Greenhouse Study on Southern Highbush Blueberries in Soilless Media Amended with Biochar to Enhance Plant Growth and Mycorrhizal Fungi Colonization

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Abstract

Amending soil with biochar, a highly porous and carbonaceous product of pyrolyzed organic material, has been found to improve blueberry growth. However, little work has been done to investigate the use of biochar as a component of soilless substrates for containerized blueberry production, a growing trend in the blueberry industry. Peat moss and perlite, two of the most commonly used components of soilless substrates, are not considered sustainable due to limited resources and high transportation costs. The purpose of this study was to determine if biochar can be a replacement for perlite and peat moss in containerized production of blueberry. A 12-week greenhouse study was conducted to evaluate the plant growth of two southern highbush blueberry cultivars (Vaccinium darrowii) 'Jubilee' and 'Jewel' in using locally sourced materials [green waste compost (C) and pine bark (Bk)] in conjunction with sphagnum peat moss (Pt), perlite (Pr), and biochar (Bi) produced from Douglas fir at 700 °C . Substrate treatments were $Bk_{30}C_{30}Pt_{30}Bi_{10}$, $Bk_{30}C_{30}Pt_{30}Pr_{10}$, $Bk_{40}C_{40}Bi_{20}$, $Bk_{40}C_{40}Pr_{10}Bi_{10}$, and $Bk_{40}C_{40}Pr_{20}$ (percentages indicated as subscripts). Across treatments, Jewel had a higher total plant dry weight than Jubilee, 10.92 and 8.69 (g/plant) respectively. When plants grown in substrates $Bk_{30}C_{30}Pt_{30}Bi_{10}$ and $Bk_{30}C_{30}Pt_{30}Pr_{10}$ were on average 60% larger than plants grown in treatments without peat moss. The low pH of the peat moss (4.5) likely buffered the high pH of the compost (7.5), allowing for greater plant growth during the first half of the study. The soil solution pH of all treatments was well above the pH recommended for blueberry at the end of the study, an affect of the compost, which was a component of all substrate treatments. Soil solution analysis across weeks indicate that the biochar did not increase the pH more than the perlite, further proof that compost was the primarily driver of pH increase. The leaf nutrient analysis revealed that all plants were low in nitrogen and phosphorous, likely a result of the higher pH values. We found no effect on mycorrhizal root colonization. Overall, biochar has potential to be a suitable replacement for perlite in containerized blueberry production when paired with low pH substrate materials such as peat moss. In this study, green waste compost did not appear to be a suitable component of substrates for containerized blueberry production.

Additional index words: perlite, soil amendments, Vaccinium corymbosum, indoor farming, plant nutrition

Table of Contents

Chapter 1: Literature Review

Chapter 2: Introduction

Chapter 3: Materials and Methods

Chapter 4: Results

Chapter 5: Discussion and Conclusions

List of Tables

List of Figures

Biochar

Farmers or plants lovers are always looking for ways to increase plant health, size, or fruit yield. Environmentalists want ways that will not harm and could potentially benefit the environment. An up and coming way to do this is the introduction of biochar. Biochar has been present on Earth for thousands of years before we started making it to benefit our crops. The process of making biochar is modeled after an old practice in the Amazonian basin of rich, fertile soils called Terra Preta ("dark earth") by the indigenous people (Spear, 2018). These soils have a clear origin involving additions of charred organics and remnants from earthen ovens use for cooking and firing pottery (Downie et al., 2011). Plants grown in Terra Preta grow faster and are more nutrient dense than those grown in neighboring soils. Terra Preta soils still hold carbon today. There is also a similar soil found in southeast Australia. Radiocarbon analysis done on the charcoal collected multiple sites on these lands showed its recalcitrance in the soil. The soils in southeast Australia exhibit the same features as Terra Preta in the Amazon.

Biochar Characteristics

Biochar is a black, highly porous, lightweight, fine grained, charcoal-like substance with a large surface area that can persist in soils for decades to millennia (Dai et al., 2013; Spear, 2018; Yu et al., 2019). Biochar is composed of single, condensed carbon rings, which has a higher surface area along with a high-density charge (Surampalli et al., 2014). It is made up mainly of carbon (about 70%) along with nitrogen, hydrogen, oxygen, and other elements. The high porosity is important for providing a habitat for beneficial soil organisms (Shaaban et al., 2018). The surface area and porosity are significant for cation and anion exchange for nutrient retention. The chemical composition and pore size vary depending on the type of feedstocks used to make it and what method is used to heat it. The organic biomass used to make biochar can come from various sources, meaning that the physical characteristics of biochar will be different depending on the chemical characteristics of the biomass. Biochar improves soil nutrient capacity, water holding capacity, soil productivity, soil quality, nutrient cycling, and an increase in soil microbial activity. Biochar that contains higher carbon percentage, along with other aromatic structures, have higher positive land amendment effects to improve soil fertility. The inorganic components also have implications on the physical structure of biochar.

Biochar does not have a defined chemical composition, but it does have a range of material that differs as a result of numerous factors, such as biomass and production (Surampalli et al., 2014). General chemical properties include pH, cation exchange capacity, atomic ratios, and elemental composition (Yu et al., 2019). Biochar's pH usually ranges from 5.9 to 12.3 and averaging about 8.9. Biochar has a high content of carbon that consists of aromatic ring structures that become larger and more condensed with increasing temperature. The biochar model contains oxygen as a heteroatom and free radicals in the conjugated aromatic ring. The carbonized biochar is a microporous carbon, and the micropores in the microcrystalline graphite structure are responsible for most of the surface area of the carbon. It is important to understand biochar's organic structural composition in order to predict the reactivity and stability for its application as a soil amendment. The condensed aromatic carbon of biochar has persisted in soil for millions of years, however, biochar with higher levels of single ring aromatic and aliphatic carbon end up mineralizing quickly.

Biochar Production

Biochar production is similar to preparation of charcoal, which has been done for thousands of years. However, in biochar, gases are allowed to escape (Surampalli et al., 2014). It is made by burning organic material from agricultural wastes or green wastes through two main ways – pyrolysis or gasification (Shaaban et al., 2018; Stefanie Spear, 2018; International Biochar Initiative, n.d.). In pyrolysis, thermal conversion occurs with a catalyst in the absence of oxygen. The material is heated above its decomposition temperature resulting in the breaking down of chemical bonds. This chemical reaction is reversible. Gasification, a thermo-chemical process, converts the biomass into a combustible gas where the materials begin decomposing in an environment with little oxygen – not enough for combustion. The main difference between pyrolysis and gasification is that pyrolysis is done in the absence of air while gasification is done in the presence of air. The more oxygen that can be excluded, the more biochar can be produced. These methods can produce clean energy through synthetic gas (syngas) or bio-oils along with biochar – the gas can be used another time or burned and released as heat (International Biochar Initiative, n.d.). During both processes, as the materials burn, there is little to no contaminating fumes released. If biochar is produced at low temperatures, the pores may become partially blocked by other organic compounds, which would decrease its nutrient retention capacity and adsorption potential. Slow pyrolysis is the preferred way to produce biochar and fast pyrolysis is the way for bio-oil. Hydrogen molecules in the plant generate syngas and bio-oil along with heat energy. The bio-oil can be used like a low-grade diesel fuel for heating and power. The process of making biochar helps to reduce contamination and safely store carbon. Biochar is more efficient at converting carbon into a stable form and is cleaner than other forms of charcoal. Biochar made from wood-based biomass is more resistant to biodegradation than biochar produced from animal manures and residues. Biochar made from the same material but produced at different temperatures have different properties of electrical conductivity (EC), pH, and phosphorous and nitrogen concentrations. The highest temperature reached during production, peak temperature, has the most impact on the yield and characteristics of the final product, but the yield decreases as the temperature continues to increase. As the peak temperature increases, there is a rise in the amount of fixed carbon in the biochar.

There is a relationship between biochar pyrolysis temperature and resistance to soil degradation (Surampalli et al., 2014). Differences in decomposition are based on the C:N ratios. Biochar has a higher C:N ratio with low concentrations of available nutrients when it is made from wood materials (Sales et al., 2020). Higher temperatures of pyrolysis produce wider C:N ratios due to the loss of nitrogen concentration compared to carbon. The wide ratio of C:N will enhance slow biochar decomposition. Even though biochar will be degraded from slow chemical and microbial decomposition, the rate of the decomposition is so slow that even large additions of biochar to soil will not significantly immobilize nitrogen. According to Surampalli et al. (2014), the high stability of biochar in soil enhances carbon sequestration as carbon is added to the soil from biochar will be removed from the atmosphere for over 1000 years. As pyrolysis temperature increases, the biochar pH and cation exchange capacity also increase.

Benefits of Biochar

Biochar has been shown to improve soil conditions, such as, nutrient and water retention, soil pH, microbial activity, and some cases have shown soil borne pathogens (Shaaban et al., 2018). Biochar allows carbon to be stored in soil over much longer periods versus unpyrolyzed biomass and lowers the risk of heavy metal uptake by plants. It is composed of recalcitrant carbon structures that prevent biochar from decomposition (Surampalli et al., 2014). Biochar has been proven to alleviate soil constraints on “problem soils” with low fertility, especially in acidic and coarse soils by increasing plant growth and productivity. Biochar helps with soil fertility by increasing the macro and micro elements. Biochar increases the pH of acidic soils, which enhance microbial populations. The porous nature also provides a favorable habitat for mycorrhizal fungi. When biochar is mixed with other organic material, such as compost, soil fertility has been shown to increase (Sales et al., 2020). Biochar alone is not known to drastically affect soil pH and is more likely affected by the amount of nitrogen present. Compared to unamended soil, biochar is known to increase shoot and root growth in multiple crops, including blueberry plants.

According to Shaaban et al. (2018), biochar decreases gaseous nitrogen loss from agricultural soils by stimulating the conversion of nitrous oxide into a nitrogen molecule. The burning and natural decomposition of biomass in agriculture contributes to large amounts of CO_2 to the atmosphere. Biochar, used sustainably, could reduce global net emissions of CO_2, methane, and nitrous oxide without endangering food security, habitat, or soil conservation (Surampalli et al., 2014). Carbon has a permanent repository in plants and soil that acts as a natural balance. Coal has obtained the status of pure carbon that is gathered by plants and sequestered through natural processes. Growing plants take in CO_2 from the atmosphere and fixes it into their cells, but about 99% of the carbon ends back up in the atmosphere when the plant is burned or consumed. Charcoal that is rich in carbon and tilled into soils, can be sequestered away for 100-1000+ years, minimizing CO_2 concentrations in the atmosphere. Research done by Surampalli et al. (2014) stated the co-production of bioenergy and biochar can lessen climate change by minimizing fossil fuel utilization. “Carbon negative” comes into play when biochar is buried into soil and bioenergy is generated during pyrolysis.

