BC:5AL substrate, the average residual N increased between before planting and at the end of the study

The addition of biochar did not have any significant effects on the concentration of P in plant tissues except in lettuce. The biochar-only amended (70M2:20MC:10BC) substrate however reduced total plant dry mass, with a consequent reduction in P content, given that there were no significant differences in dry mass between the 70M2:20MC:10BC and 80M2:20MC substrates.

The addition of alumina significantly reduced the P content of all crop tissues. Despite this, no P deficiency symptoms were seen in lettuce or peppers, which are typically expressed as stunted growth, purpling of the leaves, and reduction in root mass. A significant reduction in basil growth was however seen in the 65M2:20MC:10BC:5AL substrate (Chapter 3); basil is known to be susceptible to damage from elevated levels of N and P. Given that the substrates were freshly mixed when the basil was planted and alumina retains P nutrients, the presence of excessive N and P may have contributed to reduced plant growth rather than nutrient deficiency (Nurzyńska-Wierdak, 2012). A significant reduction in basil dry mass was not seen in the other alumina-amended (75M2:20MS:5AL) substrate.

Another potential reason for the reduction in plant dry mass could have been the concentration of aluminum in plant tissues. Aluminum toxicity could result in symptoms such as severely decreased root mass, but high Al contents (noted in Figs 4.7 – 4.9) only had a significant effect on basil root mass (Fig. 3.4), but not in lettuce or pepper (Figs. 3.5. and 3.6, respectively). It was therefore concluded that direct Al toxicity with the use of alumina amendments was unlikely the cause of any plant dry mass reduction, except perhaps in basil. Aluminum toxicity was also unlikely to occur in any of the prepared substrates in this study as it generally occurs in substrates with a pH of less than 5 (Panda, et al., 2009).

The mass balance for P demonstrates that plants use much less P than N; as such, much less P was unaccounted for, compared to N. What was notable was the very high levels of available P in all substrates, from the mushroom compost source. However, what was surprising was the relatively low amounts of P leached from all

substrates, which were reduced to under 0.2 ppm dissolved-P by the alumina amendment compared to between 1.0 to 2.0 ppm dissolved-P. It should be noted that even the lowest concentrations seen in in the alumina amended substrate leachates far exceed the recommended 10 ppb dissolved-P (0.01 ppm dissolved-P) (Florida, 2006). Unaccounted losses of P could include leaching of P in other forms that were not as dissolved P, the loss of particulate mineral and organic matter that may be saturated with P via leaching, and the very small amounts of P in fruit tissue that was not analyzed.

There were no significant differences between any of the substrates in TP after three crops (31 weeks). However, there were significant differences in TP in substrates containing biochar at the beginning of the study. Every replicate started with the same volume (mass) of compost; therefore these differences should not be seen. It is possible that this was due to a sampling error due to the fragility of dry, freshly mixed substrate and the transport of substrate samples. One major disadvantage of the substrates used in this study was their instability when disturbed. Even slight disturbances to dry substrate would cause the individual components to settle out and form layers by density. The addition of the super-fine biochar powder likely made this effect worse as it evenly coats each substrate particle while mixing. With larger substrate particles covered in a layer of biochar powder, particles of compost (the source of all P in the substrate) would have a more difficult time adhering to the M2 green roof substrate, more so than they already do in a dry substrate. Transportation of the substrate samples to the testing facility may have subjected these substrates to disturbance and may have caused the less dense and

nutrient poor M2 to rise to the surface. This may have increased the probability of this poorly homogenized layer being sampled for soil testing, causing these substrates to test artificially low for TP. If TP was tested first from the soil sample bag, then a deeper sample would have to be taken in order to test for TN, which may be why the reduction in initial TP was seen, but not initial TN from freshly mixed substrates before they were planted. While the low initial values of TP before planting cause gains in residual P, the differences in residual P were still not significantly different between all substrates.

Both the N and P mass balances show that the substrates were not depleted after the three cropping cycles (totaling 31 weeks). Small amounts of N fertilization were necessary to maintain plant health with the pepper crop, but the amount of available N remaining in the substrate was still quite high at the conclusion of the study, and could be viewed as a slowly-available source for N for future plantings. Phosphorus fertilization was not necessary as the substrates were not significantly depleted of P over the 31 weeks. Incidental phosphorus fertilization with supplemental irrigations of tap water was likely insufficient to provide enough P for adequate plant growth. The mass balances indicate that there was a relatively large reserve of N and P that could have been utilized by further crops cycles, although it is likely that supplemental fertilization might have to be used on a periodic basis to optimize crop yield.

4.5 Growth Study Nutrient Analysis Conclusions

The addition of biochar had no significant effect on plant N uptake. The amount of N removed was crop dependent, with relatively insignificant amounts

being removed by basil and lettuce crops, in comparison to that supplied by a 20% addition of mushroom compost. It is likely that half of this amount of mushroom compost would be adequate, especially during initial cropping cycles, which would avoid the potential for large leaching losses. Biochar did not have any significant effect on P uptake by any of the three crops. The addition of alumina had no significant effect on plant N uptake or tissue content, except for basil (as the first crop in the cycle). The addition of alumina significantly reduced plant P uptake and the availability of P on the amended substrates, significantly reducing the amount of P leached to almost zero. The addition of biochar and/or alumina did not have a significant effect on the amount of available N and P that was left in the substrates at the conclusion of the study.

Chapter 5: Application and Significance

5.1 Application and Significance

While alumina and biochar have been used as amendments in crop production to increase nutrient retention, they have yet to be studied in long term green roof applications. Green roof substrates may be ideal environments for using these amendments, since traditional green roof media typically have high porosity and low nutrient retention properties. This research sought to determine if compost source, combined with biochar and alumina amendments were able to increase nutrient retention of nitrate ($\text{NO}_3\text{-N}$) and available phosphorus (dissolved elemental P), and long-term availability for crop growth, while reducing leaching losses of N and P with simulated rainfall / irrigation events.

One of the things learned through this study was that the reduction of N and P leachate into the environment begins with substrate component selection, particularly of organic matter. There were significant differences seen between the amount of N and P leached by the SmartLeaf substrates versus the mushroom compost substrates. Mushroom compost substrate started with a much higher native nutrient content than SmartLeaf. Crop growth studies were not performed on any SmartLeaf substrates, but quantifying crop growth in SmartLeaf as future work would indicate whether or not it is necessary to begin with organic matter that has high nutrient loads in the substrate like those made with mushroom compost. The use of the mushroom compost represented a “worst-case” scenario in the crop growth studies to give the amendments as much potential as possible to reduce nutrient leachate, but given that much of the nutrients (especially nitrogen) leached out very early (by week 8, Figure

3.10), SmartLeaf substrates may still be viable for agricultural crop production while reducing the large initial flush of nutrients.

Biochar was chosen for this study due to its ability to increase a substrate's cation exchange capacity (CEC) which is correlated with higher soil fertility. In this study, biochar had no effect on N or P retention, nor any tangible effect on crop growth. Biochar also did not have any real effect on reducing N or P leaching from the mushroom compost amended substrate. Increasing the ratio or using a different biochar formulation could be used, but this is likely to impact water-holding capacity and air-filled porosity, which could negatively affect crop growth. There was some evidence of this in the crop growth studies, although this was not definitive. Additional research would need to be done to provide clear answers as to any nutrient retention benefits for biochar.

Nevertheless, in green roof applications where stormwater mitigation is an objective, the addition of biochar could increase stormwater retention performance. Due to biochar's low density, availability, low cost, and neutral (possibly positive) impact on crop production, the addition of biochar could provide some long-term benefits for a green roof substrate that is used in agricultural production or simply as green space.

Alumina was chosen for this study due to evidence that alumina binds P and may potentially provide a sink for soluble P when incorporated into soils. Due to the high eutrophication potential of P, the reduction of P in stormwater runoff is a priority for urban areas within the Chesapeake Bay watershed. Alumina mixed into the M2

green roof substrate at 5% (v/v) provided significant reductions (~80%) in dissolved leachate P, when compared to unamended, or biochar-only amended substrates.

There was a significant reduction in plant yield during the first cropping cycle with basil in substrates containing alumina, but this could not be attributed solely to the incorporation of alumina. As the substrate underwent further cultivation and leaching with lettuce and pepper crops, this yield reduction in alumina-amended substrates was not evident when compared to unamended substrates. While alumina did reduce the amount elemental phosphorus content in plant tissues (reduced uptake), common signs of phosphorus deficiency were not seen in any of the crops grown. This may indicate that alumina not only retains phosphorus in the substrate profile, it provides adequate available-P for crops to sustain growth. The adsorption of P by alumina is not well understood in green roof substrates, but it was evident from the crop growth studies, leachate and mass balance results that P was available for an extended period of time, even from alumina-amended substrates and no signs of P deficiency were noted in any crop. Importantly, P leaching was reduced to lower levels by alumina (though the lowest concentrations exceeded recommended dissolved-P levels), which illustrates that it could be an important tool in sequestering P from compost sources that are inherently high in P, such as mushroom compost. In states such as Maryland which strictly regulate the amount of P that can be applied to crops on an annual basis, this retention and slow release of plant-available P is invaluable.

5.2 Future Study and Recommendations

Longer-term crop growth studies are required to establish the lasting effects of P mitigation using alumina amendments. It is unclear how long alumina incorporated into green roof substrate will continue to adsorb or release available P (over long-term cultivation, freeze-thaw cycles, etc.). Adding additional seasons to the crop growth studies will better show these long term effects both on plant productivity and phosphorus leaching. There could also be other methods to use alumina in green roof systems, such as using it as a bio filter to treat downspout runoff, or in bioretention facilities at grade, before stormwater runoff entered local waterways. While results for alumina are promising, care should be taken in implementation of these results, due to some potential yield reductions just after incorporation. More crop testing with a broader variety of crops would confirm if early reductions in yield are an aspect of the alumina itself or a sensitivity of a particular crop.

Appendix

A.1. Column Study Substrate Mixing:

1. Ensure that all substrate materials are approximately air dry before mixing.
While the formulations of each substrate are mixed by volume, the presence of excess water can cause materials such as the compost to swell and be measured with less accuracy.
2. To assemble the primary mineral component M2 blend that will be used throughout the entire experiment, add by volume 75% washed M2 (3 parts) with 25% unwashed M2 (1 part) into the drum of a clean, dry electric cement mixer.
3. Once the M2 materials have been added in the correct proportions, move the cement mixer into its mixing position and blend for 2 minutes to ensure complete homogenization.
4. Dump out completed M2 blend into a clean tray and store in sealed 19L (5-gallon) buckets until needed for final substrate mixing.
5. Using a 1 gallon plastic pail, measure out the volumetric proportions of each substrate into the drum of a clean electric cement mixer. The volumetric proportions of each substrate formulation for the column study are listed in Table 1.1. Each level bucketful of material represents 10% of the volume of the final substrate volume. A total of 10 buckets of material (approximately 43L) make up a complete substrate mix. As an example, each substrate contains 20% by volume an organic compost material. This represents two full, level buckets of respective compost being added to the mixer. 5% is half the total volume of a full bucket.

6. Once all materials have been added in the correct proportions, move the cement mixer into its mixing position and blend for 2 minutes to ensure complete homogenization.
7. Dump out completed substrate into a clean tray and store in sealed 19L (5-gallon) buckets until needed.

A.2. Analyze Collected Samples for Nitrogen from Nitrate:

1. Remove samples in scintillation vials to be analyzed from the freezer and thaw at room temperature for 16 hours. Do not let samples stay out for more than 24 hours as the nitrate is not stable in a non-sterile environment at room temperature. Do not thaw more than 50 samples at a time.
2. When fully thawed, run each sample with one vial from the HANNA Nitrate kit through the spectrophotometer using the analyzing a sample for Nitrogen from Nitrate procedure.
3. Return each sample to the freezer. Do not let samples stay out for more than 24 hours.
4. Apply regression curve formula from Nitrogen from Nitrate Calibration Curve procedure to each value for highest accuracy.