There are multiple problems with the physical and chemical properties of soils that can be addressed with the use of biochar. Some soils do not have a good water holding capacity, but with biochar, can increase water retention and in turn increase yield production (Sales et al., 2019). According to research done by Asai et al. (2009), biochar has high porosity and can retain water in small pores, which would increase the water holding capacity and assist in infiltrating water from the ground surface to the topsoil. Improved physical properties, such as, bulk density and water holding capacity, could increase the retention of water and nutrients (Ding et al., 2016). Some soils have low fertility resulting in low plant growth and yield. The addition of biochar could increase the pH and cation exchange capacity, resulting in better nutrient retention of Na, K, Ca, and Mg. Biochar may also aid in the adsorption/desorption process of soils. Adsorption capacity is greatly influenced by biochar's properties. The mechanisms describing the adsorption capacity of polar and apolar compounds are attributed to hydrophobic bonding, electron donor-acceptor interactions resulting from fused aromatic carbon structures, and weak hydrogen bonds. If ammonium is used as an example of how adsorption takes place, it includes physical adsorption, ammonium attraction to negatively charged surfaces, ammonium's reaction with acidic functional groups to form amides and amines, ammonium binding to cationic species sites on the surface of biochar, and electron donor-acceptor interactions. Ding et al. (2016) states that many laboratory studies suggest biochar be used as a slow-release fertilizer because of the nutrient availability. Another factor that could be improved through biochar application the reduction of nitrous oxide emissions. This would be attributed to the content of polycyclic aromatic hydrocarbons in low biochar. Biochar produced at 200^{o}C contains large amounts of phenolic compounds that reduce nitrous oxide emission. Abiotic interactions in biochar amended soil is connected to mitigation of nitrous oxide, such as, changes of pH, water penetration and increase of bulk density, improvement of nutrients availability, soil structure, and increase of sorption capacity. Beneficial soil microbes form symbiotic relationships with plants; however, some soils have poor microbial structure. Nutrient and carbon availability can affect microbial abundance. Biochar research done by Ding et al. (2016) says that microbial abundance was increased due to the greater nutrient availability after applications of biochar. Different living conditions will be formed for microorganisms with different pH values of biochar. Researches have shown that certain criteria can influence the nutrition and carbon availability on microbial biomasses, such as, the additive amount of nutrients and carbon, properties of microorganism, and the existing nutrient and carbon availability in the soil. In return, decomposer organisms can enhance nutrient release from soil organic matter into the rhizosphere of the crop. Different microbial groups respond differently to biochar in soil. Overall, biochar is greatly beneficial as a soil additive.

Limitations of Biochar

There are still some limitations on biochar. Intermittent addition of fresh biomass might be needed when biochar is present for optimal nutrient cycling and soil-water environment (Kavitha et al., 2018). Aged biochar has a negative effect on the growth of earthworms and fungi in the soil. Aged biochar may also lead to a reduction in certain underground root biomass and thermal diffusivity in the soil. Biochar's beneficial effects are soil specific, so biochar amendment may not always play a positive role for all types of soil. Also, the effects of biochar on plant productivity depend on the plant species or specific part of the plant. For example, a study done with biochar was shown to increase vegetative growth of tomatoes, but not fruit yield. There has also been weed problems reported with the application of biochar, so repeated additions of biochar may not be good for weed control. According to Kavitha et al. (2018), another limitation of biochar is the capacity of biochar to adsorb nitrogen along with essential nutrients, such as, iron, that can be counterproductive to plant growth. Biochar can react with soil nutrients as a competitor instead of providing plants with nutrients. For example, when biochar and phosphorous fertilize are added, this could cause sorption reactions of phosphate that would contribute to the reduction of phosphorous availability to the plants. One last limitation would be the price of biochar. The cost varies on the feedstock used to produce biochar. Regulatory issues and testing of biomass feedstock could increase the costs. With all of the benefits and limitations to biochar, it is still a great amendment for soil.

Soil

Soil is the part of earth's surface consisted of disintegrated rock and humus (decaying organic materials) that provides medium for plant growth (Madaan, n.d.). It takes time for the development of soil, hundreds to thousands of years, and consists of inorganic and organic materials (Baldwin et al., 1938). The inorganic materials are composed of things like minerals and rocks, while the organic components are composed of living aspects, such as, soil microorganisms. Soil formation occurs through the rock cycle along with the integration of soil microbial and chemical activities. During decomposition of dead plants and animals, nutrients are mixed up with weathered and disintegrated rocks to form soil. Soil is considered a natural resource because of its benefits to agricultural productivity. Madaan (n.d.) mentions that different soils have different mineral and organic compositions that establish their own, specific characteristics. Our ability and capacity to maintain food production throughout the world and sustain life depends on this thin layer of soil covering the Earth (Stirling et al., 2016). Besides this, soil also filters water, detoxifies pollutants, and provides a home for many organisms that decompose the organic matter and supply nutrients to plants.

A healthy soil for crop production will provide: anchorage for plants, physical structure suitable for root growth, the capacity to absorb and infiltrate water, provides ready access of roots to available water, stores and releases nutrients, and suppresses pests and diseases

(Stirling et al., 2016). Along with all of these, agriculture is part of a wider environment, so the soils must also provide other factors that are important to the ecosystem, such as: sequestration of carbon, maintenance of biodiversity, detoxification of harmful chemicals, maintenance of water quality, prevention of nutrient and sediment loss to waterways, and minimization of greenhouse gas emissions. In addition to providing food for today, agriculture must also be able to provide for future generations – sustainable agriculture, as stated by Stirling et al. (2016). Soil health and sustainable agriculture are inseparable as the capacity to provide food for the world's increasing population depends on soil. Agriculture will only survive long term if soils are farmed in ways that repair historical damage and improve their physical, chemical, and biological properties. A whole-system approach is needed, where a range of practices are used to develop resilient field ecosystems capable of dealing with climatic stresses, pests, and diseases.

Soil Types

There are six main types of soil: loam, clay, silt, peat, sand, and chalk (Hayes, 2019; Madaan, n.d.). Loamy soil is one the richest soil types because it is composed of clay, sand, silt, and decaying organic materials (What are different types of soil, 2020). It is dark in color and has a dry, soft, and crumbly feeling. Loamy soil has good nutrient and water holding capacity. It also drains well and has pore spaces that allow air to freely move in between the soil particles to the roots. The pH of loamy soil is about 6 with high calcium contents. It has the potential of retaining water and nutrients for relatively longer periods, making it one of the richest soils for crop production. The composition of loamy soil may vary, but with the right balance of additives, it can be made almost perfect. For example, compost manure is usually added to improve the desired qualities that may be lacking. Clay soil is unique because of its exceptionally fine grains and plasticity when moist, but hard when fired. The soil particles are tightly compressed with little to no air space, making it the heaviest and densest type of soil. This characteristic also allows for the soil to hold and retain large amounts of nutrients and water, while still making it difficult for air and moisture to penetrate the soil. Madaan (n.d.) states that gardeners and farmers must know the conditions of clay soil in order to successfully use it because when wet, clay is usually difficult to garden because it is heavy, but when dry, it is smooth and soft making it easier to manage. Compost or mulch may be added to the top of the clay soil to avoid freezing in cold temperatures. Compost and mulch also allow better drainage and air flow. Silty soil is composed of clay, mud, or small rocks deposited by a lake or river. It is made of smaller particles, compared to sand, and forms a soapy slick when wet. This characteristic makes silt extremely smooth and fertile because of its ability to retain a lot of water. However, compared to other soils, silt has low nutrition. Silt is easily compacted by weight, so walking on it should be avoided when used for gardening to avoid compaction. Compaction of silt may cause required aeration. It is good for crop farming because of the miniscule particles. Peaty soil is usually dark brown but can be black as well. According to Hayes (2019), it has large quantities of organic matter and is rich in water, making it a very highly favored soil for plant growth. Peat should be drained first due to its high nutrient and water content. However, according to research by Madaan (n.d.), because of peat's high nutrient and water content, peat is able to keep plants healthy even in dry weather; it also shields plants from harm during rainy periods. Water in peat is acidic, to a small degree, but is ideal for controlling plant diseases and balancing pH levels of other soil types. Sandy soils are a pale-yellowish to brown color and are one of the poorest types of soil. It is composed of loose coral or rock grain materials and has a dry/gritty touch. Sandy soil has one of the largest particles, which prevent it from retaining water. Sand loses water content extremely fast, which makes it difficult for plant roots to be established. Plants do not usually get the opportunity to use the nutrients or water in sand because they carried away by runoff. Chalky soil is found in limestone beds with deeply rooted chalk deposits. They are very dry and known to impeded germination of plants. They are composed of and resemble calcium carbonate or calcite and have the color of chalk. Madaan (n.d.) states that chalk is not beneficial for crop farming or plant growth because it presents a lot of difficulties to work with since it has a high lime content, but low water content, giving it a pH of about 7.5. Chalk is basic and will normally yield yellow and stunted plants.

Soil Composition

Each component of the soil varies for each soil type, but in general, about 45% is composed of mineral particles, 20-30% air, 20-30% water, and 1-5% of living or dead organic matter (Stirling et al., 2016). Of course, the amount of water and air will change depending on how wet or dry the soil is. The mineral particles, or the primary soil particles, is made up of sand, silt, or clay and largely determine the physical characteristics and texture. Clay is the smallest soil particle (less than 0.002 mm), but not all are the same – there are different types of clay particles and each type determines the nutrient holding capacity and resistance to compaction or loss of structure. Since clay is usually negatively charged, it holds on to calcium and magnesium, and because of its size, organic molecules become trapped in the particles. Silt is larger than clay (0.002-0.02 mm) making them have a smaller surface area to volume ratio (surface area to volume ratio decreases as particles become larger). Silty soils do not maintain nutrients cations or bind as well as they do in clay because of silt's surface area, increasing its chances of crusting on the surface. Sand particles are the largest (0.02-2mm). Soils that have high sand content are prone to erosion because of sand's inability to bind together. Because of sand's low surface area to volume ratio, it also has poor nutrient cation and water holding capacity. Coarse sand allows air to move through the soil, but it does not protect against organic matter degradation.

The amount of air held within soil depends on the soil texture, structure, extent of compaction, and its moisture content (Stirling et al., 2016). The air in the soil, compared to atmospheric air, has 10X more carbon dioxide, lower oxygen, and greater humidity. Oxygen enters the soil and diffuses through the pores and channels where it is then absorbed by roots and organisms, which will then respire; the exchange of CO_2 and oxygen between the soil and atmosphere is important. The respiration from roots, microbes, and fauna produce CO_2, diffuses out of the soil and into the atmosphere. According to research by Stirling et al. (2016), when soil becomes compacted or saturated, soil pores are reduced, decreasing CO_2 and O_2 exchange. The inability for O_2 to enter or CO_2 to leave, can create anaerobic conditions. Oxygen concentration is higher near the surface and decreases with depth, but the rate of this depends on soil texture. High O_2 near the surface is why biological activity is the highest in the top 10 cm of soil. Small pores in soils have problems with O_2 moving down the soil profile, but soils with large pores allow O_2 to diffuse into greater depths. Besides O_2 and CO_2, there are other gases present in the soil such as nitrogen. Since very few organisms consume nitrogen, its concentration remains about the same in the soil as it is in the atmosphere. However, when O_2 is low and organic carbon is available, a group of anaerobic bacteria facilitate denitrification (removal of nitrates or nitrites by chemical reduction). During this, nitrate nitrogen gets reduced to nitrite, nitric acid, nitrous oxide, and di-nitrogen, that usually get lost into the atmosphere. According to Stirling et al. (2016), nitrous oxide is a powerful greenhouse gas, along with methane that may also be generated.

Soil water is mainly absorbed by the roots allowing the plant to grow and transpire, and contains nutrients needed for plant growth (Stirling et al., 2016). Since air is displaced when water is present, it affects aeration in the soil. Plants get their water through rainfall and irrigation, which then travels down the soil profile and fills in the pores. The rate at which the water moves through the soil profile is dependent on the size and continuity of the soil pores. Soils with small pores have a reduced infiltration rate or capacity compared to soils with large pores or where root channels remain or macropores are present from earthworm activity. As mentioned by Stirling et al. (2016), if water is unable to infiltrate the soil, then sediments and nutrients will run off. These sediments will then move into rivers and streams causing water quality problems. The soil pore size will also affect how much water is stored and how much is available to the plants. Water in large pores is easily accessed by plants, but as soil dries, the water is confined to smaller pores or as a film around soil particles. Stirling et al. (2016) states that in order for plants to get to this water, they must work harder and expend more energy, which will affect plant growth and could cause wilting or death. The texture of the soil affects the amount of water held that is accessible to plants; it is usually greater in soils with more clay or humus. All soils can reach a “permanent wilting point”, which is the point where roots can no longer extract water from the soil, but water will still remain in the soil in micropores and be unavailable to plants. Plants get nutrients by accessing nitrates, sulphates, potassium, and other dissolved elements. Nutrients like nitrogen, sulfur, and phosphate can be added through fertilizer or mineralized from organic matter, however, these nutrients will not diffuse to the roots unless there is water.

The last and smallest part of soil composition is the organic matter; it gives soil its desirable traits for agriculture (Stirling et al., 2016). The organic matter consists of living and dead parts of leaf litter, crop residues and roots (excluding root exudates), animal feces, and decomposing fauna and microbes. It can be bound to the soil particles, aggregates, or in held in between the particles. Soil organisms regulate the transformation of organic matter, the rate of this depends on the climate and soil management. Since organic matter is constantly being used by soil organisms, and a lot of carbon is respired and released in the atmosphere as CO_2, the organic matter must be replenished by inputs of organic residues. The organic matter gets cycled through the soil with inputs from plant, animal, and microbe residues. When new organic matter enters the soil, easily degraded compounds (i.e. sugars) form a carbon pool that is employed by bacteria and fungi. The microbes multiply quickly and the nutrients from the pool get transferred to a microbial biomass. When predators consume this biomass, nutrients get released into the soil and are available for the plants to use. More complex compounds are then utilized by the microbes. Compounds that are difficult to break down are transformed into humus. Soil organic matter majorly influences soil's structure, nutrient recycling, degradation of pollutants, and disease suppression.

Soil Chemical Properties

The clay and organic materials, also known as soil colloids, in soil are charged and most of the chemical interactions occur on the surfaces of these colloids (Obia et al., 2015). Nutrients can be exchanged on the surface of clay or organic material, held within the organic matter and biomass, or become dissolved in soil water. There are different ways the chemical properties can be tested, such as, pH, electrical conductivity, cation exchange capacity, and many other tests regarding the nutrients available in the soil. The pH measures the concentration of hydrogen ions, which indicates the acidity or alkalinity of the soil. When the amount of hydrogen ions increases, the pH of the soil will decrease and become more acidic. Different crops and soil organisms have different sensitivities to pH, but the optimal pH for microbial activity and crop production is around pH 7. Electrical conductivity (EC) measures the amount of salts in soils. According to Obia et al. (2015), the more salt there is in a soil, the more electricity can be moved from one electrode to another, which gives a higher EC reading.

Soil pH controls the product ratio (nitrous oxide/nitrous oxide + nitrogen) of the denitrification process (Obia et al., 2015). According to Obia et al. (2016), this could be due to low pH preventing the assemblage of nitrous oxide (N_2O) reductase, which is used to reduce N_2O to nitrogen in denitrification. Increased N_2O reductase from increased pH, because of the alkaline biochar, could be a reason why suppression of nitrous oxide emission is observed in soils treated with biochar. The rise in pH, after the addition of biochar, enabled the weakened N_2O reductase enzyme, that is usually seen at a low pH. In research done by Cayuela et al. (2013), significantly lower N_2O emissions were observed when biochar was added. In most of the soil, biochar also decreased the total nitrogen denitrified and not only the ratio [N_2O/ ($N_2O + N_2$)].

Biochars with different C/N ratios were used after their pH had been adjusted to the same pH as the soil and compared to the same biochars added without adjusting the pH to conclude that biochar buffer capacity, and not pH alone, is found to affect total emissions (Cayuela et al., 2013). Soil texture is closely related to the ability of biochar to decrease N_2O/ ($N_2O + N_2$) ratio. In fine-textured soils, biochar promoted the last step of denitrification, compared to other textures, so the mechanism of reduction is not linked to soil aeration.

Soil Problems

For plant growth to occur, roots play important roles in anchoring and supporting the plants, absorbing water and nutrients, biosynthesis, storage of chemical compounds, and interactions with abiotic and biotic factors in its surrounding environment (Yu et al., 2019). Healthy soils give access to root penetration through soil particles enabling the plants to grow to their maximum potential. However, problem soils inhibit root growth, hinder water and nutrient uptake, and reduce plant growth.

A physical constraint for plant growth is soil compaction – stress that is applied to soil causing air displacement from pore spaces, making it denser (Yu et al., 2019). Compaction can occur by vehicles or animal footprint. Physical resistance and poor aeration can occur obstructing root growth. Roots are unable to penetrate soil pores that are smaller than the diameter of the root cap, so root growth becomes hindered. The roots will be unable to explore large volumes of soil to take up the water and nutrients needed. Decrease in pore space can also reduce permeability and diffusivity of gases, which could result in anaerobic conditions.

Acidic soils (where aluminum and manganese become more soluble) occupies about half of the arable land in the world and can inhibit root elongation by destroying the root apex, affecting uptake of water and nutrients (Yu et al., 2019). Phosphorous uptake by roots gets reduced, and as a result, aluminum toxicity and phosphorous deficiency occur. Soil acidity can restrict symbiotic nitrogen fixation by limiting rhizobium (gram negative bacteria that fix nitrogen) survival and persistence in soils, then reduce nodulation. Acidic soils favored the growth of a pathogen that causes bacterial wilt in certain crops. Alkaline soils are caused by parent materials rich in calcium carbonate. They are copious with carbonates and bicarbonates, and usually have a pH of 8 or greater. The availability of iron, copper, manganese, and zinc is reduced due to the high pH, and an important problem to plants is the iron deficiency. The leaves can become chlorotic (yellowing/whitening of green plant tissue due to a decrease of chlorophyll because of a disease or nutrient deficiency) and necrotic, causing stunted plant growth and decreased harvestable yield. Alkalinity will not only cause iron (and other micronutrient) deficiencies but will also affect iron uptake regulated gene expression.

The most common nutrient deficiencies are nitrogen, phosphorous, and potassium (Yu et al., 2019). About 50%-75% of nitrogen is lost to leaching, this is concern for contamination of groundwater. Phosphorous deficiency could be caused by the slow release of inorganic phosphorous from minerals but is more likely caused by low pH in the soil. Phosphorous leaching occurs in heavily fertilized agriculture and is a main determinant of water eutrophication. Potassium is important for enzyme activity, stomatal synthesis, photosynthesis, water, nutrient, and sugar transport, and protein and starch synthesis. Soils such as sandy soils in high rainfall areas are attributed to potassium deficiency. There are eight micronutrients (iron, zinc, manganese, copper, nickel, boron, molybdenum, and chloride) important for plants, soils that are deficient or toxic to these are usually related to pH in soils.

The high concentration of dissolved salts in subsoil or irrigation water is the salinity of soil (Yu et al., 2019). If the electrical conductivity of a soil is above 4 dSm^{-1}, the exchangeable sodium percentage is below 15, and the pH is below 8.5, then it is saline. Soil sodicity occurs when sodium ions are higher in proportion to other cations, such as calcium, magnesium, and potassium. In wet soils, sodicity can cause dispersion or disintegration of clay aggregates into individual particles. Sodicity and salinity are usually found together and related to the parent materials; they are brought in with irrigation water or drainage water from nearby areas. Salinity causes a decline in plant growth because of osmotic stress due to physiological drought (plants are unable to absorb water, even if it is available), imbalance of ionic concentration, and ion effects like chlorine toxicity. Research done by Yu et al. (2019) states that sodicity reduces plant growth by slowing root growth because of high soil strength and limited gas exchange in the rhizosphere. In saline and sodic soils, all of these restrictions act together and endanger rhizosphere environments.

Soils host many organisms, including bacteria, fungi, actinomycetes, protozoa, and algae – bacteria are the most abundant (Yu et al., 2019). Soil microbes can affect soil formation, physical and chemical properties, and plant growth. Nitrogen fixation into ammonia or other molecules can be done by symbiotic and nonsymbiotic bacteria. A major fungus found in plants without roots hairs, such as blueberry plants, is mycorrhizal fungi. Mycorrhizae improve plant absorption of phosphorous and nitrogen. Rhizobacteria (root associated symbiotic bacteria promoting plant growth) stimulates crop growth directly by enabling resource attainment or regulating plant hormone levels, or indirectly by decreasing inhibitory effects of different pathogenic agents on plant growth and development. Plant roots produce compounds and molecules that can change the soil's chemical properties and support organisms.

Anthropogenic chemicals in soils that have high enough concentrations to affect human health and the ecosystem is known as soil contamination (Yu et al., 2019). This contamination is usually caused by industrial activities, agricultural chemical application, or improper disposal of wastes. High concentrations of heavy metals, from human activities like mining, smelting, disposal of metal wastes, leaded gasoline and paints, pesticides, sludges, and fertilizers, affect soil quality and biological functions due to their toxicity and persistence after entering the soil. Arsenic is a carcinogenic trace element that is present in soils from human activities and is toxic to living organisms. Plant roots can take up AS(III) and As(V), but As(V) can be converted to As (III) in plant cells. As (III) can bind to and inactivate enzymes that contain cysteine, and As(V) is a chemical analog of phosphate that can disrupt some phosphate-dependent aspects of metabolism. So, plant growth can be severely stunted by arsenic. Phosphate fertilizers, sewage sludge, mine spills, and industrial discharge are major causes of cadmium dispersion in soil. Cadmium can be easily adsorbed by roots and transported to shoots. Yu et al. (2019) states that at a high concentration, cadmium can cause phytotoxicity by decreasing nutrient uptake, inhibiting photosynthesis, inducing lipid peroxidation, and altering the antioxidant system and functioning of membranes. Lead contamination can cause inhibition of enzyme activities, alterations in membrane permeability, impaired photosynthesis, and growth inhibition. Copper contaminated soils reduces root growth because it competes with iron and can further inhibit photosynthesis be producing reactive oxygen species. Nickel contamination by mining, combustion of fossil fuels, and metal plating industries could inhibit root growth and affect photosynthesis by interacting with magnesium.

Soil and Biochar

The elevating world population and limited amount of land causes a challenge for agricultural production and food security causing high utilization of land and large amounts of chemical/organic fertilizers (Dai et al., 2020). Soil degradation, soil organic matter and nutrient depletion, and pollution have increased and have threatened sustainable agriculture production. Soil acidification and organic matter depletion can cause deterioration of soil quality, negative affect on soil microorganisms, reduction of aggregate stability, and reduced water holding capacity. These effects can limit plant growth and food production along with nutrient leaching and decreased nutrient use efficiency due to large amounts of fertilizers. Due to biochar's organic nature and active surface area, it has been used for soil quality improvement (Yu et al., 2019). Biochar is seen as a positive soil amendment to improve crop growth because of large surface area, pore structure, abundant oxygen containing functional groups, and high cation exchange capacity. However, the efficiency of plant productivity is dependent on biochar properties and soil conditions. Plant productivity with biochar varies under different soil conditions because of the soil physicochemical properties. The properties of biochar should be selected carefully before their application to soils, according to the soil's conditions and specific problems, to increase biochar's effect.

Biochar can help reduce bulk density and particle density in soils because biochar has lower bulk and particle density, thus improving aggregation and porosity in problem soils (Yu et al., 2019). Sandy soil is affected by this more than clayey soils are. Since biochar can aid in lowering bulk density, it therefore will help with compaction. According to Omondi et al. (2016), biochar increases soil porosity by 8.4% because of the porosity of biochar, reduced bulk density, increased soil aggregation, interaction with mineral soil particles, and reduced compaction. Movement of water, heat, and gases in soils also increases with rising soil porosity and decreasing bulk density. Once gravitational water has drained down, biochar is filled up and holds onto water in its pores, reducing water permeability and increasing water retention – huge improvement for sandy soils. Biochar alters hydraulic conductivity (ease of which fluid can move through pore spaces or fractures) and reduces saturated water flow in coarse soils and increases flow in fine soils. Physical properties of problem soils could benefit from biochar rather than highly fertile or productive soils.

Along with physical improvement, biochar is known to improve the chemical properties of soil, as well (Yu et al., 2019). Biochar can help alleviate soil acidity because of biochar's alkalinity, high buffering capacity, function groups, and its silicon effects. Silicon can help neutralize soil acidity (Owino-Gerroh and Gascho, 2011). Calcium, potassium, magnesium, sodium, and silicon in feedstocks form carbonates/oxides during pyrolysis that can react with hydrogen and aluminum in acid soils to reduce acidity and increase pH. Certain biochar functional groups also contribute to biochar alkalinity, specifically when pyrolyzed at lower temperatures. Increased buffering capacity in soils is due to an increase in cation exchange capacity after application of biochar. Depending on carbonization temperatures, acidic biochar could be produced to decrease high alkalinity in soils, but not much research has been completed on this. Compared to other soil neutralizers, biochar persists in soils for a long time maintaining suitable soil pH levels.