A.3. Analyzing a Sample for Nitrogen from Nitrate:

1. Each nitrate kit provides the consumable materials to test 50 samples. These materials included 50, 13 mm vials filled with chromotropic acid and 50 sachets of nitrate reagent powder.

2. Set the spectrophotometer to the proper factory installed program stored on the device for testing nitrogen from nitrate.
3. Using 1 vial from the Hanna Nitrate kit, carefully remove the lid and place 1.00 mL of a sample using a 1.000 mL pipette with a new disposable tip for each sample.
4. Screw the lid back on the vial and slowly invert the vial 10 times. The vial will begin to heat up when the sample is dissolving into the solution in the vial.
5. Place the vial into the spectrophotometer and zero the machine. No reactions have taken place and the vial is colorless at this stage.
6. After zeroing, remove the vial from the spectrophotometer and add all of the contents of one sachet of nitrate reagent powder to the vial.
7. Invert the vial 10 times to dissolve the reagent powder. The color will change to yellow indicating the presence of nitrate.
8. Insert the vial into the spectrophotometer and allow it to stand and react undisturbed for 5 minutes. The spectrophotometer has a built in timer set for 5 minutes in the program on the machine.
9. Once 5 minutes has passed, the button for measuring the sample can be pressed and in a few seconds, the spectrophotometer displays the detected concentration as ppm nitrogen from nitrate.

A.4. Nitrogen from Nitrate Calibration Curve:

1. Prepare a 1.0L solution of 100 ppm nitrogen using potassium nitrate, DI water, and a 1.0L volumetric flask.
2. Use this 100 ppm nitrogen stock solution to create a panel of diluted solutions measuring 25 ppm, 20 ppm, 10 ppm, 5 ppm, 2 ppm, 1 ppm, 0.5 ppm, 0.2 ppm, and 0.1 ppm nitrogen.
3. Using the A.3 procedure, run two vials of each serial dilution through the spectrophotometer.
4. Average the values of the two vials for each dilution.
5. Plot these measured values on the x-axis of a scatter plot by the expected values on the y-axis.
6. Calculate a linear regression curve and R² for the measured data by the expected data.
7. Apply linear regression curve to measured values from the spectrophotometer.

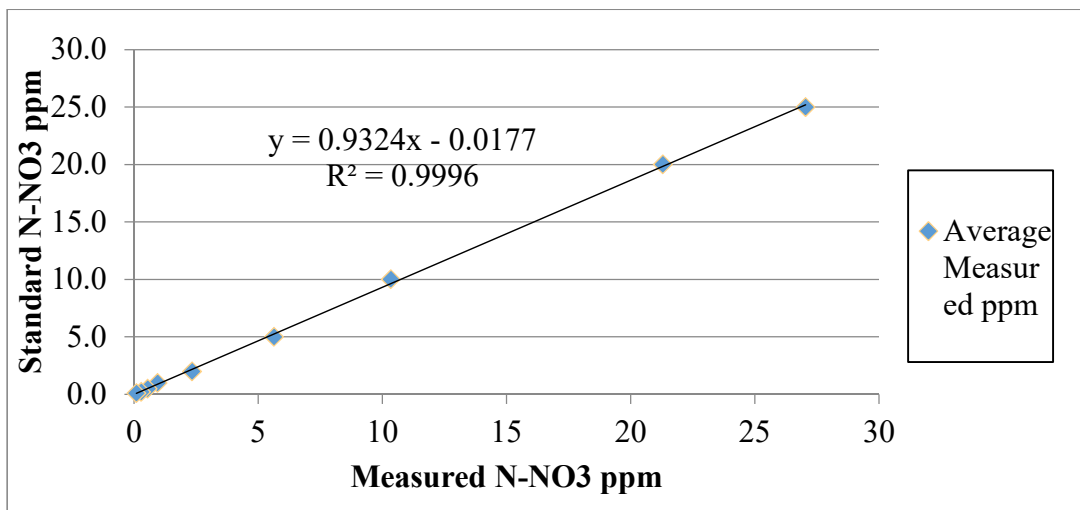


Fig. A.4.1 Calibration curve of HANNA Spectrophotometer.

Table A.4.1 Repeated Measurements of column study samples.

	Sub 14, column C, WN7	Sub 4, column L, WC2	Sub 13, column A, WC3
Sample 1	4.2 ppm NO ₃ ⁻ -N	13.9 ppm NO ₃ ⁻ -N	6.6 ppm NO ₃ ⁻ -N
Sample 2	5.1 ppm NO ₃ ⁻ -N	13.3 ppm NO ₃ ⁻ -N	5.9 ppm NO ₃ ⁻ -N

A.5. Dissolved Phosphorus Sample Preparation for Column Study:

1. Samples for phosphorus analysis were set offsite to be analyzed by AgroLabs in Harrington, Delaware where they were analyzed by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP).
2. Remove each analysis sample from the freezer and allow to thaw for 16 hours.
3. Place 10 mL of each sample into a new, clean 20 mL scintillation vial.
4. Label the scintillation vials for the AgroLabs sample submission sheet.
5. Package the scintillation vials so that they will not spill or be damaged in transit. The cardboard trays that the scintillation vials come in are well suited for this.
6. Send vials via FedEx to AgroLabs in Harrington, DE.
7. After several days, AgroLabs emails a document of the results for the dissolved phosphorus concentration of each sample.

A.6. Physical Properties Determination

1. Clean out five identical plastic jars with tightfitting lids
2. Completely fill each jar with water to determine the total volume of each jar.
Record the volume as V_{Jar} .

3. Drill eight 3mm holes in the bottom of each jar through the surfaces that touch the table when set upright. This ensures that the holes are at the lowest point of the jar.
4. Weigh each of the five empty jars with the lid attached and record each as W_{Jar}
5. Completely fill each jar with the substrate to be tested. Gently tap each jar five times on the table to settle the substrate. Add more substrate and tap again if head space is revealed. Do not put on the lid.
6. With the jars open, place them into the pan that can be filled to cover the jars.
7. Very slowly fill the pan with water over the course of three hours to push out all of the air in the voids in the substrate. The perforated holes allow the jar to fill from the bottom up.
8. Fill the pan until the water level is about 0.5 cm below the top surface of the substrate.
9. Let the water stand for another 15 minutes to fully conduct into this last layer of substrate.
10. Tightly screw on the lids to each jar while disturbing the jar as little as possible.
11. Carefully remove each full jar from the water bath and dry the outside with a towel.
12. Weigh each of the saturated jars and record their weight as W_{Sat}

13. Over a container, loosen the lid and allow excess water to drain out of each jar through the holes in the bottom for a minimum of 30 minutes or until the substrate stops draining.
14. Weight each drained jar and record it as W_{Drain}
15. Weigh five foil pans large enough to hold the contents of each jar. Record this weight as W_{Pan} .
16. Empty the jars into the pans ensuring all substrate is scraped out. Oven dry the substrate for at least 48 hours or until the weight stops changing between days.
17. Weight the substrate in the pan and record this weight as W_{Oven}
18. Calculate water holding capacity (WHC), air filled porosity (AFP), total porosity, and bulk density (wet and dry) for each jar.
19. The calculations for the physical property analysis portion of this experiment are as follows:

W_{Jar} =Weight of empty jar with lid

W_{Sat} =Weight of fully saturated jar of substrate

W_{Drain} =Weight of jar of substrate after draining from fully saturated

W_{Pan} =Weight of foil pan for oven

W_{Oven} =Weight of oven dried substrate in foil pan

V_{Jar} =Volume of jar

Thus:

Water Holding Capacity (%WHC) = $\frac{((W_{\text{Drain}} - W_{\text{Jar}}) - [W_{\text{Oven}} - W_{\text{Pan}}])}{V_{\text{Jar}}} \times 100$

Percent Air Filled Porosity (%AFP) = $\frac{(W_{\text{Sat}} - W_{\text{Drain}})}{V_{\text{Jar}}} \times 100$

Total Percent Porosity = (%WHC + %AFP)

Bulk Density at Container Capacity = $\frac{(W_{\text{Drain}} - W_{\text{Jar}})}{V_{\text{Jar}}}$

Bulk Density at Oven Dry = $\frac{(W_{\text{Oven}} - W_{\text{Pan}})}{V_{\text{Jar}}}$

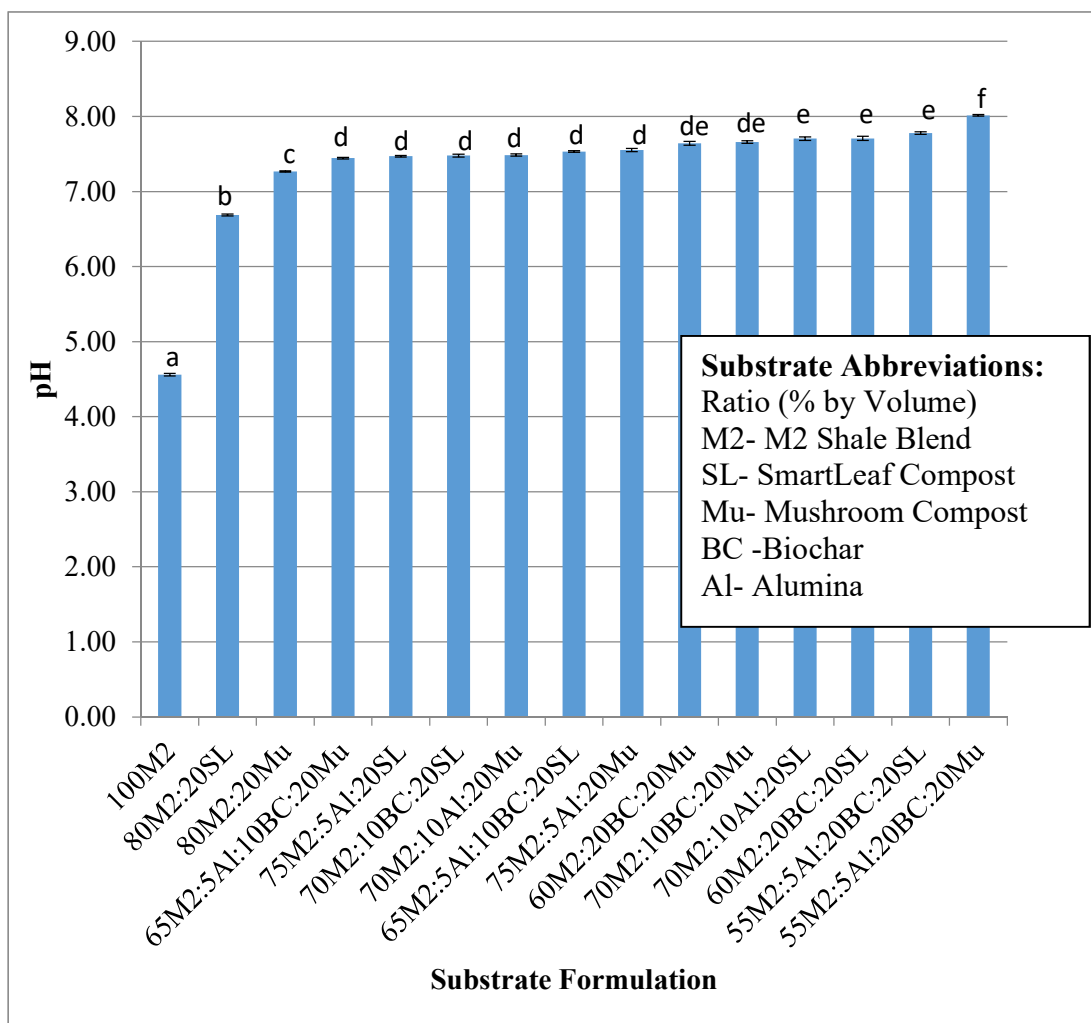


Fig. A.6.1 Histogram showing the average active acidity values for each substrate in pH extracted using DI water. Letters upon bars indicate significance levels about the mean (Tukey's HSD, $P < 0.05$). Error bars show standard error.