Since biochar is considered an organic fertilizer, it can improve soil fertility (Yu et al., 2019). Depending on the soil's deficiency of nutrients, biochar can be catered to meet that need (nitrogen, phosphorous, potassium, calcium, magnesium, sulfur, iron, manganese, copper, zinc, and silicon). For example, biochar produced from rice or other grass is known to have high silicon; biochar from soybean contains high nitrogen; biochar from eggshell contains high calcium; and biochar from manure is rich in multiple nutrients. Even if there is not a fitting feedstock to produce a specific biochar, then biochar can be engineered to meet the needs of the soil. Nutrients in biochar are released slowly, mediated by biochar's unique properties and the sorption-desorption process. The pores and networks within biochar create structural obstacles and other unique connections, like chemical bonding to carbon materials, interfere with the easy release of nutrients. Functional groups on biochar that have strong sorption capacity are able to concentrate nutrients in problem soils and allow for slow desorption for plant uptake.

Biochar can relieve the negative effects of salts, to help with saline and sodic soils (Yu et al., 2019). As mentioned above, biochar reduces pH that can be linked to salt. High cation exchange capacity biochars could improve root uptake of more cations, like potassium, calcium, and magnesium, resulting in a release of hydrogen, which will balance the charge in the rhizosphere. Biochar also increases surface charges causing a substation in ions (sodium by potassium, calcium, and magnesium) and reducing sodium levels. Sodium sorption increases with increased surface area and pore volume, but more research is needed on this topic for improving saline soils.

Biochar and Plant Production

With biochar added to soil, plant size increases and nutrition changes (Sales et al., 2020). Studies have shown biochar as a strong amendment for improving crop yields, especially in nutrient-poor soils, but only a small improvement in nutrient-rich soils (Hussain et al., 2016). Plants responses to biochar are correlated to the type of biochar, rate of application, soil properties, and climate. The liming effect and increase in water holding capacity are seen as the main reasons to why there is an improvement in crop production. Water retainment improvement at field capacity was greater with biochar addition than with water held at permanent wilting point (the increased plant available water). So, the rise in plant available water increases with biochar. According to Haider et al. (2014), the application of biochar to poor sandy soils expanded plant growth because it improved soil-plant water relation, by improving relative water content and leaf osmotic potential, and photosynthesis, by reducing stomatal resistance and increasing electron transport rate of photosystem II, under drought and well-watered conditions. Biochar with fertilizers has a synergistic effect on crop yield. Biochar enhances crop productivity under normal conditions along with yield under unfavorable conditions, like salinity and drought. It can alleviate adverse effects of salt stress for plant growth, for example, plants receiving salt and biochar had growth rates similar to plants that had no addition of salt. Research by Hussain et al. (2016) shows that biochar improved salt stress by adsorbing sodium and increasing potassium content. Biochar has the ability to lessen salinity-induced reductions in mineral uptake. Most research has been based off of short-term studies (about 1-2 years), so long term studies should be done to get the full effect of biochar on productivity. Overall, biochar is seen to improve plant production, mainly by improving soil quality.

Highbush Blueberry Plant

Vaccinium corymbosum (Ericaceae family) is a highbush, perennial shrub native to eastern and northeastern United States (Sales et al, 2020). Different types of blueberry plants are grown based on their chilling requirement and winter cold hardiness (Retamales and Hancock, 2018). All blueberries require well-drained, acidic soils, and lots of moisture. Highbush blueberries get further separated into northern or southern highbush, depending on the chilling requirements and winter hardiness. Southern highbush blueberries do not tolerate winter temperatures below freezing and require about 550 or less hours of chilling. Most commercial blueberry production comes from highbush and lowbush types. Highbush has also become a major international crop. Many wild, edible highbush blueberry plants have been harvested for thousands of years by indigenous people. Highbush and rabbiteye blueberries were domesticated at the end of the 19^{th} century; plants were dug from the wild and transplanted.

Climate and Production

Highbush blueberries are grown across a wide variety of climates, such as, mild and moist summers with very cold winters, mild and moist summers with moderate winders, hot and wet summers with mild winters, and hot and dry summers with mild winters (Retamales and Hancock, 2018). Most plantings are done on naturally acidic soils with high organic matter.

Overheard irrigation is more common than trickle irrigation, but some do not get irrigated at all. Highbush are generally grown at closer spacings. Pruning is done when the plants are dormant and performed annually or biannually by removing the least productive canes. Growth regulators are used in the southern US to increase leaf development during the spring and heighten ripening in southern highbush. Highbush is usually hedged to control plant size, encourage branching, and increase fruit set.

According to Retamales and Hancock (2018), most blueberry production comes from cultivars from highbush (*V. corymbosum*), rabbiteye (*V. ashei*), and native strands of lowbush (V. angustifolium). Some of the most important characteristics that breeders look for are flavor, large fruit size, light blue color, small scar where the pedicel detaches, easy fruit detachment, firmness, and long storage life. Reducing chilling requirements, expands the range of adaptations for highbush blueberries is an important breeding goal, along with extending seasons and winter cold tolerance. Chilling requirement can be reduced by integrating genes from *V. darrowii* into *V. corymbosum.* Most breeders have relied on pedigree breeding, so elite parents can be selected for each generation for intercrossing. All new blueberry cultivars get patented and licensed.

Once blueberries enter dormancy, a period of low temperature for normal growth and development is needed. There is controversary over what temperatures are most effective for the chilling requirement of highbush blueberries. Optimal temperatures are thought to be higher in southern highbush than in norther highbush.

Blueberry Anatomy

Blueberry plants can be grown in different types of soils along with organic amendments to increase nutrient retention, drainage, and soil water retention (Sales et al., 2020). All

Vaccinium species are woody perennials (Retamales and Hancock, 2018). Highbush blueberries can grow up to 4 meters tall. Blueberry shrubs are composed of shoots that grow from new buds or previously formed buds that are dormant. Shoots that emerge from the base of the plants are called canes. The canes become woody in the second season of growth. Flower buds on blueberries are large and round, but vegetative buds are smaller, narrow, and pointed. The number of flowers found in an inflorescence bud is negatively correlated to the distance from the tip. The number of buds on a shoot is related to the shoot thickness, cultivar, and light penetration.

Blueberry leaves are simple, serrated and arranged alternately along the stem (Retamales and Hancock, 2018). Most highbush plants are deciduous, but some that have lower chilling temperature ranges can be evergreen, as long as the temperatures remain above freezing. Leave shapes can vary from elliptic, spatulate, oblanceolate, to ovate. Highbush varieties have different amounts of pubescence and glands under the leaves.

There are two major types of roots in highbush blueberries, thick storage roots and fine roots (Retamales and Hancock, 2018). The storage roots help with anchorage and storage, while the fine roots are used for water and nutrient absorption. Blueberries do not have root hairs, so they have developed a symbiotic relationship with mycorrhizal fungi (Sales et al., 2020). Mulching tends to concentrate the roots near the surface of the soil. According to Abbott and Gough (1987), high rates of irrigation increases the depth of the roots.

The blueberry fruit is a true fruit – a fruit that develops from the mature and ripened ovary after fertilization – with many seeds (Retamales and Hancock, 2018). The fruit ripens after two to three of pollination, depending on the cultivar and environmental conditions. High temperatures will promote fruit ripening. The fruit color can range from light blue to black, found in the epidermal and hypodermal layers, with a waxy cuticle layer, however the flesh is white.

Growth and Development

Vegetative buds swell in the early spring as the leaves begin to develop within the buds (Retamales and Hancock, 2018). Vegetative bud break usually occurs sooner than floral bud break depending on the cultivar, chilling duration, and temperatures in the spring. When the vegetative buds open, leaves cluster closely around the stem and then separate as the internodes expand. Shoots will grow rapidly to begin with then stop from apical abortion ("black tip"). Growth is renewed when the axillary bud is released, and the black tip is discarded – usually only one axillary bud is released from dormancy, leaving the shoot bare. In the first year, a new shoot breaks from the base and remains unbranched and all growth arises from a single vegetative bud. In the second year, two or more vegetative buds will break dormancy and begin to grow – the first branching. In the years after, multiple vegetative buds will break every year after fruiting. Increased branching and twiginess of the shoot occur over time, causing fruit size and yield per cane to diminish as the canes become twiggier.

All blueberry fruits present a double sigmoid growth curve (Retamales and Hancock, 2018). In the first stage, rapid cell division and dry weight gain occurs (Birkhold et al., 1992). There is a little bit of fruit growth that occurs in Stage II, but mainly active because of seed development. In Stage III, very rapid fruit growth occurs through cell enlargement; sugars accumulate, and berries turn blue from anthocyanin accumulation. According to field studies done by Woodruff et al. (1960), the intensity of color in blueberries increases over the first six days after the fruit begins to color and stabilize. Lipids and waxes decrease in the early stages of ripening and then remain constant. Starch and other complex carbohydrates remain stable throughout maturation. Total sugars increase for about nine days after color change, then being to level off. Sugar accumulation stops when berries detach. Blueberries become softer as they ripen because of enzymatic digestion of the cell wall, pectin, cellulose, and hemicelluloses. Cultivars vary greatly on their ability to maintain firmness after ripening.

Acidity decreases continually during berry ripening, causing an increase in sugar to acid ratio (Retamales and Hancock, 2018). Increased crop load decreases the fruit sugar levels but does not affect acidity levels or food storage quality. Increased nitrogen decreases acidity but has little effect on sugar levels. When the numbers of days between harvests are lengthened, the sugar levels are increased while acidity is decreased, resulting in decreased shelf life. Third harvest fruit have higher sugar levels and lower acidity levels, with reduced shelf life.

Blueberry Nutrition

Blueberry plants have low nutrient demands compared to other fruit trees (Retamales and Hancock, 2018). In most situations, regular fertilizer application is needed for commercial fields. There are different conditions in the plant and soil that explain the low nutritional requirement for blueberries. They are calcifuge plants – adapted to acidic soils. When blueberries are grown in soil pH between 4.0 and 5.5, optimum growth and productivity are obtained. Blueberries have shallow roots that lack root hairs, limiting the surface area in contact with the soil. Because of this, the roots are colonized by mycorrhizae fungi. With significant expansion of blueberry growth, they are being grown in soils not optimal for blueberry production, so amendments are needed to provide adequate conditions. In many areas, nitrogen is the most frequent or only nutrient applied to blueberries. Soils that are high in organic matter have higher nitrogen supplies, so fertilization is not as needed. According to Retamales and Hancock (2018), when organic mulch is added, then additional nitrogen is needed since nitrogen is used by microbes to decompose those materials. Calcium is also important because it impacts the fruit quality. Soil analysis is recommended before planting blueberries to determine the nutritional status and pH. Leaf analysis and soil pH monitoring is done after being planted for nutrient management.

Soil pH Requirements for Blueberry Plants

According to Retamales and Hancock (2018), the recommend soil pH for highbush blueberries ranges from 4.5 to 5.5. The pH affects the availability of nutrients for plants, and high pH is a problem that is usually seen in new blueberry sites. When blueberries are grown in high pH, their leaves turn yellow and sometimes will have green veins. The leaves are generally small and will turn brown and fall from the plant before the season finishes. With high pH, little growth occurs, and some plants may die. If plants become stunted from high pH, they do not usually recover and will need to be replanted (Hart et al., 2006). High pH soils usually have iron, manganese, and copper deficiencies, so fixing pH is more helpful than adding these elements to the soil. Soils can be acidified using sulfur before planting or with sulfuric acid through irrigation. There are two variables that affect the amount of sulfur needed for acidification: initial pH and CEC of the soil. The higher the difference between these two, the more sulfur needed to adjust the acidity. Sulfuric acid on drip irrigation acidifies soils faster than elemental sulfur, especially in soils with low CEC.