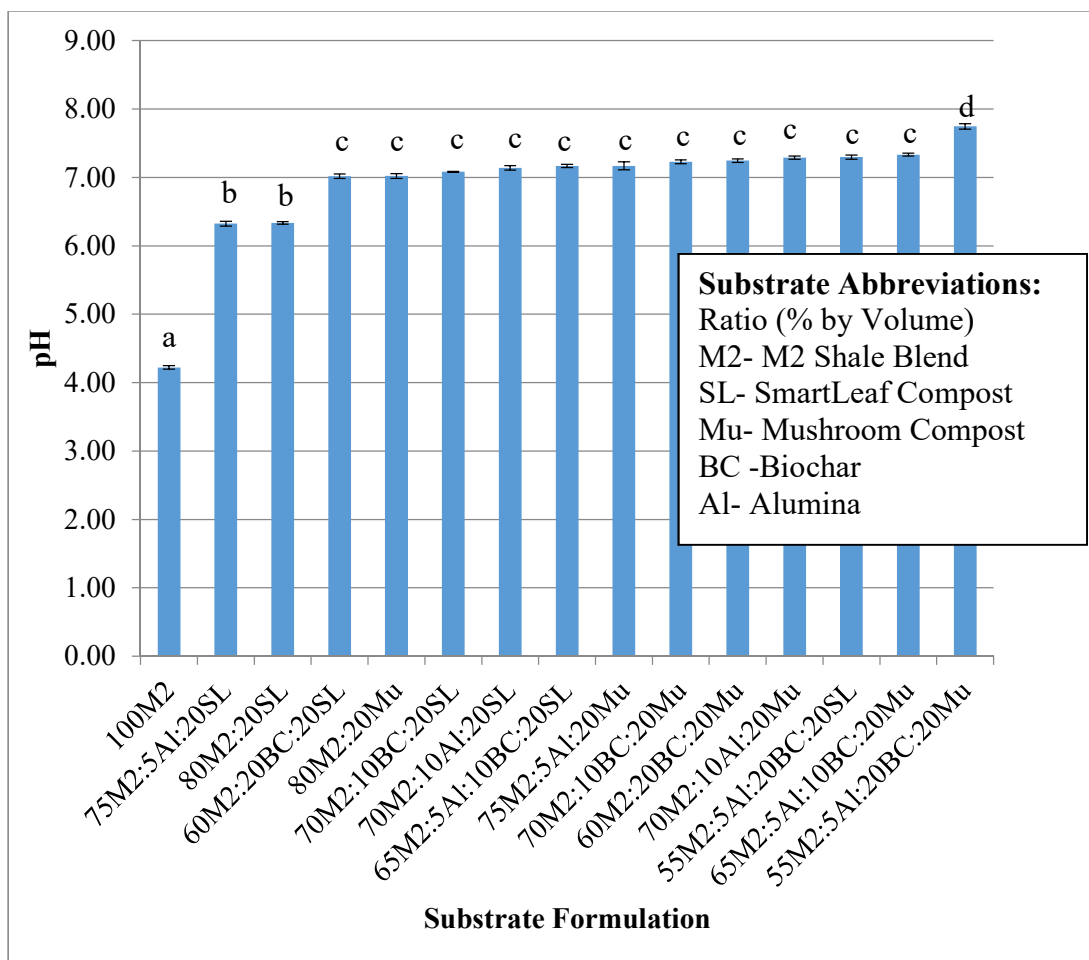


Fig. A.6.2. Histogram showing the average exchangeable acidity values for each substrate in pH extracted using a KCl solution. Letters upon bars indicate significance levels about the mean (Tukey's HSD, $P < 0.05$). Error bars show standard error.