Mycorrhizal Fungi in Blueberry Plants

Mycorrhizal fungi and blueberry roots have formed a symbiotic relationship to help them grow in soils with low pH, low nitrate, low calcium, and high organic matter (Retamales and Hancock, 2018; Vega et al., 2009). If mycorrhizal was inoculated into the blueberry plant, then plant, root, and shoot dry weight will increase. Leaf photosynthetic rate, transpiration, and water use efficiency is not affected by mycorrhizal inoculation. Mycorrhizae increase soil nutrient uptake, efficiency of fertilizers, improve water use, and protect the plant from toxic elements, like aluminum – aluminum's concentration increases as soil pH decreases. The fungi can take in ammonium and nitrate to transfer them to the plant, along with increased uptake of nitrogen and phosphorous. Mycorrhizae can transfer carbon and nitrogen simultaneously to the plant whenever organic sources of nitrogen are applied, however, this offsets the carbon drain that is required to sustain fungal growth.

Mycorrhizal fungi are more abundant in natural environments but can be more important in nursery and commercial plants (Retamales and Hancock, 2018). Levels of fungi colonization can be doubled if the plants are inoculated with mycorrhizae in the nursery. Inoculation in the nursery (container grown blueberries) will increase the total plant biomass. However, there may be some host-fungus specificity due to report on the variation of mycorrhizal isolates and their ability to increase nutrient uptake. Roots of highbush blueberry plants that fruit early in the season usually have higher levels of colonization than those that fruit later in the season. Colonization of the fungi depends on the cultivar, rate of fertilizer application, and the amount and type of soil organic matter present. Increasing amounts of fertilizer usually decreases mycorrhizal colonization. Soil amended with organic materials also reduces mycorrhizae.

Mycorrhizal Fungi and Biochar

Studies have shown that biochar can have positive effects on mycorrhizal fungi (Warnock et al., 2007). Biochar additions can change the nutrient availability by affecting the physicochemical properties of soil. As nutrient availability increases, elevated plant performance and tissue nutrient concentrations occur, which in turn will increase colonization rates of mycorrhizae. Biochar can also increase the ability of mycorrhizal fungi to help the plant resist infection by pathogens. Research has shown that biochar or activated carbon (AC), which has many similarities to biochar, increases plant root colonization. However, according to Warnock et al. (2007), some studies showed negative effects of biochar or AC on the prevalence of mycorrhizal fungi due to nutrient effects. In some studies, mycorrhizal responded better to biochar than to other types of organic material. It is possible that the positive responses are due to the amount of carbon in the material being added to the soil.

Biochar amendments alter nutrient levels and other soil physicochemical properties that affect plants and mycorrhizal fungi by increasing bioavailable nutrients like nitrogen, phosphorous, and metal ions (Warnock et al., 2007). Since biochar alters pH, an increase in the nutrients would occur that would be available to soil biota and plants roots, including mycorrhizal fungi. The addition of biochar to soil can positively or negatively affect soil microbes, such as mycorrhization helper bacteria (MHB), which promote the establishment of the root-fungus symbiosis (Rigamonte et al., 2010). MHB secrete metabolites that can facilitate the growth of fungal hyphae and colonization of plant roots. Other bacterial species that could be affected by biochar are phosphate solubilizing bacteria (PSB) that solubilize important nutrients, especially phosphate, making it available to mycorrhizal fungi and the host plant. Biochar could serve as a source of carbon for any soil bacteria, including MHB and PSB, resulting in heightened benefits of mycorrhizal fungi. Biochar also alters plant-mycorrhizal fungi signaling or detoxifies chemicals leading to altered root colonization by mycorrhizal fungi. Biochar's particles could adsorb signal molecules that are not immediately intercepted by mycorrhizal hyphae or spores or consumed by another biota. The stored signals could be desorbed later on by water reaching the biochar particles, causing them to be re-dissolved into the soil water and become available again to stimulate mycorrhizal colonization. However, if biochar permanently instead of temporarily removes the signals from the soils, then this would cause a decrease in the number of signal molecules reaching mycorrhizal hyphae and result in mycorrhizal decrease. Biochar can also adsorb compounds that are toxic to mycorrhizal fungi. Biochar serves as refuge from hyphal grazers. Due to particle size of biochar and mycorrhizal size, the particles are large enough to accommodate MHB and mycorrhizae (Blackwell et al., 2015). A negative effect that biochar has on mycorrhizal fungi could be the decrease in the fungi due to decreasing availability of nutrients in soils; especially with biochar's very high carbon to nitrogen ratio along with a portion of biochar decomposing and leading to nitrogen immobilization. However, most of the time biochar is beneficial to plant productivity.

Indoor Farming

With the global population rapidly increasing, the demand for food also increases. Greenhouses can play an important role in providing fresh food that are still high in vitamins and minerals (Hamming et al., 2019). Greenhouses can have high crop production along with high water use. Resources are becoming scarcer, so there is an urgency for maximum resource efficiency. One difficulty with greenhouse farming, is the finding enough skilled workers to manage crops. Greenhouse managers must have high levels of knowledge and experience to control the crops.

Greenhouses protect crops from rain, wind, low temperatures, or pests (Hamming et al., 2019). Modern greenhouses come equipped with active control machines, such as heating, lighting, and irrigation to make an admirable environment for plants. Growers must determine the climate and irrigation needed. In some cases, outside weather conditions and weather forecasts were used for climate simulations. Crop growth simulations were carried out with cropping cycle to predict future growth and development setpoints. Computations can be done daily to ensure crops are grown in an optimum control strategy.

Vertical farming can also help where plants are produced in vertically stacked layers (Gnauer et al., 2019). This optimizes plant growth and soilless farming techniques, such as hydroponics, aquaponics, and aeroponics. Vertical farming provides the ability for gardening in places where the environment is too harsh for agricultural production. In hydroponics, plants are grown in liquids containing essential nutrients without any soil applied. Aeroponics, a subgroup of hydroponics, allows roots to be completely exposed to the air and are frequently supplied with nutrient enriched spray or mist. Pumps circulate nutrient enriched water. In vertical farming, space is used more efficiently than in other technologies. Arrays are also much lighter since no soil is used and can be built cheaper. It also uses less water and nutrients since it is recirculating. No pesticides are necessary. Fertilizers can be reduced and reused. However, if an error stops the nutrient supply to the roots, then the crops are at risk to failure in little time.

The goal of climate and crop management is to optimize crop growth rate by finding the best balance of climate and crop characteristics so that the maximum amount of fruit per m^2 is achieved without affecting the photosynthetic capacity (Hamming et al., 2019). If fruit load is too high compared to the photosynthetic capacity, young fruits will abort causing a negative impact and total fruit yield. If fruit load is too low, production will be low. Photosynthetic capacity is mainly determined by light and carbon dioxide. If light levels remain low compared to fruit load, abortion and uneven distribution will occur. Light and carbon dioxide interact in a non-linear way, but both factors have a stronger positive effect at higher levels of each other. Light and carbon dioxide management are an important of greenhouse and crop management.

With artificial intelligence (AI) control in greenhouses, more explicit and better combinations can be made for optimum growing conditions (Hamming et al., 2019). According to research done by Hamming et al. (2019), AI -assisted or AI-managed greenhouse production can improve crop production in locations where knowledge may be limited. Robotic elements in vertical farming are often used for harvesting or surveillance of the systems (Gnauer et al., 2019). A robotic extension for an existing indoor farming system can identify and harvest ripe plants. A camera visually detects the plants and a robotic frame moves a manipulator that harvests the plants. Another robotic system that was developed can monitor and adapt humidity, temperature, plants seeds, and water a greenhouse. With increasing knowledge of technology and advances in AI, indoor farming can be greatly beneficial for future food production with the growing global population.

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Introduction

The annual world production of blueberry (Vaccinium sp.) has essentially doubled since 2014 (Brazelton and Aust, 2020). Total worldwide production in 2019 was between 900,000 – 1 million tons, with U.S. and Canada contributing 435,000 tons, an increase of 27% from 2018 (Kramer, 2020). However, North America's share of the global highbush blueberry market fell from 50% to 30% from 2018 to 2019, a result of increased production in South America, China, and South Africa (Perkowski, 2020). Increased production is attributed to new horticultural systems, including substrates, that are changing where blueberries can be grown (Perkowski, 2020).

Blueberry production has been limited by blueberry's specific edaphic requirements. Blueberry is an acid loving plant and optimal growth occurs when the soil has a pH between 4.5 and 5.5 (Strik et al., 1993; Retamales and Hancock, 2018). As a result, blueberry primarily acquires the ammonium (NH_4^+) species of Nitrogen, the chemical form of nitrogen that predominates in low pH soils (Alt et al., 2017). Furthermore, blueberry sensitive to high salinity, a characteristic often found in high pH soils (Bryla and Machado, 2011). Blueberry has shown a positive growth response to increasing additions of organic matter, in part due to the symbiotic relationship with ericoid mycorrhizal fungi, which aide in nutrient and water uptake (Cheng et al., 2012). Indeed, the desired physiochemical conditions of soils are managed with the addition of organic matter for blueberry production, including peat moss and sawdust (Sales et al., 2020). Furthermore, blueberry is a long-lived perennial shrub, requiring soil conditions to be maintained throughout the life of the planting (Retamales and Hancock, 2018). In addition to soil requirements, there are also soil borne plant pathogens that reduce yield in blueberry such as Phytophthora and Armillaria root rot (Pscheidt, 2015). The use of containers to produce blueberry has not only allowed more control over the environmental conditions but has also allowed new markets to rise in countries traditionally not suited for blueberry production, further increasing the need for sustainable approaches to soilless media (Perkoswki, 2020).

Peat and perlite are common components in soilless media for containerized production several horticulture crops, including blueberry (Kingston et al., 2017). Peat moss is an ideal substrate for growing blueberry due to its low pH (4.5) and high nutrient and water holding capacity (Spiers, 1986). However, Peat is a costly, finite resource, that requires replenishment due to decomposition, thus is not considered a sustainable production practice (Sendi et al., 2013). Furthermore, the harvesting of peat from natural bogs rapidly increases CO_2 emissions and degrades natural habitats, resulting in harvesting restrictions in some countries (Huang and Gu, 2018). On the other hand, perlite is a lightweight and porous mineral, produced from volcanic glass, used for aeration (Nelson, 2012). However, it is manufactured using energy-intensive processes at high temperatures and has become increasingly expensive due to transportation costs (Kennard et al., 2020). Furthermore, perlite has been associated with eye and lung irritation and recent research indicates that heavy exposure may have long-term health concerns (Weldon, 2012). For these reasons, growers have been looking for alternatives to these commonly used substrates.

Biochar, a carbon rich material by-product of bioenergy production via pyrolysis or gasification of agricultural wastes, increases plant growth when incorporated into mineral soils (Dai et al., 2020; Ding et al., 2016; Jiang et al., 2020; Yu et al., 2019). Biochar can be produced from any form of organic matter, allowing production to occur in more regions, potentially reducing transportation costs (Surampalli et al., 2014). Furthermore, the application of heat increases the stability of the organic material, with estimates of biochar remaining in the soil for more than a millennium (Glaser et al., 2001; Spokas et al., 2010) The recalcitrant nature of biochar reduces the costs associated with replenishment, a requirement of peat moss (Nelissen et al., 2015). Biochar has several of the characteristics associated with peat moss and perlite such as a high CEC and porosity and low bulk density (Spiers, 1986). Sales et al. (2020) found that plants grown in sandy soil amended with 20% biochar (v/v) had more than 70% greater plant dry weight than those grown in unamended soil.