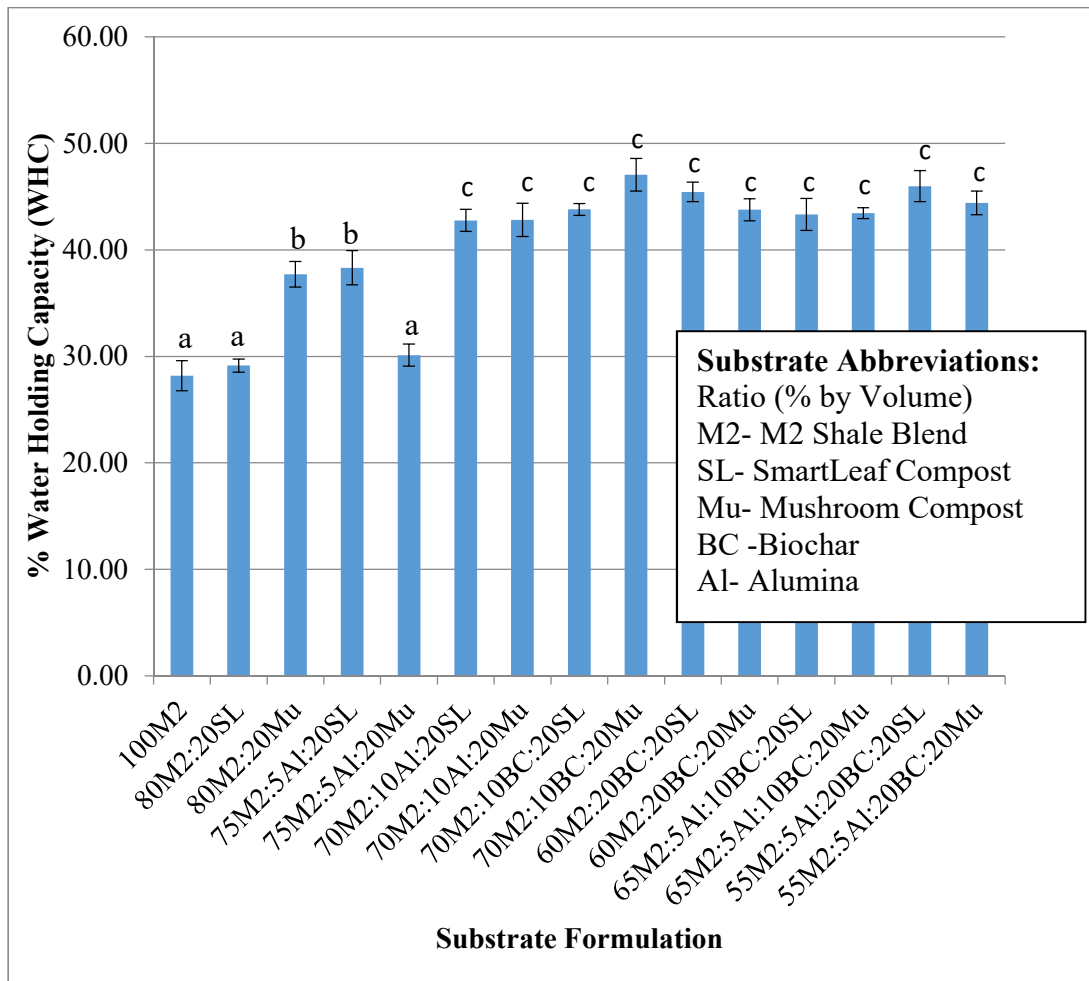


Fig. A.6.3. Histogram showing the average percent water holding capacity (%WHC) for each substrate. Letters upon bars indicate significance levels about the mean (Tukey’s HSD, P<0.05). Error bars show standard error.

A.7 ICPE-9000 Dissolved Phosphorus Methods

1. Remove samples in scintillation vials to be analyzed from the freezer and thaw at room temperature for 16 hours. Do not let samples stay out for more than 24 hours. Do not thaw more than 100 samples at a time.
2. Install one of the MCE filter membranes into a clean, reloadable syringe filter.

3. Using a 50 mL syringe compatible with the assembled syringe filter, pour the contents of both vials of tub study leachate samples to be analyzed into the syringe and filter into two new scintillation vials.
4. Label and seal the scintillation vials of filtered sample
5. Discard the filter membrane and wash the syringe, plunger, and syringe filter with low residue (phosphorus free) soap. Rinse with DI water and soak in an acid bath for 3-24 hours. Rinse the syringe, plunger and syringe filter in DI water and let air dry.
6. Repeat steps 2 through 5 for every sample to be tested.
7. Ensure the ICPE-9000 is ready for testing by ensuring the cooling pump is on and the machine is supplied with argon gas. Check the rinse water tanks and ensure they are filled with DI water.
8. Create a serial dilution curve in order to calibrate the ICP by preparing 10 mL of the following concentrations (ppm) of mg-P/L: 0.0, 0.01, 0.02 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0. Place 10 mL of each concentration in a test tube and place in respective order into the auto sampling carousel, 13 vials in all.
9. Using the ICP analytical software package, set the ICP to test for elemental phosphorus associated with dissolved-P and enter the calibration curve into the Calibration section of the phosphorus methodological program. Enter the number of samples to be tested after the calibration plus the number of check samples that occur every 15 samples.

10. Using the software, turn on the plasma torch in the ICP and begin the testing.
The auto sampler will begin taking samples from the first vial and use it for the calibration curve. Each vial takes approximately 6 minutes to test.
11. While the calibration curve test tubes are running, pour 10 mL of each of the filtered samples into a test tube and add them in their numbered order to the carousel. Every 15th sample should be a check sample that contains 10mL of 1.0 mg-P/L.
12. When a sample has been completed, it can be removed from the carousel.
Since the carousel is continuous, more samples can be added in its place to the end of the run.
13. When the run is complete, post processing will most likely need to occur.
This entails checking the calibration curves to ensure a high R-squared value to ensure the most accuracy and the removal of any points on the curve.
14. Interference post processing entails checking the readings to ensure that the ICP is reading the correct peaks of the signals it receives from testing each sample. This is done through manually checking the cumulative readings the ICP took and ensuring the peak signals are within the minimum and maximum range of detection of the ICP for phosphorus testing.

A.8 AQ300 SEAL Colorimetry Chemical Digestion of Samples

1. Due to financial constraints, weeks 7 through 31 were tested using the AQ300 SEAL spectrophotometer via colorimetry.

2. Prepare a solution of 1.0 mg-P/L and 0.5 mg-P/L from a stock solution. These stock solutions will be used by the SEAL to calibrate the machine before each run and at intervals to check the readings as the run is being tested. The SEAL can test 57 digested samples at a time and requires 2 mL of digested sample in order to run.
3. To prepare the calibration and check solutions, place 25 mL of 1.0 mg-P/L solution into a 50 mL graduated HotBlock tube provided by Environmental Express (Cole-Parmer), Charleston SC. The preparation of the 0.5 mg-P/L solution follows the same steps as the 1.0 mg-P/L solution.
4. Set the Hotblock sample heater to 110 degrees Celsius. The hot block can boil 36 samples at a time.
5. Add 0.25g of potassium persulfate and 0.5 mL of 10N concentrated sulfuric acid to each tube.
6. Place the tube with the sample, potassium persulfate, and sulfuric acid into one slot in the HotBlock heater and gently boil for 45 minutes.
7. Remove the sample from the hot block and cool at room temperature for 30 minutes
8. Add two drops of phenolphthalein (indicator solution) to the cooled tube and swirl to dissolve.
9. While gently swirling, add 2N sodium hydroxide solution to the tube until the indicator solution turns a light pink. This will take approximately 4.5 mL of 2N sodium hydroxide solution.