Biochar has been successfully used in soilless media trials as a replacement for both peat moss and perlite for horticulture crop production (Northup, 2013; Steiner andartung, 2014; Blok, 2017). Tian et al. (2012) reported that biochar used in combination with peat increased total plant biomass and leaf surface area of Calathea rotundifolia cv Fasciata more than peat alone. Choi et al. (2018) reported higher fresh and dry weights of chrysanthemum grown in 80% biochar and 20% pine bark than those grown in the control. Awad et al. (2017) reported that biochar paired with perlite and peat moss resulted in increased plant growth of Chinese cabbage (Brasssica rapa ssp. Pekinensi), dill (Anthenum garveolens), and red lettuce.

Study Objectives:

1. Evaluate the growth response of two cultivars of southern highbush blueberry (Jewel and Jubilee) for containerized production in soilless substrates.
2. Determine the effects of biochar replacement on the pH and EC of the soil solutions.
3. Quantify mycorrhizal root colonization of blueberry roots in response to various soilless substrate components.
4. Determine the benefits, if any, of replacing peat moss and perlite with biochar as a component of soilless substrates.

The substitution of biochar for perlite is suspected to either increase or have no discernable effect on blueberry plant growth. Mycorrhizal root colonization is also expected increase in biochar amended substrates. The high pH of the biochar and the compost is anticipated to reduce plant growth in all treatments.

Materials and Methods

Soilless Media Amendments. The amendments used in this study were: biochar, pine bark mini nuggets, green-waste compost, sphagnum peat moss, and perlite. The biochar was produced through gasification at about 750 °C from Oregon Biochar Solutions in Central Point, Oregon (Sales et al., 2020). The biochar was produced at about 750 °C by gasification. The biochar is manufactured commercially from mixed conifers. Bark, peat moss, and perlite were all purchased from a local hardware store. The green-waste compost was donated from the City of Greensboro Landfill (Greensboro, NC). The compost is made from residential yard waste, tree and grass clippings, and leaves. No manure is used in this compost. The process occurs on ten acres of land where the material gets grounded into two-inch pieces. The pieces are put into windrows and the temperature is monitored for about three days to kill any seeds and pathogens present. Their entire curing process takes 8-10 months. After curing, the product is put through a screening process to separate finer particles for compost.

Soilless Media Treatments. There were five amendments used in this study: $Bk_{30}C_{30}Pt_{30}Bi_{10}$: 30% bark, 30% compost, 30% peat moss, 10% biochar, and 0% perlite; $Bk_{30}C_{30}Pt_{30}Pr_{10}$: 30% bark, 30% compost, 30% peat moss, 10% perlite, 0% biochar; $Bk_{40}C_{40}Bi_{20}$: 40% bark, 40% compost, 20% biochar, 0% peat moss, and 0% perlite; $Bk_{40}C_{40}Pr_{10}Bi_{10}$: 40% bark, 40% compost, 10% perlite, 10% biochar, and 0% peat moss; $Bk_{40}C_{40}Pr_{20}$: 40% bark, 40% compost, 20% perlite, 0% perlite, and 0% biochar. The amendments were mixed by the L to ensure that the percentages were correct. They were mixed in a large plastic tub by hand until all parts were evenly distributed. After every treatment was mixed, 4 L pots were filled evenly, leaving about 3-5 cm on top to avoid losing material.

Experimental Design. Each treatment had four reps and two cultivars, Jewel and Jubilee, bringing the total number of plants to forty. The cultivars were obtained from a nursery in Oregon (Fall Creek Farm and Nursery, Lowell, OR). Blueberry plants were received as one-year old liners that were propagated from tissue culture. The plants were kept cold in a fridge before being shipped off, and once arrived, they were stored in the greenhouse. The blueberries had purplish-reddish colored leaves due to the temperature shock.

Once all pots were filled with each treatment type, the blueberry plants were planted in the pots, 20 Jewel and 20 Jubilee. After planting, about 10 mL of acid-loving fertilizer (10N-8P-8K) was sprinkled around the top of the media. The plants were watered and most of the fertilizer immediately was dissolved into the media. Each pot was labeled according to the cultivar type and amendment. Red labels were used for Jewel with T1, T2, T3, etc. on them, while orange labels were used for Jubilee with the treatment types also labeled. The pots were spread out evenly in a randomized order on a lab bench in the campus greenhouse. The greenhouse experiment began on 15 June 2020 (week 0) and ended on 8 Sep 2020 (week 12) in a temperature and moisture-controlled greenhouse at the University of North Carolina at Pembroke.

The plants were watered every day, once a day, with overhead sprinkle irrigation in the greenhouse. Once a week, additional watering occurred by hand (500 mL) in order to collect leachate for data. Drip pans were placed under the pots before the additional 500 mL of water was added to collect leachate without the overhead watering system affecting the leachate to be tested.

Measurements. Weekly pH and electrical conductivity (EC) were tested using the leachate from the additional 500 mL of water. In week 1 (22 June 2020) of the study, about 10 mL of fertilizer was added and pH and EC were taken before and after the fertilizer addition. No fertilizer was added on 15 June 2020 when plants were first potted to ensure the plants survived the media. The leachate in the drip pans were poured into a plastic beaker that was used to pour into tests tubes. A pH meter and EC meter were used to measure each. The pH and EC were averaged out according to treatment alone and cultivar with treatment (i.e. $Bk_{40}C_{40}Pr_{20}$ Jubilee and Jewel).

After the 12-week greenhouse study finished, all the leaves were removed from the 40 plants and placed into separate paper bags to be oven dried. The stems were cut down to the tops of the roots and placed in labeled paper bags to be oven dried, as well. The leaves and stems were placed in an oven for about 6 days at about 65.5 °C until they were completely dry and the stems had no green color on the inside. The leaves' and stems' dry weights were then taking individually and placed back in the bags. The leaves were sent off to a university lab for analysis for nutrients.

For the roots, the pots were turned upside down and loose dirt was shaken off. The roots were not pulled out of the pots to avoid tearing of any of the small/weaker roots. The roots were rinsed and placed in labeled Ziploc bags in a fridge to be washed. For washing, a sprayer was used to spray off all media with a sieve behind the roots to catch any loose roots. About 2 g of each root was taken for the mycorrhizal count. The rest of the roots were placed in labeled paper bags to be oven dried. The roots were oven dried for about 7 days at 65.5 °C. The dry weights of the roots were then taken.

Mycorrhizal Fungi. The 2 g weighed out were split into half, so about 1 g was used for mycorrhizal testing. The other 1 g was saved in case a second batch was needed. A 10% KOH mixture was made using 180 g of KOH to 1800 mL of distilled water. The roots were placed the 10% KOH for about 4 h in labeled test tubes. Roots were originally placed in 10 % KOH for about 2 h, then an additional 1 h, however, clearing did not occur, so the 10% KOH was poured out and new 10% KOH was added for an additional h. The clearing took place in a hot water bath set to 90 °C. The 10% KOH was rinsed from the roots using tap water. Then, the roots were rinsed 5% HCl for about a minute to ensure binding of the trypan blue. The roots were stained overnight using a mixture consisting of 300 mL glycerol, 300 mL distilled water, 15.6 mL acetic acid, and 0.06 g trypan blue. The roots were stored in 50% glycerol mixture (200 mL glycerol and 200 mL distilled water).

The mycorrhizal count was done using the gridline method (Brundrett, 2008). A 100 mm^2 square petri dish with 1 cm gridlines were used. The roots were spread out over the petri dish and viewed under a dissecting microscope. A double-sided tally clicker, from Amazon, was used to keep track of any colonization that was present or not over each gridline.

Statistical Analysis

SigmaPlot (Systat Software Inc., San Jose, California) database was used to run analyses on all data collected. Dry weights, weekly pH and EC, mycorrhizal fungi, and leaf nutrition were run on SigmaPlot using a two-way ANOVA with cultivar and amendment. The results showed any significance between amendments, cultivars, and amendment x cultivar interactions with a p value of ≤ 0.05. Along with the p values, amendment and cultivar averages were also computed.

The averages were in SigmaPlot to make graphs for pH and EC. Tables were used to show averages and significance for dry weights, leaf nutrient analysis, and mycorrhizal fungi.

Results

Chemical properties of artificial media

Initial characteristics of artificial media components (Table 1). The media consisted of pine bark nuggets, green-waste compost, peat moss, perlite, and biochar. Biochar had the highest pH at 9.49, while peat moss had the lowest at 4.04. Compost had the highest total amount of N with 0.84 $mg\cdot kg^{-1}$ of NH_4-N and 64.7 $mg\cdot kg^{-1}$ of NO_3-N; biochar had 0.56 $mg\cdot kg^{-1}$ NH_4-N and 0.75 $mg\cdot kg^{-1}$ NO_3-N; peat moss had 3.53 NH_4-N and 0.4 NO_3-N; and bark had 0.99 NH_4-N and 0.3 NO_3-N. Only compost had copper at 0.08 $mg\cdot kg^{-1}$ Biochar had the highest amount of S and K. Compost had the highest amount of Ca, Mg, S, Fe, Zn, and B.

Plant growth

Dry Weight (Table 2). Jewel had a greater total dry weight (10.92 g) than Jubilee (8.69 g). All amendments were very significant ($p<0.001$). Amendment $Bk_{30}C_{30}Pt_{30}Bi_{10}$ biochar had the greatest amount of total dry weight (13.8 g), while $Bk_{40}C_{40}Bi_{20}$ had the lowest total dry weight (6.44 g). Leaves had no significance in cultivar, but stems, roots, and total all had significance ($p<0.05$). There was no significance for amendment by cultivar for all treatments. Treatment with greatest total dry weight had the greatest dry weight for stems (4.47 g) and leaves (6.39 g), $Bk_{30}C_{30}Pt_{30}Pr_{10}$ had the greatest dry weight in roots (3.05 g).

Leaf nutrient analysis

Macronutrients (Table 3). Jubilee had the highest amount of all macronutrients, except for calcium. N (17 $mg\cdot g^{-1}$) and K (10.31 $mg\cdot g^{-1}$) were highest in $Bk_{40}C_{40}Pr_{10}Bi_{10}$. P (0.85

mg·g^{-1}) and S (1.19 mg·g^{-1}) were highest in $Bk_{40}C_{40}Pr_{20}$. Both amendments that contained no biochar had the highest, and the same, amount of Ca (5.25 mg·g^{-1}). There was a significance ($p<0.001$) of cultivar seen in 4 out of the 6 macronutrients (P, K, Ca, S). There was also a significance seen for amendments ($p<0.01$) in N, P, K, and S. There was no significance seen for amendment by cultivar for all macronutrients.

Micronutrients (Table 4). Jewel had the greatest amount of Fe, Mn, and Zn, while Jubilee had the greatest amount of Cu and B. Amendment $Bk_{30}C_{30}Pt_{30}Pr_{10}$ had the highest amount of Fe (83.88 µg·g^{-1}) and Mn (297.38 µg·g^{-1}). Amendment $Bk_{40}C_{40}Pr_{10}Bi_{10}$ had the highest amount of Zn (19.25 µg·g^{-1}) and B (63.58 µg·g^{-1}). Cu was highest in $Bk_{30}C_{30}Pt_{30}Bi_{10}$. There was a significance ($p<0.05$) of cultivar seen in Mn, Zn, and B. There was a significance of amendment ($p<0.001$) seen only in Mn. There was only a significance of cultivar by amendment seen in B at (p 0.001-0.01).

Weekly readings

Weekly pH (Figure 1). The pH of $Bk_{30}C_{30}Pt_{30}Pr_{10}$ started with the lowest of less than 6, while $Bk_{40}C_{40}Bi_{20}$ started off as the highest over 7.5. $Bk_{30}C_{30}Pt_{30}Pr_{10}$ finished off as the lowest at the end of the 12-week study, but briefly passed $Bk_{30}C_{30}Pt_{30}Bi_{10}$ at week 4. $Bk_{40}C_{40}Pr_{10}Bi_{10}$ and $Bk_{40}C_{40}Pr_{20}$ ended around the same pH after 12 weeks. $Bk_{30}C_{30}Pt_{30}Bi_{10}$ changed the most during the study by increasing to above 7; all the others ended between 7-7.5 as well.