10. Using DI water, fill the tube to the 50 mL mark. Cap the tube and gently shake to homogenize the solution.
11. Remove the cap and add, dropwise, 5N sulfuric acid while gently swirling the tube until the samples return to clear and lose their pink color. This usually requires 2-3 drops. The samples are now fully digested and are ready for analysis.
12. To digest a tub study water sample, use a syringe filter and syringe fitted with a .45 micrometer pore size MCE membrane. Filter 25 mL of a sample to be tested for dissolved phosphorus.
13. Place 25 mL of the sample to be tested into a 50 mL graduated HotBlock tube.
14. Follow steps 4 through 11 to digest each sample from the tub studies.
15. Analyze immediately or move 20-25 mL of the digested sample to a labeled, clean 20 mL scintillation vial and freeze at -10 degrees C.

A.9 AQ300 SEAL Colorimetry Reagent Preparation

1. Place 4.0g of ammonium molybdate tetrahydrate into a 100 mL volumetric flask.
2. Add approximately 40-50 mL of DI water and swirl until dissolved. Add enough DI water to fill the flask to the 100 mL line. Place Para-film over the mouth of the flask and invert several times to fully dissolve the solute. This is the prepared ammonium molybdate solution. This solution is viable for 21 days. After this time, discard and remake this solution. Refrigerate in a sealed container.

3. Place 0.3g of potassium antimonial tartrate into a 100 mL volumetric flask.
4. Add approximately 40-50 mL of DI water and swirl until dissolved. Add enough DI water to fill the flask to the 100 mL line. Place Para-film over the mouth of the flask and invert several times to fully dissolve the solute. This is the prepared potassium antimonial tartrate solution. This solution is viable for 21 days. After this time, discard and remake this solution. Refrigerate in a sealed container.
5. Place 1.5g of ascorbic acid into a 100 mL volumetric flask.
6. Add approximately 40-50 mL of DI water and swirl until dissolved. Add enough DI water to fill the flask to the 100 mL line. Place Para-film over the mouth of the flask and invert several times to fully dissolve the solute. This is the prepared ascorbic acid solution. This solution is viable for only 1 day. After this time, discard and remake this solution. Refrigerate in a sealed container.
7. To make 100 mL of the coloring reagent, mix together in an opaque container 22.0 mL of the ammonium molybdate solution, 65 mL of 5N sulfuric acid, 7.5 mL of potassium antimonial tartrate solution, and 5.5 mL of DI water. This solution is viable for 21 days. After this time, discard and remake this solution. This coloring reagent is also sensitive to light. Always keep in an opaque container or store in darkness. Refrigerate in a sealed container.

A.10 AQ300 SEAL Colorimetry Sample Analysis

1. The SEAL stores all of the necessary reagents and calibration solutions within the machine to pull from during use. These solutions are stored in 40 mL, pie slice shaped “reagent segments” around a carousel adjacent to the sampling carousel.
2. Empty and rinse out the four reagent segments that will be needed to run the methodology.
3. Fill one reagent segment with each of the respective solutions and place in the correct slot on the reagent carousel; the digested 1.0 mg-P/L solution, the digested 0.5 mg-P/L solution, the coloring reagent, and the ascorbic acid solution.
4. Empty and refill the DI water container connected to the SEAL and empty the waste container into the appropriate disposal container in the lab.
5. On the sample carousel, place 57 (or however many samples are being tested) 2 mL sample vials in each slot labeled 1 through 57.
6. Fill each sample vial with 2 mL of digested sample to be tested
7. In a ring around the sample vials, there are plastic blocks (reaction segments) that serve as reaction chambers for the coloring reagents to work. Ensure that all of the used reaction segments are removed and replaced with new ones before running any test. They cannot be washed and reused.
8. Once all of the materials are in place, run the daily startup procedure in the SEAL Analytical software package that comes with the AQ300. If necessary, run the weekly or monthly procedures as well if the date calls for it.

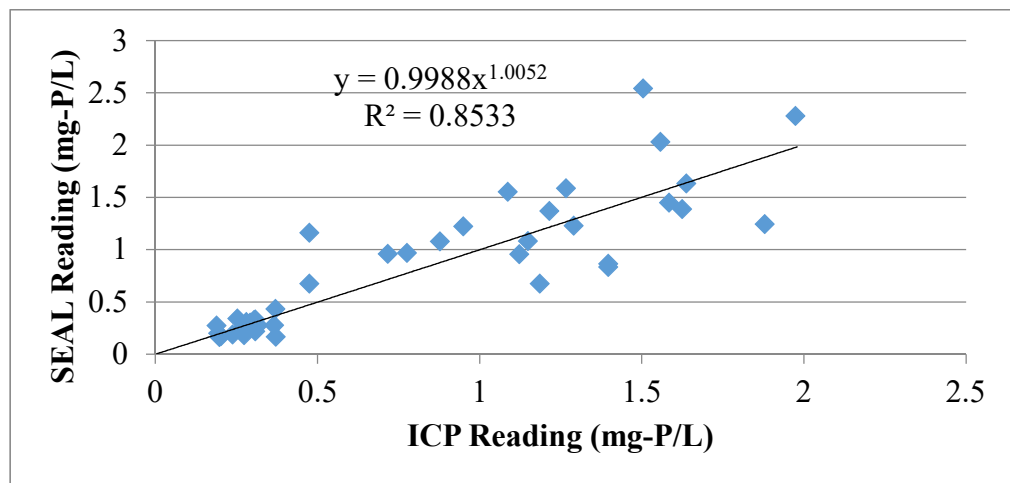
9. The daily procedure involves running the SEAL through its cleaning, zero calibration with DI water, and testing all of the pumps and lines to ensure there are no air bubbles causing problems for the system. This procedure is automated by the software.
10. Weekly and monthly tasks include checking tubes, lamps, and pumps for wear and damage, and rising out the waste disposal system.
11. Using the software, begin the run for phosphorus analysis and the SEAL will automatically calibrate using the digested stock solutions installed in step 3. The SEAL also uses these solutions to check the readings every 15 samples and will trigger an error if they fall outside of +/-10%. After auto-calibration, the SEAL will begin sampling, reacting, analyzing, and cleaning out the digested samples.
12. Once the run is complete, any used plastic ware from the sample carousel can be discarded.
13. Remove and refrigerate any reaction segments containing extra reagents.