Weekly EC (Figure 2). $Bk_{30}C_{30}Pt_{30}Bi_{10}$ started with the lowest EC (~650 µS·cm^{-1}), while $Bk_{40}C_{40}Pr_{20}$ started with the highest (~1770 µS·cm^{-1}). All treatments seemed to follow the same path by having a drop in EC around week 1, then peaking around week 2 and 3, except for $Bk_{30}C_{30}Pt_{30}Bi_{10}$ that did not have a dip and only peaked at week 3. After this peak, all amendments began to decrease and start to level off around week 8 until week 12. All were very close in readings after leveling off (~200-350 $\mu S \cdot cm^{-1}$).

Mycorrhizal fungi colonization

Table 5. Jewel that the highest amount of mycorrhizal colonization at 19.11%. Amendment $Bk_{40}C_{40}Pr_{10}Bi_{10}$ had the highest amount of colonization at 22.52%, while amendment $Bk_{30}C_{30}Pt_{30}Bi_{10}$ had the lowest at 9.57%. There was no significance seen for cultivar, amendment, or cultivar by amendment.

Table 1. Initial chemical characteristics of artificial media components

Characteristics	Bark	Compost	Peat moss	Biochar
pH	4.72	7.86	4.04	9.49
EC	60	2300	200	2910
NH_4-N (mg・kg^{-1})	0.99	0.84	3.53	0.56
NO_3-N (mg・kg^{-1})	0.3	64.7	0.4	0.75
P (mg・kg^{-1})	2.9	2.56	0.32	20.4
K (mg・kg^{-1})	14.7	582	2.97	682
Ca (mg・kg^{-1})	0.86	88.8	4.65	3
Mg (mg・kg^{-1})	0.47	24.9	3.37	2.19
S (mg・kg^{-1})	0.68	24.7	3.84	13.6
Fe (mg・kg^{-1})	0.14	1.79	0.27	0.1
Mn (mg・kg^{-1})	0.02	0.09	0.04	0.02
Zn (mg・kg^{-1})	0.02	0.12	0.02	0.01
Cu (mg・kg^{-1})	—	0.08	—	—
B (mg・kg^{-1})	0.08	0.51	0.1	0.15

Table 2. Dry weights of two cultivars of blueberries [z]

Cultivar	Stems	Leaves	Roots	Total
Jubilee	2.54 a	4.05	2.11 a	8.7 a
Jewel	3.9 b	4.4	2.62 b	10.92 b
Amendment				
$Bk_{30}C_{30}Pt_{30}Bi_{10}$	4.47 a [x]	6.39 a	2.94 ab	13.8 a
$Bk_{30}C_{30}Pt_{30}Pr_{10}$	4.25 ab	6.15 a	3.05 a	13.45 a
$Bk_{40}C_{40}Bi_{20}$	2.15 b	2.46 b	1.83 b	6.44 b
$Bk_{40}C_{40}Pr_{10}Bi_{10}$	2.54 b	3.11 b	1.94 b	7.59 b
$Bk_{40}C_{40}Pr_{20}$	2.69 b	3.02 b	2.08 b	7.74 b
Significance				
Cultivar	**	NS	*	**
Amendment	***	***	***	***
Amendment x Cultivar	NS	NS	NS	NS

[z] Stems, leaves, and roots were separated at the end of the 12-week study

[Bk, C, Pt, Pr, Bi] bark, compost, peat, perlite, and biochar, respectively

[x] Means followed by the same letter within a column are not significantly different at $P \leq 0.05$
[NS, *, **, ***] Not significant, significant at p<0.001, 0.001-0.01, and 0.01-0.05, respectively

Table 3. Amendment effects on concentration of macronutrients in leaves in two cultivars of blueberries

Cultivar	N (mg·g^{-1})	P (mg·g^{-1})	K (mg·g^{-1})	Ca (mg·g^{-1})	Mg (mg·g^{-1})	S (mg·g^{-1})
Jubilee	12.91	0.86 a	10.26 a	4.73 a	1.67	1.23 a
Jewel	12.83	0.69 b	6.5 b	5.32 b	1.65	0.87 b
Amendment						
$Bk_{30}C_{30}Pt_{30}Bi_{10}$	11.4 b [x]	0.8 a	8.85 ab	4.91	1.55	1.08 ab
$Bk_{30}C_{30}Pt_{30}Pr_{10}$	10.61 b	0.76 a	7.89 b	5.25	1.61	1.01 ab
$Bk_{40}C_{40}Bi_{20}$	10.03 b	0.64 b	7.58 b	5	1.76	0.84 b
$Bk_{40}C_{40}Pr_{10}Bi_{10}$	17 a	0.8 a	10.31 a	4.71	1.61	1.14 a
$Bk_{40}C_{40}Pr_{20}$	15.29 a	0.85 a	7.26 b	5.25	1.75	1.19 a
Significance						
Cultivar	NS	***	***	***	NS	***
Amendment	***	***	**	NS	NS	**
Amendment x Cultivar	NS	NS	NS	NS	NS	NS

[x] Means followed by the same letter within a column are not significantly different at $P \leq 0.05$
[Bk, C, Pt, Pr, Bi] bark, compost, peat, perlite, and biochar, respectively
[NS, *, **, ***] Not significant, significant at p<0.001, 0.001-0.01, and 0.01-0.05, respectively

Table 4. Amendment effects on concentration of micronutrients in leaves in two cultivars of blueberries

Cultivar	Fe (µg·g^{-1})	Mn (µg·g^{-1})	Zn (µg·g^{-1})	Cu (µg·g^{-1})	B (µg·g^{-1})
Jubilee	77	163.36 a	15.43 a	5.27	68.81 a
Jewel	80.42	204.78 b	20.21 b	4.01	48.25 b
Amendment					
$Bk_{30}C_{30}Pt_{30}Bi_{10}$	79.01	240 c	18.19	6.55	59.45
$Bk_{30}C_{30}Pt_{30}Pr_{10}$	83.88	297.38 a	16.6	4.53	56.26
$Bk_{40}C_{40}Bi_{20}$	76.3	85.23 d	16.6	3.51	56.68
$Bk_{40}C_{40}Pr_{10}Bi_{10}$	73.26	146.88 b [x]	19.25	3.95	63.58
$Bk_{40}C_{40}Pr_{20}$	81.09	150.88 b	18.45	4.64	56.68
Significance					
Cultivar	NS	*	**	NS	***
Amendment	NS	***	NS	NS	NS
Amendment x Cultivar	NS	NS	NS	NS	**

[x] Means followed by the same letter within a column are not significantly different at $P \leq 0.05$

[Bk, C, Pt, Pr, Bi] bark, compost, peat, perlite, and biochar, respectively
[NS, *, **, ***] Not significant, significant at $p<0.001$, 0.001-0.01, and 0.01-0.05, respectively

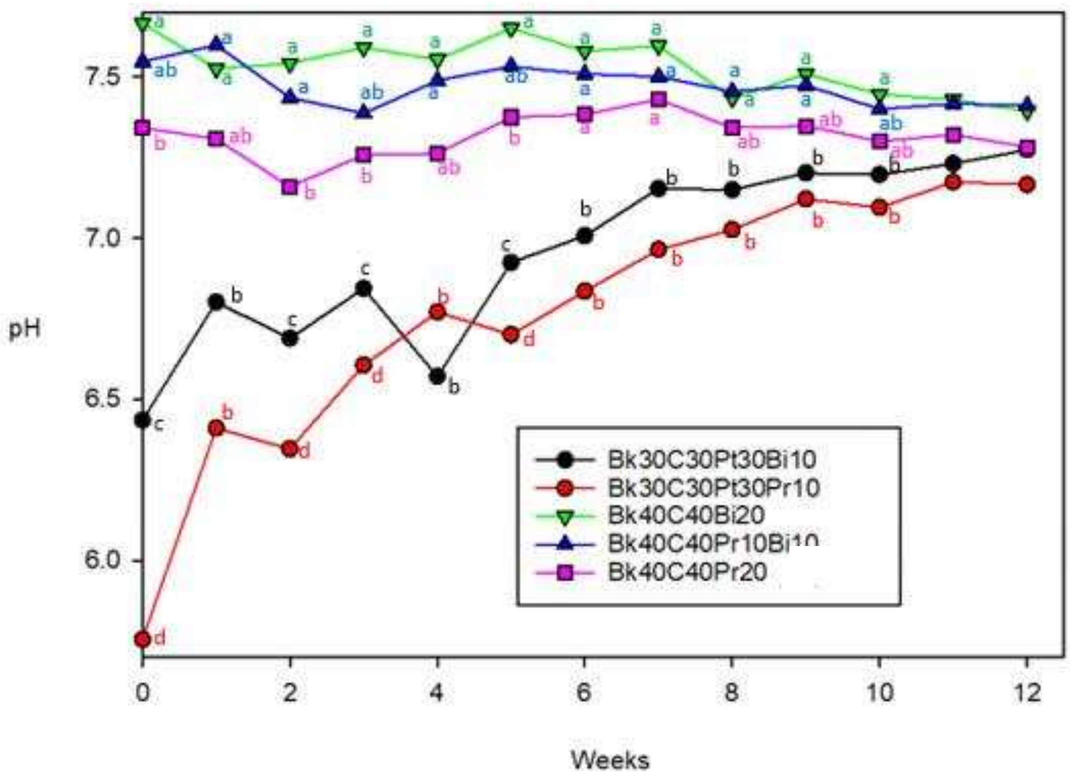

Figure 1. *Amendment effects on weekly pH of 'Jubilee' and 'Jewel' blueberry plants over* 12-week greenhouse study.
Bk, C, Pt, Pr, and Bi indicate amount of bark, compost, peat, perlite, and biochar, respectively
Means followed by the same letter within a week *are not significantly different at* $P \le 0.05$

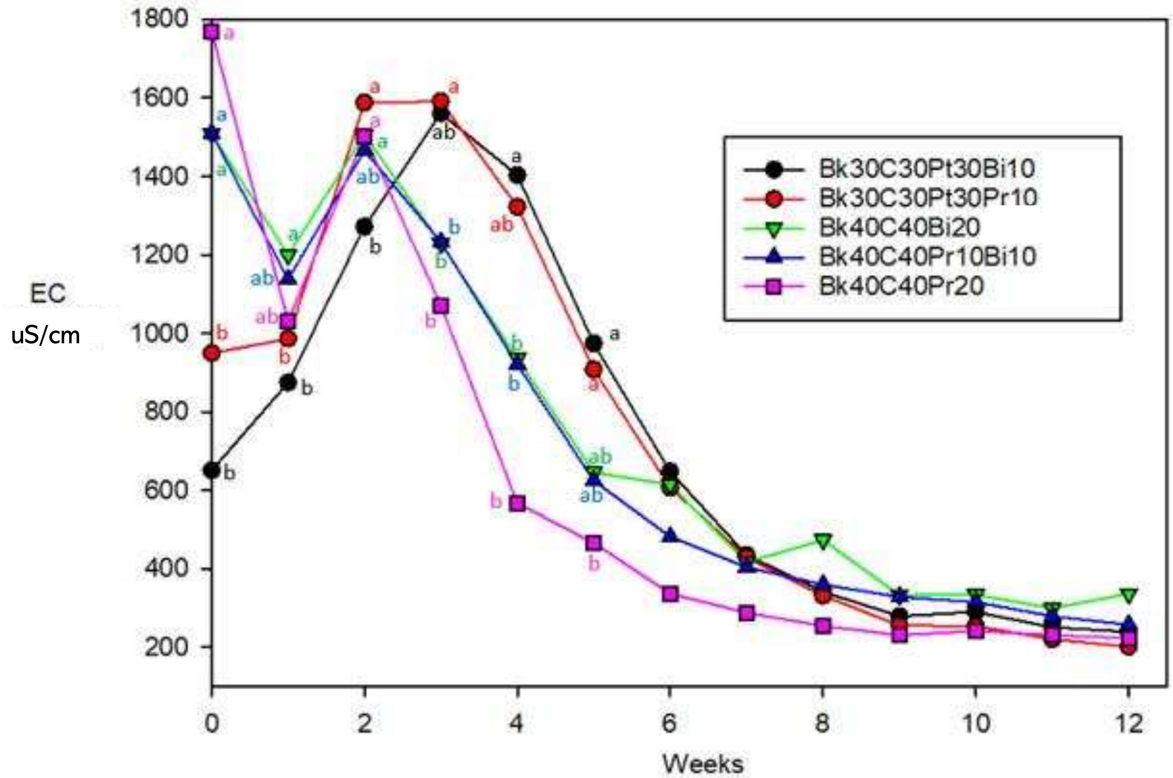

Figure 2. *Amendment effects on weekly EC of 'Jubilee' and 'Jewel' blueberry plants* over 12-week greenhouse study.

Bk, C, Pt, Pr, and Bi indicate amount of bark, compost, peat, perlite, and biochar, respectively

Means followed by the same letter within a column are not significantly different at P *≤ 0.05*

Table 5. Effects of amendments on root colonization by mycorrhizal fungi in two cultivars of blueberries

Cultivar	Mycorrhizal colonization (%)
Jubilee	18.15
Jewel	19.11
Amendment	
$Bk_{30}C_{30}Pt_{30}Bi_{10}$	9.57
$Bk_{30}C_{30}Pt_{30}Pr_{10}$	18.5
$Bk_{40}C_{40}Bi_{20}$	22.28
$Bk_{40}C_{40}Pr_{10}Bi_{10}$	22.52
$Bk_{40}C_{40}Pr_{20}$	20.28
Significance	
Cultivar	NS
Amendment	NS
Amendment x Cultivar	NS

[Bk, C, Pt, Pr, Bi] bark, compost, peat, perlite, and biochar, respectively
[NS] Not significant – no significance was found in colonization

Discussion

Blueberry is commonly grown in substrates composed of peat moss, coir, bark, and perlite in containerized production (Kingston et al., 2017). Components for soilless substrates should create a favorable balance between air porosity and water holding capacity, promote rooting development and nutrient uptake, while also being economically viable (Matt, 2015). We found no significant difference between plants grown in substrates with 10% biochar than those grown in 10% perlite. Plants grown in substrates containing pine bark (30%), compost (30%), peat moss (30%), and either 10% biochar or 10% perlite had, on average, twice the dry weight of plants grown in the other treatments. Indicating that the effect biochar had on plant growth was similar to that of perlite. We also found no discernable difference in the dry weight of plants grown in substrates where peat was removed and replaced with perlite or biochar. While plants grown in these treatments were significantly smaller than those where peat moss was present, the reduced growth was not discernable between treatments, suggesting the effect perlite had on growth was similar to the effect of biochar. Northup (2013) reported that when marigold (Tagetes patula L. French M.), petunia (Petunia x hybrida), impatiens (Impatiens walleriana Hook. f.), broccoli (Brassica oleracea L. Italica group), and pepper (Capsicum annuum L.) were grown in substrates containing biochar, they had equal or greater plant dry weight than those grown in a control containing perlite. Matt (2015) also reported no difference in the growth of Northern Rocky Mountain native plants when grown in increasing amount of biochar vs those grown in the control with perlite.

Plants grown in substrates with peat moss resulted in more plant growth of southern highbush blueberry than those grown in substrates without peat moss. These findings suggest that peat moss provided physiochemical properties to the substrates that were critical to blueberry plant growth. The physical characteristics of substrate materials effect moisture retention, air space, and total porosity. However, Mendez et al. (2015), showed that the addition of 50% biochar to peat moss increased moisture retention than 100% peat moss, indicating that growth inhibition was not due to a lack of moisture. Additionally, Zhang et al. (2008), reported that biochar improved container moisture retention when mixed with compost at rates of 20% and 30%. Other studies have found that biochar had no effect on moisture retention when used as a replacement for peat moss (Steiner and Hartung, 2014; Vaughn et al., 2015). Additionally, biochar has been reported to increase the porosity of both compost and peat moss (Sales et al., 2020). The high porosity of biochar has been found to increase air space in substrates where biochar was added to peat moss (Yan et al., 2020). Therefore, it is unlikely that the physical characteristics of the soilless components had an impact on blueberry plant growth.

The chemical characteristics of soilless substrate components have large impact on nutrient availability, pH, EC, and as a result, plant growth. The cation exchange capacity (CEC) of a substrate represents the substrates net negative charge, and ability to retain nutrients and prevent leaching. The CEC of the biochar is often reported as similar to or greater than peat moss and compost (Vaughn et al., 2015; Kern et al., 2017). Functional groups on the surface of biochar serve as exchange sites for nutrient absorption and are responsible for its CEC. While feedstock and production temperatures determine the CEC of biochar, it is generally considered to have a high CEC. Haedlee et al., (2014) showed that a mixture of biochar (25%) and peat moss (75%) had a higher CEC than that of 100% peat moss. Therefore, it is unlikely that the removal of peat moss and increase of biochar would result in less growth due to nutrient retention.

Plants grown in treatments with peat moss and biochar had similar leaf nutrient content concentrations. In contrast, plants grown in other treatments had significantly higher nutrient concentrations. The lower leaf nutrient values in plants with higher dry weights is due to nutrient dilution from increased growth. Sales et al. (2020) reported very high leaf nutrient levels in plants with stunted growth due to phytophthora root rot. These results indicate a limiting growth factor other than nutrient availability. However, according to the Nutrient Management Guide to Blueberry in Oregon, the leaf nitrogen concentrations of the large plants grown with peat moss were below the recommended levels for blueberry. Pine bark has a low C:N ratio, which has been known to reduce nitrogen availability is soils. Kingston et al. (2017) reported reduced blueberry plant growth with increasing amounts of bark. Therefore, low leaf nitrogen levels were likely a result of nitrogen immobilization. Furthermore, at higher pH levels, nitrogen exists as nitrate (NO_3) instead of ammonium (NH_4), the preferred form of nitrogen for blueberry.

Plant growth can be restricted in soilless media when the EC is above recommended levels for each plant type. Substrates with a high EC, caused by high amounts of salts, hinders nutrient uptake by increasing the osmotic pressure of the nutrient solutions (Samarakoon et al., n.d.). Blueberry is sensitive to salinity and the recommended salinity of substrates for blueberry < 2.0 (mS/cm). Compost can have EC values that are above those recommended for blueberry due to the high amounts of nutrients associated with composts. The EC of biochar also varies with production methods and feedstock (Li et al., 2017; Liang et al., 2016). Several studies indicate that increasing rates of biochar is correlated with increased EC. Vaughn et al., (2015) showed that the addition of 5, 10, and 15% biochar to peat moss and vermiculite increased EC. The biochar and compost used in our study were 2.9 and 2.3 respectively. However, we monitored weekly EC values of the soil solutions and the EC found the EC values of all treatments across the 12-week study was below the recommendation for blueberry. Therefore, is unlikely that elevated EC values decreased plant growth.

Since blueberry is an acid loving plant, the high pH values of the compost and biochar are a concern for blueberry production. Plants grown in treatments where peat moss was present had a much lower soil solution pH than the soil solutions of other treatments through week 9 of the study. Therefore, reduced growth in treatments without peat moss, are likely due to elevated pH levels of the soil solution (Kingston et al., 2017). This is likely due to elevated pH levels in substrates due to the high pH of the compost. Compost has been found to increase pH above levels recommended for optimal blueberry (Sullivan et al., 2012). The recommended substrate pH for blueberry is between 4.5-5.5 and composts are typically neutral to alkaline (7 to 8). The pH of the compost in our study was 7.9, well above the recommended pH for blueberry. Peat moss is often used to reduce the pH of soils for blueberry production (Kingston et al., 2017). The low pH (4.5) of the peat moss in our study likely acted as a buffer to the high pH of the compost. However, substrates without peat moss lacked the ameliorating effect of the peat moss, resulting is less than optimal growth conditions. Pine bark, which has a low pH (4.7), low buffering capacity, and was likely unable to influence pH to the degree of peat moss. It could be argued that peat moss also acted as a buffer to the high pH of the biochar (9.5).

Biochar is not known to drastically affect the pH of soil, the amount present in the soil affects pH (Surampalli et al., 2014). However, biochar is still known to increase pH depending on the rate of application and according to Cornelissen et al. (2018), there have been some documentations of decreases in pH after the addition of biochar (Molnar et al., 2016; Sales et al., 2020). In this study, amendments with more biochar (20%), perlite (20%), or had a biochar-perlite (10% each) mix started with a higher pH. These amendments leveled off and did not change much at the end of the 12-week study. However, those with only 10% biochar or 10% perlite started off more acidic, then became more neutral at the end of the study, supporting that biochar alleviates constraints in acidic and coarse soils by increasing plant growth and productivity (Conte, 2014; El-Naggar et al., 2019). Studies have shown that there is an increase in soil pH immediately after the application of biochar (Shah et al., 2017). Biochar produced below 400 °C had a low pH and low EC (Li et al., 2013). Research has shown that biochar increases pH and EC (Shah et al., 2017). However, the effect varied with the salt contents of biochar since its characteristics can vary between different biochars (Spokas, 2010). This is inconsistent with this study where amendments with biochar did not affect the EC compared to all the other amendments. Amendments that reached the highest EC contained perlite instead of biochar.

The porous nature of biochar provides an advantageous habitat for mycorrhizal fungi colonization by providing a niche for hyphae and protection against fungal grazers (Hockaday et al., 2007; Jaafar et al., 2014; Surampalli et al., 2014). Previous research done on fungal growth on biochar in petri dishes have shown that biochar surfaces could be colonized by fungal hyphae along the cracks (Ascough et al., 2010). According to Jaafar et al. (2014), pore connectivity and pore size could influence microbial colonization of biochar, but there has been little research done on this. Woody biochar has the potential for fungal colonization because of its pore size, therefore enhancing root colonization by mycorrhizal fungi (Solaiman et al., 2010; Warnock et al., 2007). In one study, the level of fungal colonization was greater in soilless media than biochar in soil (Jaafar et al., 2014). In this study, mycorrhizal colonization was at its highest in treatment with 10% perlite and 10% biochar (40% bark, 40% compost) at 22.52%, while treatments with 20% biochar (40% bark, 40% compost) closely followed at 22.28%. However, when perlite or biochar were interacting with the soilless media separately $Bk_{30}C_{30}Pt_{30}Bi_{10}$ or $Bk_{30}C_{30}Pt_{30}Pr_{10}$), the rate of colonization decreased, especially with only biochar. This is inconsistent with previous research done showing that mycorrhizal increases with the application of biochar (Sales et al., 2020). Mycorrhizal colonization is usually lower in blueberry plants when they are grown in fertile soils, this could be why colonization rates were so low in the amendment containing only 10% biochar (Yang et al., 2002). Mycorrhizal fungi are known to form symbiotic relationships with blueberries and other members of the Ericaceae family due to their shallow root systems and lack of root hairs (Cheng et al., 2013; Smith and Read, 2008).

Conclusion

The artificial media in this study interacted with each other to produce the results given, however the exact interactions are not known. Some results were consistent with past experiments done, for example biochar alleviating acidic soils, while some data was inconsistent with previous studies, for example biochar having little effect on plant nutrient concentrations and biochar increasing mycorrhizal fungi colonization. The pH was also higher than expected for blueberries, however, the blueberries still grew very well. Blueberry plants grown in containers have an advantage for temperature regulation, pest control, and moisture. This study can be continued by comparing the interactions of each media substrate to see the significance of the interactions compared to the plant growth, leaf nutrition, and mycorrhiza count of the plants. A longer experiment could be done since it took about 7-8 weeks for everything to stabilize (pH and EC) to give the mycorrhizal fungi time to grow more. Also, an experiment with more plants could be done: 80 plants – 40 grown to about 14-16 weeks, other 40 grown longer and both sets compared. More research is needed on blueberry plants grown with biochar, especially in artificial media through indoor farming.

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