A.11 ICP to SEAL Regression Curve:

1. The first six weeks of leachate samples were analyzed via ICP. However, due to financial constraints the rest of the weeks from 7 through 31 had to be analyzed via the SEAL spectrophotometer. Due to their differences in analysis methods and sample preparation, a regression curve needed to be constructed to relate the two methods.

2. Pick 36 samples at random from the first six weeks to be used to create the regression curve.
3. Run these 36 samples through the ICP and record the values measured by the machine for each sample. This was done by going through the steps detailed in **ICPE-9000 Dissolved Phosphorus Methods**.
4. Run the 36 samples through the SEAL Spectrophotometer and record the values measured by the machine for each sample. This was done by going through the steps detailed in **AQ300 SEAL Colorimetry Chemical Digestion of Samples and AQ300 SEAL Colorimetry Sample Analysis**.
5. Plot the data from both machines and generate a regression curve to unify the data with the lowest R-squared value.
6. Apply the regression curve to the samples collected over the first six weeks to minimize transformation of data. This will convert ICP reading to SEAL readings.

Fig. A.11.1. Regression curve between ICP measured data and SEAL measured data.



A.12 Compost Analysis



Account No. : 1910

Compost (TMECC) Analysis Report

ALEXANDER, BRENDA
CITY OF COLLEGE PARK
4500 KNOX RD
COLLEGE PARK MD 20740

Invoice No. : 1117089
Date Received : 01/23/2020
Date Analyzed: 01/24/2020

Lab No. : 10592

Results For : CITY OF COLLEGE PARK
Sample ID : 9217 51ST AVE COLLEGE PARK
#1 EAST

	Analysis Dry Basis	Analysis As Is Basis	Lbs / Ton		Available First Year
			Dry Basis	As Is Basis	
Organic N, % N	1.84	0.68	36.7	13.6	3.4
Ammonium, % N	0.170	0.0630	3.4	1.3	1.2
Nitrate, % N	0.015	0.0060	0.3	0.1	0.1
Total N, % N	2.02	0.75	40.4	15.0	4.7
Phosphorus, % P ₂ O ₅	0.68	0.25	13.6	5.0	3.5
Potassium, % K ₂ O	0.73	0.27	14.6	5.4	4.9
Sulfur, % S	0.38	0.14	7.6	2.8	1.1
Calcium, % Ca	4.73	1.76	94.5	35.1	24.6
Magnesium, % Mg	0.86	0.32	17.1	6.4	4.5
Sodium, % Na	0.08	0.03	1.6	0.6	0.6
Zinc, ppm Zn	180.4	67.0	0.4	0.1	0.1
Iron, ppm Fe	4057.8	1506.3	8.1	3.0	2.1
Manganese, ppm Mn	889.6	330.2	1.8	0.7	0.5
Copper, ppm Cu	61.9	23.0	0.1	0.0	0.0
Aluminum, ppm Al	636.9	236.4	1.3	0.5	0.3
Boron, ppm B	40.7	15.1	0.1	0.0	0.0
Soluble Salts, (EC 1:5) dS/m		0.68			
pH		7.9			
Moisture, %	62.88				
Dry Matter (TS), %	37.12				
Ash, %	50.38	18.70			
Organic Matter LOI 550C, %	49.62	18.42			
Organic Carbon, %	28.78	10.68			
Organic C:N Ratio	14.2				
Bulk Density, lbs / cubic foot		30			
Human Inerts & Plastic Film, %		< 0.1			

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1/31/2020

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101 Clukey Dr.
Harrington, DE 19952

Fig. A.12.1. Compost Analysis of SmartLeaf compost used in the column studies as an organic substrate component.



Account No. : 879

Compost (TMECC) Analysis Report

WYE RESEARCH CENTER
 PO BOX 169
 QUEENSTOWN MD 21658

Invoice No. : 1117023
 Date Received : 01/17/2020
 Date Analyzed: 01/20/2020

Lab No. : 10565

Results For : ANDREW RISTVEY
 Sample ID : COMPOST 1
 UMD

	Analysis Dry Basis	Analysis As Is Basis	Lbs / Ton		Available First Year
			Dry Basis	As Is Basis	
Organic N, % N	4.70	3.60	94.0	72.1	18.0
Ammonium, % N	0.704	0.5400	14.1	10.8	10.3
Nitrate, % N	0.005	0.0040	0.1	0.1	0.1
Total N, % N	5.41	4.15	108.2	83.0	28.4
Phosphorus, % P ₂ O ₅	2.49	1.91	49.8	38.2	26.7
Potassium, % K ₂ O	3.54	2.71	70.7	54.2	48.8
Sulfur, % S	2.14	1.64	42.8	32.8	13.1
Calcium, % Ca	1.85	1.42	37.0	28.4	19.9
Magnesium, % Mg	0.71	0.54	14.2	10.9	7.6
Sodium, % Na	0.60	0.46	12.0	9.2	9.2
Sodium Adsorption Ratio (SAR)	9.47				
Zinc, ppm Zn	471.6	361.6	0.9	0.7	0.5
Iron, ppm Fe	901.5	691.3	1.8	1.4	1.0
Manganese, ppm Mn	581.3	445.7	1.2	0.9	0.6
Copper, ppm Cu	462.2	354.4	0.9	0.5	0.7
Aluminum, ppm Al	1917.0	1470.0	3.8	2.9	2.1
Boron, ppm B	4.4	3.4	0.0	0.0	0.0
pH		7.7			
Moisture, %	23.32				
Dry Matter (TS), %	76.68				

Note: The available first year Ammonium-N is calculated based on maximum availability, or incorporation within 24 hours. Advise a nutrient consultant for adjustments beyond 24 hr incorporation.

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1/20/2020

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Fig. A.12.2. Compost analysis of mushroom compost used in the column studies and crop growth studies as an organic substrate component.

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